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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Magnetotactic bacteria selectively synthesize membrane-bound, nanometer-sized, single-domain magnetic particles known as magnetosomes. Because these bacteria have complex nutritional requirements, only one species, Aquaspirillum magnetotacticum has been grown in pure culture. This bacterium produces approximately twenty intracellular magnetic particles per cell of single-domain size. To synthesize these particles, A. magnetotacticum must possess a highly efficient system(s) to remove iron from the environment. To investigate the mechanism of iron-uptake and the synthesis of magnetic particles in this microorganism, we will construct and screen genomic libraries of A. magnetotacticum for the iron-uptake and magnetosome-synthesizing genes. We will also use the available information on the mechanisms of iron-uptake in other bacteria to identify and characterize analogous systems, related genes, or homologous sequences in this magnetotactic bacterium. We have determined already that the genes of A magnetotacticum are functionally expressed in E. coli. Furthermore, we have identified in

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this bacterium a sequence homologous to the tonB gene of E. coli. The tonB gene is known to be required for iron assimilation in enteric bacteria. The long-term goal of this project is to clone the identified genes in a suitable host organisms that would make the large-scale, regulated production of single-domain magnetic particles possible. The large-scale biological production of these particles will have a significant impact on various technologies that are important for Navy applications.

PROGRESS REPORT ON CONTRACT N00014-89-C-0085

TITLE: Genetic Engineering of Single-Domain Magnetic Particles

PRINCIPAL INVESTIGATOR: Nahid S. Waleh. Molecular Biology Department
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START DATE: March 1, 1989

RESEARCH OBJECTIVES

1. Screen the genomic library of *A. magnetotacticum* for iron-uptake and magnetosome synthesizing genes.
2. Clone and sequence the *tonB* homologous sequence.
3. Determine the function of "*tonB*-like" gene in *A. magnetotacticum*.
4. Identify and clone sequences of *A. magnetotacticum* that are homologous to known iron-uptake genes in other microorganisms.

PROGRESS AND PLANNED ACTIVITIES

Screening the Genomic Library

We have screened the genomic library prepared from the DNA of *A. magnetotacticum* for the genes of a siderophore-mediated iron-uptake system. Such a system had previously been reported to exist in this bacterium. The DNA fragments of *A. magnetotacticum* were cloned into the cosmid vector c2RB, and the recombinant cosmids were propagated in an iron-uptake-deficient *E. coli* host strain. Library clones were plated on a medium containing a dye-iron complex (developed at J. B. Neilands' laboratory at UC Berkeley) that turns from blue to orange in the presence of a chelating molecule.

In spite of our extensive screening, we were unable to identify a siderophore-producing colony. Out of 10,000 recombinant plasmid-carrying colonies tested, none changed the color of the medium from blue to orange. (We have already established and reported that there is a representation of every functional sequence in about 300 colonies of this library.)

One possibility is that the iron-uptake genes are scattered in the chromosome of *A. magnetotacticum*. In that case, one should be able to detect the siderophore or its iron-binding activity in the supernatant culture fluids of *A. magnetotacticum*. We used the Csaky test for the detection of a hydroxamate-type siderophore. Hydroxylamine and benzohydroxamic acid were used as positive controls and water was used as negative control. In this test, we were unable to detect any hydroxamate-type molecule in the supernatant culture fluids of *A. magnetotacticum*, even when the supernatant was concentrated by about 20-fold. We used the color assay medium of Neilands to detect any iron-binding activity in the supernatant fluid cultures of *A. magnetotacticum*. The supernatant of a culture of *E. coli* grown under iron-limiting conditions and the uninoculated medium of *A. magnetotacticum* were used as positive and negative controls, respectively. In this test also, we did not observe any iron-binding activity, and the color of the assay solution remained blue in the presence of the culture supernatant and the uninoculated medium. A color change was detected with *E. coli*'s culture supernatant, however. A color change was also detected when the culture supernatant of *A. magnetotacticum* and the uninoculated medium were concentrated by about 20-fold. This color change apparently is due to high salts present in the concentrated medium (Neilands personal communication).

We are planning to screen the genomic library of *A. magnetotacticum* using a different selection medium. This medium contains the chelating agent 2,2'-dipyridyl and has been used successfully for identifying a novel iron-uptake system in *Serratia marcecens*. We are also planning to screen the library under microaerophilic conditions in case some of the iron-uptake gene products are oxygen-sensitive (*A. magnetotacticum* is a microaerophilic bacterium).

Cloning the *tonB* Gene

We have identified a few positive library clones in the hybridization experiments using a high-specific-activity, single-stranded *tonB* sequence as probe. We have started sequencing these clones.

We have also constructed synthetic primers complementary to the 5' and 3' ends of the *tonB* gene of *E. coli* and have used them in a PCR reaction to amplify the "*tonB*-like" gene of *A. magnetotacticum*. The results of PCR reaction indicate the presence of three major fragments, one of which has approximately the same molecular weight as the *tonB* gene of *E. coli*. One of the other two fragments is larger and the other smaller than the amplified *tonB* sequence of *E. coli*. We are currently in the process of cloning these fragments into the M13 bacteriophage for sequencing.

Identification of *A. magnetotacticum* Sequences that are Homologous to the Other Iron-Uptake Genes in Other Microorganisms

We have conducted Southern blot experiments with the digested DNA of *A. magnetotacticum* using a number of iron-uptake or iron-uptake-associated genes of *E. coli* as probe. The sequences that we have examined so far include the entire aerobactin operon of *E. coli*, the receptor gene of ferrichrome-mediated system (*fhuA*), a ferrichrome-mediated iron-uptake gene (*fhuB*), the consensus FUR binding site, the *tonB* gene, and the *btuB* gene. The probe for the consensus Fur binding site was a 21-mer synthetic oligonucleotide. Plasmid pABN1, which carries the entire aerobactin operon, was nick-translated and used as probe. The remaining probes were high-specific-activity, single-stranded DNAs that were prepared by cloning the genes into M13 bacteriophage and synthesizing a complementary copy by primer-extension method.

In these experiments, we have identified in *A. magnetotacticum* sequences homologous to the *tonB* and *btuB* genes of *E. coli*. BtuB protein interacts directly with TonB in the cell's periplasmic region. This protein, like TonB, is a multifunctional protein; one of its functions is the transport of vitamin B_{12}.

Using similar techniques, we are planning to prepare high-specific-activity probes from enterochelin-mediated iron-uptake genes and the *exbB* gene. The homologous sequences identified will be cloned and sequenced.

Identification of Codon Usage in *A. magnetotacticum*

Because our initial library screening tests were negative, we decided to clone and sequence a gene unrelated to the iron-uptake genes of *A. magnetotacticum* in order to obtain information about the codon usage and the promoter and terminator sequences in this bacterium. We screened the library for a *recA*-like function in *A. magnetotacticum*. The *recA* gene-product in *E. coli* is involved in homologous recombination and DNA repair. It also regulates the expression of a number of genes scattered in the chromosome. Because of its importance to the cell we assumed that this gene must have been preserved evolutionarily among bacterial species. We have identified and isolated a clone with a functional *recA*-like sequence among the library clones of *A. magnetotacticum*. One of the intriguing features of this gene is that it is regulated by LexA, the SOS repressor molecule, which also regulates the *recA* gene *E. coli*. The sequencing of this gene is in progress.

PUBLICATIONS

A.E. Berson, D.V. Hudson, and N.S. Waleh. Cloning and Characterization of the recA gene of *A. magnetotacticum*. Arch. Microbio. (Submitted.)

TRAINING ACTIVITIES

One undergraduate student (American, Caucasian) will be supported by this contract during the months of July and August, 1989 .