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Magnetosome Genes in the *Gammaproteobacterium* Strain BW-2

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Abstract

Magnetotactic bacteria (MTB) biomineralize intracellular nanometer-sized, magnetic crystals surrounded by a lipid bilayer membrane known as magnetosomes. These crystals, which consist of magnetite (Fe₃O₄) or greigite (Fe₃O₄·Fe₂O₃), cause the cell to align along the geomagnetic field lines as they swim, a phenomenon known as magnetotaxis. Strain BW-2 is a magnetite-producing magnetotactic bacterium isolated from Badwater Basin, Death Valley National Park (California) and is one of only two species of MTB that are known to phylogenetically belong to the *Proteobacteria* class of the *Gammaproteobacteria* phylum. The biomineralization of magnetite in magnetotactic bacteria is mediated by a series of genes that include the *mam*, *mms*, and *mtx* genes that presumably control the production of and the size and shape of the magnetosome crystal within the magnetosome. Magnetosome genes have not yet been found in the genomes of newly discovered magnetotactic *Gammaproteobacteria*.

In this study, we use polymerase chain reaction with degenerate primers designed from mom genes found in other MTB, and DNA sequencing to search for and amplify possible mom genes in the genome of strain BW-2. In addition, with enough DNA sequence, we may be able to find evidence of the presence of a magnetosome gene island in this organism. Positive results from this study will be instrumental in determining evidence for lateral gene transfer of the magnetosome gene island to the *Gammaproteobacteria* and the evolution of magnetotaxis based on magnetite biomineralization in general.

Introduction

Magnetotactic bacteria (MTB) are Gram-negative prokaryotes that display a phenomenon known as magnetotaxis where these organisms align along geomagnetic field lines as they swim (LeFebvre et al. 2011). MTB experience a torque in a magnetic field that causes them to passively align along magnetic field lines: cells are not being pulled in any direction (as shown on Figure 1) and even dead cells align but don’t swim. This phenomenon is caused by the ability of MTB to biomineralize intracellular magnetic crystals (Figure 2) that consist of either an iron oxide or iron sulfide surrounded by a lipid bilayer known as magnetosome (Bazzlinski and Frankel, 2004). Most of these organisms organize magnetosomes in one or more chains (Bazzlinski and Frankel, 2004). The control of production and specific characteristics of these crystals display significant diversity that is mediated by a series of genes known as the *mam*, *mnt*, and *mms* genes. These genes are organized in genomes of MTB of the *Alphaproteobacteria* and *Deltaproteobacteria* within a genomic island, known as the magnetosome island. Genomic islands are typically surrounded by transposable elements such as insertion sequences, transposases, and pseudogenes (Bazzlinski and Frankel, 2004). Because of this, magnetosome genes and the trait of magnetotaxis is thought to have been transmitted to many different bacteria through horizontal gene transfer.

The purpose of this study is to search for and amplify mom genes in the newly-isolated *Gammaproteobacterium* strain BW-2. If enough DNA sequence is obtained, it may be possible to show evidence for the existence of the magnetosome island in this organism thus also providing evidence for horizontal gene transfer of the magnetosome island to the *Gammaproteobacteria*.

Methods

The polymerase chain reaction (PCR) using degenerate primers (Table 1) for specific magnetosome genes was employed to amplify possible mom genes in strain BW-2. Primers were designed from mom genes found in magnetotactic *Alphaproteobacteria* and *Deltaproteobacteria*. PCR products were sequenced (Functional Biosciences, Inc., Madison WI) while some PCR products were cloned into pGEM-T Easy Vector (Promega) prior to sequencing.

**Table 1. Degenerate PCR primers used to amplify magnetosome genes in strain BW-2.**

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<thead>
<tr>
<th>Primer Name</th>
<th>5’ Primer Sequence</th>
<th>3’ Primer Sequence</th>
<th>Product Size (bp)</th>
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<tr>
<td>mamO</td>
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<td>mamK</td>
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<td>5’-cgttggaaatgtacgcac-3’</td>
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<td>5’-cgttggaaatgtacgcac-3’</td>
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<tr>
<td>mamI</td>
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<td>5’-gccgtgagcttcaacggca-3’</td>
<td>374</td>
</tr>
</tbody>
</table>

Figure 1. The effect of the Earth’s inclined geomagnetic field lines on a magnetotactic bacterium. Cells align themselves in a chain (A) that tends to align them along the field lines while they swim, if they were a compass needle. Cells are not being pulled in any direction.

Figure 2. Transmission electron micrograph of a cell of strain BW-2 (A) and a magnetosome chain from the organism (B).

Discussion

Here we show strong evidence that the magnetosome genes, *mamK* and *mamO*, are present in the genome of BW-2. Although, no evidence for the presence of a large number of other magnetosome genes was found in strain BW-2, the genes may still be present. This most likely due to the fact that the degenerate primers we used were designed from sequences of magnetotactic *Alphaproteobacteria* and *Deltaproteobacteria* genes, which are very different enough that most of the primers we used were not effective. We also expected to amplify a fragment of 4 kb, based on data from magnetotactic *Alphaproteobacteria* and *Deltaproteobacteria*, between the genes *mamK* and *mamO* with primers designed directly from strain BW-2. However, we did not, and it may be that the fragment could not be amplified for a number of reasons, one likely possibility being that the organization of the magnetosome genes is different in strain BW-2 compared to other MTB (Figure 3).

Our results make strain BW-2 an excellent candidate for a whole genome sequencing.

Future Directions

Studying magnetite biomineralization in magnetotactic bacteria is important because magnetite magnetosomes have been shown to have unique magnetic, physical and optical properties that can be exploited in a very large number of scientific, commercial, and medical applications (Lang and Schiller, 2006) including magnetic cell separation and the diagnosis and treatment of cancerous tumors.

Acknowledgements

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References