Small Magnetism, Big Attraction: The Magnetotactic Bacteria

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Small Magnetism, Big Attraction: The Magnetotactic Bacteria

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III. Significance of the Magnetosome Chain

IV. Why Make Magnetosomes?

V. Genomics, Genetics and Proteomics

VI. Las Vegas??? Magnetotactic Bacteria in the Desert?

VII. A Magnetotactic Menagerie

VIII. What Does It All Mean?
The magnetotactic bacteria were first reported in 1963 by Salvatore Bellini in an obscure publication of the Instituto di Microbiologia of the University of Pavia, Italy.

He microscopically observed bacteria that swam consistently towards the North Pole and hence called them “baterri magnetosensibili”. Believed cells had an internal magnetic compass.

They were rediscovered by Richard Blakemore in 1974 and described in Science in 1975.
There are (at least) Two Types of Magneto-aerotaxis...

(Frankel et al., (1997) Biophys. J.)

Axial Magneto-aerotaxis

* e.g., *Magnetospirillum magnetotacticum*

Polar Magneto-aerotaxis

* e.g., *Magnetococcus marinus* strain MC-1
IMPORTANT FEATURES OF THE MAGNETOTACTIC BACTERIA

They are diverse with regard to morphology and physiology: “Magnetotactic Bacteria” has no taxonomic meaning

• All known are Gram-negative members of the Domain Bacteria and belong phylogenetically to various subgroups of the Proteobacteria, the Nitrospirae phylum and now the candidate division OP3 of the PVC “superphylum” (monophyletic clade consisting of OP3 together with the candidate division Poribacteria and the Planctomycetes, Verrucomicrobia, Chlamydiae and Lentisphaerae)

• All known are motile by means of flagella

• All display a negative tactic and/or growth response to atmospheric levels of O$_2$ (~21% O$_2$); they are all anaerobes or microaerophiles or both

• They all have a respiratory form of metabolism; only one is known to be capable of fermentation (pyruvate to acetate and H$_2$)

• They have great potential in the biogeochemical cycling of several key elements in natural environments (N, S, C and Fe etc.)

• They are ubiquitous in aquatic habitats and cosmopolitan in distribution. Locally confined to or slightly below the oxic-anoxic interface.
That internal compass of Bellini…?

Magnetotactic Bacteria Biomineralize Magnetosomes

Defined as an intracellular magnetic mineral crystal, either magnetite ($\text{Fe}_3\text{O}_4$) or greigite ($\text{Fe}_3\text{S}_4$), surrounded by a lipid-bilayer membrane (the “magnetosome membrane”)

From: Gorby, Beveridge & Blakemore, J. Bacteriol. 1988
Magnetosomes

Note consistent species-specific magnetosome crystal morphology:
First suggestion of genetic and crystallochemical control over magnetosome biomineralization

Uncultured rod-shaped bacterium from Little Styx River, New Zealand
Scale Bar = 0.5 µm (from R.P. Blakemore)
## Minerals Produced by Magnetotactic Bacteria

<table>
<thead>
<tr>
<th>Mineral (magnetism)</th>
<th>Formula</th>
<th>Produced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetite (+)</td>
<td>Fe₃O₄; Fe²⁺Fe³⁺₂O₄</td>
<td>Cocci, Spirilla, Rods</td>
</tr>
<tr>
<td>Greigite (+)</td>
<td>Fe₃S₄</td>
<td>Many-celled Prokaryote (MMP), Rods</td>
</tr>
<tr>
<td>? (-)</td>
<td>Sphalerite-type FeS*</td>
<td>MMP, Rods</td>
</tr>
<tr>
<td>Mackinawite (-)</td>
<td>Tetragonal FeS*</td>
<td>MMP, Rods</td>
</tr>
<tr>
<td>Pyrite (-)**</td>
<td>FeS₂</td>
<td>MMP</td>
</tr>
<tr>
<td>Pyrrhotite (+)**</td>
<td>Fe₇S₈</td>
<td>MMP</td>
</tr>
</tbody>
</table>

Intermediates in greigite formation. **Probably errors in mineral identity.**

Magnetosome crystals are generally of high structural perfection...

Idealized Crystal Morphologies from HRTEM Studies

a-d: Fe$_3$O$_4$; a, cubo-octahedron (equilibrium form); b-c, hexahedral prisms; d, elongated cubo-octahedron

e-f: Fe$_3$S$_4$; a, cubo-octahedron (equilibrium form); b, rectangular prismatic crystal

The elongated and tooth-shaped crystals of magnetite are unique and have been used as biomarkers or “magnetofossils” in sediments and meteorites
Magnetosomes Contain Crystals that are Stable Single-Magnetic-Domains

- Magnetotactic bacteria produce crystals that are the smallest crystals that can be formed of Fe$_3$O$_4$ or Fe$_3$S$_4$ and still be permanently magnetic at ambient temperature

- Thus, these organisms, by forming SMDs, have maximized the magnetic remanence of the individual magnetosome crystals
Magnetotactic Bacteria Generally Arrange Their Magnetosomes in (a) Chain(s) Along Long Axis of Cell

In the chain motif, the total cell magnetic dipole moment is the sum of that of the individual crystals - thus by forming chains, the bacterium has maximized its magnetic dipole moment (remanence)
The magnetosome chain acts like a single magnetic dipole!

From: Dunin-Borkowski, McCartney, Frankel, Bazylnski, Posfai & Buseck, Science 1988
So Magnetosomes are Clearly a Masterpiece of Microbial Engineering…

But Why
Make
Magnetosomes?
The Big Question…
Magnetotactic Bacteria Need to Find and Maintain an Optimal Position in Gradients

Magnetotaxis appears to increase efficiency of chemotaxis in vertical chemical gradients (e.g., Fe$_3$O$_4$-producers and O$_2$ gradients) by reducing a 3-dimensional search problem to a 1-dimensional search problem.

But is this the complete story? Role of magnetite, possibly.

But why do cells take up and process so much Fe in the first place?
South-seeking Magnetotactic Bacteria in the Northern Hemisphere??!!

A. Small rod-shaped magnetotactic bacterium

B. The “barbell”

The MMP →

Scale Bars = 5 μm

From: Simmons, Bazylinski & Edwards, (2006) Science
How Do Magnetotactic Bacteria Construct the Magnetosome Chain?

Appears to be a complex process that involves a number of steps…

1) Magnetosome vesicle formation

2) Uptake and transport of Fe into the cell

3) Transport of Fe into the magnetosome vesicle

4) Biomineralization of Fe$_3$O$_4$ in the magnetosome membrane vesicle

Keep in mind… Not all steps are temporally ordered within the cell

Magnetotactic bacteria pump lots of Fe! 1-3% Fe on a dry weight basis

(Compare to *Escherichia coli…* 0.025% Fe (Madigan et al., 2003))
Genes and Proteins Involved in Construction of the Magnetosome Chain

Protein Profiles of Fractions of strain MV-1

SF: Soluble Cell Fraction

MF: Membrane Fraction (without magnetosomes)

MM: Magnetosome Membranes

MW: Molecular Weight Markers

Magnetosome Membranes Contain Unique Proteins
The Magnetosome Membrane Originates as an Invagination of the Cell Membrane

Use of Electron Cryotomography

From: Komeili et al., (2006) Science
Genes and Proteins Involved in Construction of the Magnetosome Chain

Magnetosome membrane proteins and genes are referred to as:

- Mam or *mam*
- Mms or *mms*
- Mtx or *mtx*
Role of Some of the Magnetosome Proteins in the Construction of the Magnetosome Chain

MamK – actin-like protein similar to MreB
Appears to be essential for construction of magnetosome chain

Magnetospirillum magneticum
From: Komeili et al., Science 2006
Role of Some of the Magnetosome Proteins in the Construction of the Magnetosome Chain

MamJ – an acidic protein associated with a “filamentous structure”

From: Scheffel et al., Nature 2006

*Magnetospirillum gryphiswaldense*
MamJ (continued)

Blue: Cell Membrane
Red: Magnetite Crystal
Yellow: Magnetosome Membrane
Green: “Filamentous Structure” (MamK)

Magnetospirillum gryphiswaldense

Other Magnetosome Proteins...

MamB, MamM: cation diffusion facilitator (CDF) proteins… heavy metal transporters

MamC, MamD (Mms13 and Mms7, respectively) – Size of magnetite crystal (Scheffel et al. 2008)? MamC appears to be the most abundant magnetosome membrane protein in several strains

MamE: HtrA-like serine proteases… serine protease induced by heat shock in *Escherichia coli*… action in periplasm

Mms16: GTPase activity… formation of magnetosome membrane vesicle (Okamura et al. 2001) … but probably not

Mms5, Mms6, Mms7, Mms13: tightly bound to Fe$_3$O$_4$… (Arakaki et al. 2003)

Mms6: Purified has an effect on Fe$_3$O$_4$ crystal morphology (Arakaki et al. 2003; Prozorov et al. 2007; Amemiya et al. 2007)

MagA: Found in magnetosome membrane and cell membrane… a putative Fe$^{2+}$ pump
Does Mms6 Have an Effect on Magnetite Crystal Morphology?

Scale bars = 200 nm

Figure 1. TEM images of magnetite nanoparticles obtained by co-precipitation of FeCl$_2$ and FeCl$_3$: A) without protein, B) with Mms6, C) with ferritin (Note that ca 5 nm iron oxide nanoparticles seen as darker small dots appear embedded in surrounding globular bodies, most likely protein), D) with Lnc2, and E) with BSA. F) Selected-area electron diffraction pattern of Mms6-derived magnetite cluster with random orientation showing varying intensities of the diffraction rings; G) selected-area electron diffraction pattern of Lnc2-derived magnetite cluster with random orientation, showing weaker intensity and a larger number of the electron diffraction peaks; and H) the selected-area electron diffraction pattern from ferritin-derived magnetite nanoparticles. Here the individual electron diffraction peaks overlap and form electron diffraction rings, which is consistent with the presence of a large number of small and randomly oriented nanoparticles. Scale bars: 200 nm.

Organization of the Magnetosome Genes…

*Magnetococcus marinus* MC-1

In *Magnetospirillum* species, *Magnetococcus marinus*, *Desulfovibrio magneticus* and the vibrio *Ca. Magnetovibrio blakemorei* strain MV-1, the magnetosome genes appear to be organized in clusters... and the clusters themselves are in close proximity.

The genomes of *M. magnetotacticum*, *M. magneticum* and *Magnetococcus* are available at various websites. (That of *M. magneticum* and strain MC-1 are completed).

In *M. gryphiswaldense* the magnetosome genes are found in a stretch of DNA about 35 kb long within a 130 kb segment of DNA surrounded by transposons... called a magnetosome gene island... spontaneous non-magnetotactic mutants have been shown to lack this latter 130 kb section (Schübbe et al. 2003; Ullrich et al. 2005).

Putative magnetosome islands have been found in other strains... Significance... strong possibility of lateral transfer of magnetosome synthesis genes... may explain broad diversity of magnetotactic bacteria...
Magnetosome Gene Island (MAI) in *Magnetospirillum gryphiswaldense*

**Magnetospirillum spec.**

- Core genome
- Mobile element(s)
- tRNAs
- Genes with accessory functions
- Mobile element(s)
- Genes with accessory functions
- Mobile element
- Core genome

**Operons**

- *mms* operon
- *mamGFDC* operon
- *mamAB* operon


“General” Gene Island

Dobrindt et al., 2004

Dobrindt et al., 2004

Dobrindt et al., 2004
UNLV - Some Changes in Lab...
~2008

New Postdoc Christopher Lefèvre
With Chris Came Some New Ideas…

OK. Who's thinking outside the box again?
And New Approaches…

Genetics versus Ecology and Phylogeny?

Why not look for magnetotactic bacteria on our own backyard?

We had Lake Mead but not many other sources of water.

How do we find these places…?

1) Google Earth maps looking for green!
2) Driving around looking for green!
What did we expect to find?

But magnetotactic bacteria in the desert?
And back to some big questions…

Evolution of magnetotaxis… and associated questions

Was there any evolutionary link based on the known phylogeny of the magnetotactic bacteria, i.e., could this information give clues as to whether magnetotaxis is monophyletic or polyphyletic?

Why greigite-producers only found in marine environments?

Were there any magnetotactic bacteria that could be considered extremophilic? Were any phylogenetically affiliated with the Archaea?
New Magnetotactic Bacteria of the *Nitrospirae* Phylum (1)

Strain: LO-1

Status: Uncultured

Morphology: Ovoid, 3.5 ± 0.5 µm by 2.7 ± 0.3 µm (n = 53) in size

Habitat: Found in a number of freshwater to brackish springs and from Lake Mead

Temperature Relationship: Mesophilic

Oxygen Relationship: Microaerobic and/or Anaerobic?

Magnetosomes: Bullet-shaped crystals of magnetite
Strain LO-1 (cont.)
Strain LO-1 (cont.)
New Magnetotactic Bacteria of the *Nitrospirae* Phylum (2)

Strain: HSMV-1

Morphology: Vibrioid to helical, 1.8 ± 0.4 by 0.4 ± 0.1 µm (n = 59) in size

Habitat: Found in a series of brackish hot springs in the Great Boiling Springs geothermal field near Gerlach, NV

Temperature Relationship: Facultatively moderately thermophilic with a probable maximum temperature limit of ~63°C

Oxygen Relationship: Probably Anaerobic

Magnetosomes: Bullet-shaped crystals of magnetite
Strain HSMV-1 (cont.)
Strain HSMV-1 (cont.)

Strain HSMV-1 = *Candidatus* Thermomagnetovibrio paiutensis

New Magnetotactic Bacteria of the *Proteobacteria* Phylum: *Deltaproteobacteria* Class

Focus was highly alkaline habitats…

Mono Lake, CA: salinity 68 ppt pH = 9.5

Soda Spring, Zzyzx Road CA salinity 3 ppt, pH = 9.5
New Magnetotactic Bacteria of the *Proteobacteria* Phylum: *Deltaproteobacteria* Class

Uncultured

Strain ML-1 from Mono Lake

Cultured
New Magnetotactic Bacteria of the *Proteobacteria* Phylum: *Deltaproteobacteria* Class

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Strain HB-1</td>
<td>Cultured</td>
</tr>
<tr>
<td>(From alkaline spring at Death Valley Junction)</td>
<td></td>
</tr>
<tr>
<td>2) Strain ML-2</td>
<td>Cultured</td>
</tr>
<tr>
<td>(From Mono Lake)</td>
<td></td>
</tr>
<tr>
<td>3) Strain ZZ-1</td>
<td>Cultured</td>
</tr>
<tr>
<td>(From Soda Lake)</td>
<td></td>
</tr>
</tbody>
</table>

All strains: HB-1, ML-2 and ZZ-1

Morphology: Vibrioid to helical, single polar flagellum

Temperature Relationship: Mesophilic

Oxygen Relationship: Anaerobic (sulfate-reducers)

pH: Optimum pH 9.5, no growth at pH < 9.0
New Magnetotactic Bacteria of the *Proteobacteria* Phylum: *Deltaproteobacteria* Class

Sequence identity $\geq 97\%$: same species  
$<97\%$ but $>95\%$: different species  
$>5\%$: different genus

New Magnetotactic Bacteria of the Proteobacteria Phylum: Gammaproteobacteria Class

Bacterium                     Status

1) Strain BW-2               Cultured
   (BW = Badwater Basin, Death Valley National Park, CA)

2) Strain SS-5               Cultured
   (SS = Salton Sea, CA)
Strains BW-2 and SS-5

A, B: Strain BW-2
Morphology: Rod-shaped, polar
bundle of 7 flagella

Habitat: Found in brackish springs of
Badwater Basin (salinity ~28 ppt)

Temperature Relationship: Mesophilic
Oxygen Relationship: Microaerobic

C-E: Strain SS-5
Rod-shaped, single polar flagellum

Found in hypersaline water and sediment
of Salton Sea (salinity ~52 ppt)

Mesophilic
Microaerobic

Magnetosomes of Strains BW-2 & SS-5

A,B: Strain BW-2
Cuboctahedral crystals of magnetite

C-F: Strain SS-5
Elongated prismatic crystals of magnetite
Strains BW-2 and SS-5

Grows in $O_2$ gradient medium

$S^{2-}$ or $S_2O_4^{2-}$ as electron donors oxidized to $SO_4^{2-}$

Bicarbonate is sole C source: autotrophic
Phylogeny of Strains BW-2 and SS-5
Greigite-producing Magnetotactic Bacteria

Two Types – Both thought to be restricted to marine environments. None of either type had been cultured.

1) Magnetotactic Multicellular Prokaryotes (MMPs)

2) Large rod-shaped bacteria
Fe₃O₄ and Fe₃S₄ in the Same Bacterium
Mineral Composition Affected by External Conditions?

Uncultured rod from Pettaquamscutt Estuary (RI)... only marine?

Greigite-producing Rods

Not restricted to marine environments…

Gram-negative rods all with single polar flagellum

Some produce Fe₃O₄ as well as Fe₃S₄
Greigite-producing Rods Phylogeny

These organisms represent a previously unrecognized group of sulfate-reducing bacteria
Greigite-producing Rods
Isolation of Strain BW-1

Because of the large amount of cells we could collect, we went after the greigite-producing rod from Badwater Basin, Death Valley National Park... Mineral composition affected by external chemical and redox conditions!!
We are currently sequencing the genome of BW-1... what have we found thus far?

There are two cassettes (islands?) of mam genes... one in which genes have the higher identities with those of Fe₃O₄-producing magnetotactic bacteria and the other, higher identities with those of the greigite-producing MMP, Candidatus Magnetoglobus multicellularis!

First remember that magnetotactic bacteria are phylogenetically associated with the Candidate OP3 superphylum and the *Nitrospirae* and *Proteobacteria* phyla.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mineral</th>
<th>Crystal Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidate OP3</td>
<td>Fe$_3$O$_4$</td>
<td>Bullet-shaped</td>
</tr>
<tr>
<td><em>Nitrospirae</em></td>
<td>Fe$_3$O$_4$</td>
<td>Bullet-shaped</td>
</tr>
<tr>
<td><em>Deltaproteobacteria</em></td>
<td>Fe$_3$O$_4$, Fe$_3$S$_4$</td>
<td>Bullet-shaped, Mixed morphologies</td>
</tr>
<tr>
<td><em>Alphaproteobacteria</em></td>
<td>Fe$_3$O$_4$</td>
<td>Cuboctahedral &amp; Elongated Prismatic</td>
</tr>
<tr>
<td><em>Gammaproteobacteria</em></td>
<td>Fe$_3$O$_4$</td>
<td>Cuboctahedral &amp; Elongated Prismatic</td>
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</tbody>
</table>

What is the most primitive form of magnetite crystal?
Origin of Magnetotaxis and Magnetosome Formation
Origin of Magnetotaxis and Magnetosome Formation

What does this all mean?

What was the first magnetosome mineral produced by the magnetotactic bacteria?
Based on known Bacterial phylogeny, Fe$_3$O$_4$

What was the first magnetosome crystal morphology?
Based on known Bacterial phylogeny, bullet-shaped

Where did greigite magnetosome formation (genes) originate?
Originated in the *Deltaproteobacteria*... gene duplication and mutation?

Why no magnetotactic members of the Beta-, Epsilon- and Zetaproteobacteria?
??? Maybe there are and just not discovered yet. Genes lost?

Must take this new information into consideration on the interpretation of magnetosfossils as biomarkers
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Fe₃O₄ particles are promiscuous!

Fe₃O₄ particles (magnetosomes) are in higher organisms including salmon, trout, sea turtles, bees, birds... cows?

And possibly humans!

Are similar genes for magnetite biomineralization present in these organisms?