

Generation and Analysis of a *fur* (Ferric Uptake Regulator) Mutant of *Shewanella oneidensis* MR-1 Using Genomic and Proteomic approaches.

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Shewanella oneidensis MR-1 is capable of utilizing a variety of compounds as terminal electron acceptors, including ferric iron. However, little is known about the genetic basis and regulatory mechanisms controlling iron metabolism in this bacterium. To determine whether the putative *fur* gene is involved in iron reduction, a *fur* mutant of MR-1 was generated by insertional mutagenesis and characterized by DNA microarrays and two-dimensional (2D) PAGE. In *E. coli* and other organisms, the Fur protein represses transcription in the presence of high iron by binding to specific sequences in the promoters of iron-regulated genes. Comparative sequence analysis revealed that MR-1 Fur exhibits a high degree of sequence identity (>70%) at the amino acid level to Fur proteins of *E. coli* and *Vibrio cholerae*. The MR-1 *fur* gene was disrupted by cloning a 179-bp internal *fur* fragment into the suicide vector pKNOCK-Km and then transferring the construct into MR-1 cells. Physiological studies indicated that the *fur* mutant was similar to wild-type MR-1 when compared for anaerobic growth and reduction of various electron acceptors. To define genes regulated by Fur, we used partial microarrays containing approximately 1,000 MR-1 genes thought to be involved in energy metabolism, transcriptional regulation, environmental stress, and siderophore production. Preliminary results suggested that disruption of the *fur* gene affected the expression of siderophore-related genes as well as other genes. This agreed with the finding that the *fur* mutant produced 3-fold higher levels of siderophore than the wild-type strain. Analysis of the *fur* mutant by 2D-gel electrophoresis indicated that at least 4 major proteins increased significantly ($P < 0.005$) in abundance in *fur* mutant cells relative to MR-1 cells. Efforts are underway at identifying these proteins. Although MR-1 Fur is not directly involved in metal reduction, it does appear to play an important regulatory role in pathways leading to iron acquisition.

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