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The Characterization of two diverse magnetotactic bacteria: LEMS and MMS-1

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Magnetotactic bacteria (MTB) are a diverse group of prokaryotes that biomineralize membrane-bound magnetic crystals known as magnetosomes. The magnetosomes are aligned within the cell and consist of either magnetite (Fe$_3$O$_4$) or greigite (Fe$_3$S$_4$). The biomineralization of magnetosomes consists of several processes including: invagination of the cytoplasmic membrane, iron uptake into the cell and then into the magnetosome membrane vesicle, and crystallization of the mineral phase inside the vesicle. Mam genes control magnetosome biomineralization with most of the genes present in an island called a magnetosome island. Many of the mam genes are conserved between different species of MTB. The genes that are in the island have suggested that they play a significant role in the organization of the magnetosomes and how they align within the cell. The focus of this investigation is to determine if certain conserved mam genes are found in two isolated and metabolically diverse magnetotactic spirillums: LEMS and MMS-1.
The Characterization of Two Diverse Magnetotactic Bacteria: LEMS and MMS-1

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Abstract

Magnetotactic bacteria (MTB) are a diverse group of prokaryotes that biomineralize membranes-bound magnetic crystals known as magnetosomes. The magnetosomes are aligned within the cell and consist of either magnetite (Fe₃O₄) or greigite (Fe₃O₄). The biomineralization of magnetosomes takes place through a complex set of processes: synthesis of the cytoplasmic membrane, iron uptake into the cell and then into the magnetosome membrane vesicle, and crystallization of the mineral phase inside the vesicle. Membrane control magnetosomes biomineralization with most of the genes present in an island called a magnetosome island. Many of the main genes are conserved between different species of MTB. The genes that are in the island have suggested that they play a significant role in the organization of the magnetosomes and how they align within the cell. The focus of this investigation is to determine if certain conserved main genes are found in two isolated and metabolically diverse magnetotactic epibionts: LEMS and MMS-1.

PCR was the primary tool used to determine the similarity of DNA of other MTB using Mam primers to amplify with LEMS and MMS-1. The information gained from the successful sequencing of these genera for MMS-1 and LEMS will provide a piece of the complex and difficult task of organizing and completing a genomic library.

Introduction

Magnetotactic Bacteria are a highly diverse group of prokaryotes that biomineralize membrane-bound magnetic crystals known as magnetosomes. Magnetosomes are intracellular structures comprising magnetic iron mineral crystals enveloped by a phospholipid bilayer membrane known as the Magnetosome membrane (Barby et al., 1985; Komlev et al., 2006; Bazylinski et al., 2007). The MMM contains proteins that are unique to the MMM and that it is likely that these proteins play the key roles in magnetic biomineralization in magnetosomes. The proteins and the genes that encode for these are called the Mam (magnetosome membrane) or Mms (magnetic particle membrane specific) proteins (in mam or mms genes (Bazylinski et al., 2007).

The magnetosomes in every magnetotactic bacteria genome examined showed they are in close proximity. The genomic region that contained the Magnetosome genes in M. gppakondoense also contained 22 mobile elements annotated as transposases of the insertion sequence type and integrases (Bollschweiler et al., 2005). These mobile elements are common and important features in genomic islands (Mahillon and Chandler, 1998; Mahillon et al., 1999). The similar organization of the magnetosome operons in different magnetotactic bacterial strains assumes that the magnetosome gene island might have been transferred via horizontal gene transfer to many different types of bacteria. This would explain the great diversity of the group. The isolated magnetosomes under different conditions able to retain many of the properties of the MTB and the diversity of magnetotactic bacteria not every single gene of the island is preserved in every species. The goal of this study is to determine what many genes are essential to those which are predicted, and thus found in common species of magnetotactic bacteria.

Data/ Results

Current is the only mam and mms1 gene has been successfully sequenced and have been shown similarity to the other magnetotactic bacteria from LEMS, the samples of MMS-1 which were mmaM and mms1 remain without contamination and after blanding the sequences return results of Arabidopsis. These are currently biologically sequenced and should result in the distribution of these results.

Methods

1. In order to characterize magnetotactic bacteria, the first thing that must be done is to compare the genes of our magnetotactic spirals with other magnetotactic bacteria which have been predicted to have the same gene by running a homology search.
2. Try if any of these genes match with any of the other magnetotactic bacteria via the genome database and not the individual cell. Try if the gene is growing or being a single cell. A single cell in the reaction up to 16 pl and repeat PCR and electrophoresis.
3. The PCR product will provide sufficient DNA for direct sequencing or direct sequencing, but the amount of DNA can be increased using the genomic technique and can be transferred to a filter E. coli cells.
4. The PCR product is first extracted from the gel, using the methods of the Qiagen DNA extraction kit.
5. The DNA is mixed with the Oligo dT primer, and 10X Buffer, and 10X dNTP buffer.
6. The competent cells are first heat shocked to introduce the new strain of DNA into the cell.
7. The E. coli cells will grow over 12 to 24 hour to allow the DNA to be enriched in growing cells.
8. Upon isolation the following day the cells are plated and stained to introduce colonies of the transformed E. coli cells.
9. Several colonies are cloned into a plasmid for growth for no more than 16 hours, when e. coli is in its exponential phase growth.
10. The cells are then grown in the presence of the appropriate antibiotic and plated and grown overnight to produce clones of the transformed E. coli cell.
11. Several colonies are selected to grow in Liquid media for no more than 16 hours, when e. coli is in its exponential phase growth.
12. The cells are then grown overnight in the presence of the appropriate antibiotic and plated and grown overnight to produce clones of the transformed E. coli cell.
13. Several colonies are cloned into a plasmid for growth for no more than 16 hours, when e. coli is in its exponential phase growth.
14. The sequence is then used to Applied Bioscience for sequencing.
15. When the sequencing results are received, vector MTN is used to get a consensus and continue the sequence.
16. The early data is then input into the HCR Biotech Local Alignment Sequencing Tool (HCR-BLAST) to check for its similarity to marine bacteria that are already in the database to check for similarity between the genes.

Conclusion/Future Research

In conclusion, the research was beneficial not only because mam and mms1 are conserved between these methanospirilla spirals, but also because the new primers offer amplification. Further characterization of these primers could be expected to have within the conserved region of the magnetotactic bacteria are expected to confer less close constraint to provide much more detailed results.

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