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Mur Regulates the Gene Encoding the Manganese Transporter MntH in *Brucella abortus* 2308

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MntH is the only high-affinity manganese transporter identified in *Brucella*. A previous study showed that MntH is required for the wild-type virulence of *Brucella abortus* 2308 in mice (Anderson ES, et al., Infect. Immun. 77:3466–3474, 2009) and indicated that the *mntH* gene is regulated in a manganese-responsive manner in this strain by a Mur homolog. In the study presented here, the transcriptional start site for *mntH* in *B. abortus* 2308 was determined by primer extension analysis. Specific interactions between Mur and the *mntH* promoter region were demonstrated in an electrophoretic mobility shift assay (EMSA), and a Mur binding site was identified in the −55 to −24 region of the *mntH* promoter by DNase I footprint analysis. The specificity of the interaction of Mur with the putative Mur box was further evaluated by EMSA employing oligonucleotides in which the consensus nucleotides in this region were substituted. These studies not only confirm a direct role for Mur in the Mn-responsive regulation of *mntH* expression in *Brucella abortus* 2308 but also identify the *cis*-acting elements upstream of *mntH* that are responsible for this regulation.

A substantial number of bacterial proteins require metal ions for their activity and proper function. However, the accumulation of metals beyond the level at which they are needed can be toxic due to incorrect metal-protein interactions (39) and the capacity of certain metals, such as iron and copper, to participate in the production of toxic oxygen radicals (38). To ensure that they only accumulate the levels of metals they need to meet their physiologic requirements, bacteria produce transporters that mediate both the influx and efflux of specific metal ions (39). The expression of the genes encoding these transport systems typically is tightly controlled by transcriptional regulators whose activities respond to the levels of specific metal ions in the bacterial cell (11). This specific and differential regulation of metal transport genes enables bacteria to actively adapt to different and sometimes rapidly changing levels of available metals in the external environment (39).

Manganese is an essential cofactor for a variety of bacterial proteins (25). Bacterial genes encoding manganese transporters typically are regulated by MntR- or Mur-type transcriptional regulators (12, 30). Mur is a structural homolog of the iron-responsive ferric uptake regulator (Fur) (13, 14), which controls the expression of iron uptake genes in many bacteria (20). Although Mur was first described in *Rhizobium leguminosarum* (7) as a suspected iron-responsive regulator (40), genetic and biochemical studies have clearly shown that Mur is a manganese-responsive transcriptional regulator of manganese uptake genes (7) in *R. leguminosarum* and other alphaproteobacteria (5, 28). Mur does not, however, appear to directly participate in the regulation of iron-responsive genes in these bacteria. Instead, the iron-responsive transcriptional regulators Irr and RirA control the expression of the iron metabolism genes in the alphaproteobacteria (18, 30, 33).

The *Brucella* spp. are members of the alphaproteobacteria and are the causative agents of brucellosis (32). Brucellosis causes sterility and abortion in wild and domestic animals and a severe febrile illness in humans (24). *Brucella* strains rely upon MntH as their sole high-affinity manganese transporter (1), and MntH plays a critical role in the virulence of *B. abortus* 2308 in experimentally infected mice. The expression of the *mntH* gene is regulated in a manganese-responsive manner in this strain, and genetic studies have implicated Mur in this regulation. The purpose of the studies described in this report was to determine whether or not Mur plays a direct role in the manganese-responsive regulation of *mntH* expression in *B. abortus* 2308, and if so, to identify the nucleotide sequences to which Mur binds in the *mntH* promoter region.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains and plasmids used in this study are listed in Table 1. *Brucella* strains were cultivated on Schaedler agar supplemented with 5% defibrinated bovine blood (SBA) at 37°C with 5% CO_2 or in brucella broth at 37°C with shaking. Low-manganese minimal medium was prepared as previously described (1). Ampicillin (25 μg/ml) and kanamycin (45 μg/ml) were included in these growth media as appropriate for the selection of *Brucella* strains carrying antibiotic resistance markers. *Brucella* stock cultures were stored at −80°C in brucella broth supplemented with 25% glycerol. *Escherichia coli* strains were grown at 37°C on LB agar or in LB broth or these media supplemented with 100 μg/ml ampicillin or 45 μg/ml kanamycin as needed. *E. coli* stock cultures were maintained in LB supplemented with 25% glycerol at −80°C.

Production of recombinant *Brucella* Mur. The oligonucleotide primers rMur Forward and rMur Reverse (see Table S1 in the supplemental material), which encode BsaI restriction sites, were used with Pfx DNA polymerase (Invitrogen) to amplify a 426-bp DNA fragment containing the *mur* gene from *B. abortus* 2308 genomic DNA by PCR, and this fragment was cloned into the expression vector pASK-IBA6 (IBA GmbH, Göttingen, Germany). The resulting plasmid, designated pEAM2, which contains a gene fusion encoding an N-terminal Strep-tag II version of the

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TABLE 1 Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Reference or source
Strains		
Escherichia coli		
DH5α	Laboratory cloning strain	Invitrogen
BL21(DE3)	Laboratory cloning strain	37
Brucella abortus		
2308	Virulent challenge strain	Laboratory stock
Plasmids		
pUC19	ColE1-based cloning vector; Ap^r	41
pASK-IBA6	ColE1-based expression plasmid for production of *Strep*-tagged recombinant proteins; Ap^r	IBA GmbH
pEAM2	pASK-IBA6 derivative containing a cloned copy of the *mur* gene from *B. abortus* 2308	This study
pMRS	Derivative of pUC19 containing the hybridized Murbox control F and R oligonucleotides cloned into the EcoRI/BamHI site[a]	This study
pMRS1	Derivative of pUC19 containing the hybridized Murbox mutation 1 F and 1 R oligonucleotides cloned into the EcoRI/BamHI site[a]	This study
pMRS2	Derivative of pUC19 containing the hybridized Murbox mutation 2 F and 2 R oligonucleotides cloned into the EcoRI/BamHI site[a]	This study
pMRS3	Derivative of pUC19 containing the hybridized Murbox mutation 3 F and 3 R oligonucleotides cloned into the EcoRI/BamHI site[a]	This study
pMRS4	Derivative of pUC19 containing the hybridized Murbox mutation 4 F and 4 R oligonucleotides cloned into the EcoRI/BamHI site[a]	This study
pMRS5	Derivative of pUC19 containing the hybridized Murbox mutation 5 F and 5 R oligonucleotides cloned into the EcoRI/BamHI site[a]	This study

[a] The sequences of the Murbox control and Murbox mutation oligonucleotides are listed in Table S1 in the supplemental material.

Brucella Mur, was introduced into *E. coli* DH5α by chemical transformation for screening purposes and the propagation of the plasmid. Once the authenticity of the gene fusion was confirmed by nucleotide sequence analysis, this plasmid was introduced into *E. coli* strain BL21(DE3) by electrotransformation. For recombinant protein production, *E. coli* cultures were grown overnight at 37°C in LB broth, and 10 ml of these cultures was used to inoculate 1 liter of LB in a 4-liter Erlenmeyer flask. These cultures were grown at 37°C with shaking at 250 rpm to an optical density at 600 nm of approximately 0.5, at which time anhydrotetracycline (AHTC) was added to the cultures to obtain a final concentration of 0.2 μg/ml and the incubation continued. Three to 4 h after the addition of AHTC, bacterial cells were harvested by centrifugation at 2,700 × *g* for 15 min at 4°C and lysed with CelLytic B-cell lysis reagent (Sigma) in the presence of the protease inhibitor phenylmethylsulfonyl fluoride (PMSF) on ice for 30 min. The cell lysates were subjected to centrifugation at 17,510 × *g* for 15 min at 4°C, and the supernatant was collected and passed through a Strep-Tactin gravity flow column (IBA GmbH, Göttingen, Germany) following the manufacturer's instructions. Protein fractions were pooled and dialyzed in electrophoretic mobility shift assay (EMSA) binding buffer (10 mM Tris-HCl, 40 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 5% glycerol [pH 7.5]) and concentrated by centrifugation through an Amicon Ultra-15 centrifugal filter (molecular weight cutoff of 3,000) following the manufacturer's instructions. Total protein was quantified by the Bradford assay (4), and the degree of purity was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Aliquots of the recombinant *Brucella* Mur protein were maintained at −80°C in the presence of 10% glycerol.

Identification of the *mntH* transcriptional start site by primer extension analysis. Total RNA was isolated from *B. abortus* 2308 grown in low-manganese medium and this medium supplemented with 50 μM $MnCl_2$ employing the methods described by Mohanty et al. (23). The primer *mntH*-PE (see Table S1 in the supplemental material) was annealed to 50 μg of total RNA, and the primer extension reaction was carried out using a modification of the methods described by Sambrook et al. (34). Briefly, the reaction was performed using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) in 5× first-strand buffer (250 mM Tris-HCl [pH 8.3], 375 mM KCl, 15 mM $MgCl_2$, 100 mM dithiothreitol) plus 0.5 mM deoxynucleoside triphosphates (dNTPs). The 5′ end of the *mntH* transcript was identified by electrophoresis in a denaturing 6% (wt/vol) acrylamide and 7 M urea sequencing gel in glycerol-tolerant buffer (0.089 M Tris base, 0.0285 M taurine, and 0.5 mM Na_2EDTA) alongside a DNA sequence ladder generated using the same primer. The PCR product used as a template for the sequencing ladder was a 1-kb DNA fragment encompassing the coding region of *mntH* and upstream sequence amplified from *B. abortus* 2308 genomic DNA with the primers *mntH*-Forward and *mntH*-Reverse (see Table S1 in the supplemental material). DNA sequence analysis was performed using the SequiTherm Excel II DNA sequencing kit following the instructions provided by the manufacturer (Epicentre, Madison, WI).

EMSA. The methods described by Platero et al. (28) were employed for the EMSAs, with the exception that the nonionic detergent NP-40 was excluded from the reaction mixture. The DNA fragment representing the *mntH* promoter region was amplified by PCR from *B. abortus* 2308 genomic DNA using Pfx polymerase and the primers designated *mntH*-MurboxF and *mntH*-MurboxR (see Table S1 in the supplemental material) and end labeled with [γ-^{32}P]ATP (Perkin Elmer, Waltham, MA) using T_4 polynucleotide kinase (Promega, Madison, WI). An unlabeled version of this DNA fragment was used as a specific competitor in this reaction mixture, and an unlabeled version of a 150-bp DNA fragment, representing the upstream region and 20 bp of the coding region of the *recA* gene (BAB1_1224) amplified from *B. abortus* 2308 genomic DNA by PCR with the primers recAF and recAR (see Table S1 in the supplemental material), was used as a nonspecific competitor.

EMSA analysis employing nucleotide fragments with mutated forms of the Mur binding site was performed using the methods described by Stojilkkovic et al. (36). Synthetic and complementary oligonucleotides (35 nucleotides in length), within which selected nucleotides in the putative Mur binding site in the *mntH* promoter region were substituted, were synthesized by Integrated DNA Technologies (Coralville, IA). EcoRI and BamHI sites were also incorporated at the ends of these oligonucleotides, which are labeled Murbox mutations 1 to 5 F and Murbox mutations 1 to 5 R in Table S1 in the supplemental material. Complementary oligo-

nucleotides were annealed, phosphorylated, and cloned into BamHI-EcoRI-digested pUC19, resulting in the construction of plasmids designated pMRS1 to pMRS5 (Table 1). Double-stranded DNA fragments containing the mutated Mur binding sites were removed from the pUC19 derivatives by digestion with BamHI and EcoRI and used for EMSA analysis using the procedures described in the previous paragraph.

DNase I footprint analysis. The DNase I footprint assay was performed as described previously (8), with slight modifications. Briefly, the oligonucleotide primers *mntH*-MurboxF and *mntH*-MurboxR (see Table S1 in the supplemental material) were individually labeled with [γ-^{32}P]ATP (Perkin Elmer) using the T_4 polynucleotide kinase reaction (Promega, Madison, WI) prior to their use in PCRs with Pfx polymerase to generate 110-bp DNA fragments encompassing the putative Mur box and flanking regions of the *mntH* promoter. The resulting PCR products were subjected to agarose electrophoresis and purified by gel extraction (Fermentas, Glen Burnie, MD). DNA probes corresponding to 15,000 cpm of the forward labeled and reverse labeled templates were incubated separately in EMSA binding buffer supplemented with 100 ng/ml bovine serum albumin (BSA) and 50 ng/ml salmon sperm DNA (nonspecific competitor) in the presence of 100 μM $MnCl_2$ and increasing concentrations of the recombinant *Brucella* Mur protein. The reaction mixture was incubated at room temperature for 30 min prior to treatment with 0.05 U of DNase I freshly diluted in 10× DNase I buffer (400 mM Tris-HCl [pH 8.0], 100 mM $MgSO_4$, 10 mM $CaCl_2$) for 1 min. This reaction was stopped by the addition of 5 mM EDTA and heating at 65°C for 10 min. Reaction mixtures were ethanol precipitated and resuspended in 4 μl of formamide loading buffer (98% formamide, 10 mM EDTA [pH 8.0], 1 mg/ml xylene cyanol FF, 1 mg/ml bromophenol blue). Digested DNA fragments were separated on a denaturing 6% (wt/vol) acrylamide and 7 M urea sequencing gel in glycerol-tolerant buffer (as described above). Gels were dried in a vacuum dryer and subjected to autoradiography. The sequence protected by Mur was determined by comparing the nucleotide sequences generated for a 100-bp region of the *mntH* promoter region using the SequiTherm Excel II DNA sequencing kit (Epicentre, Madison, WI) and *B. abortus* 2308 DNA preparations exposed to DNase I treatment with and without recombinant Mur as templates.

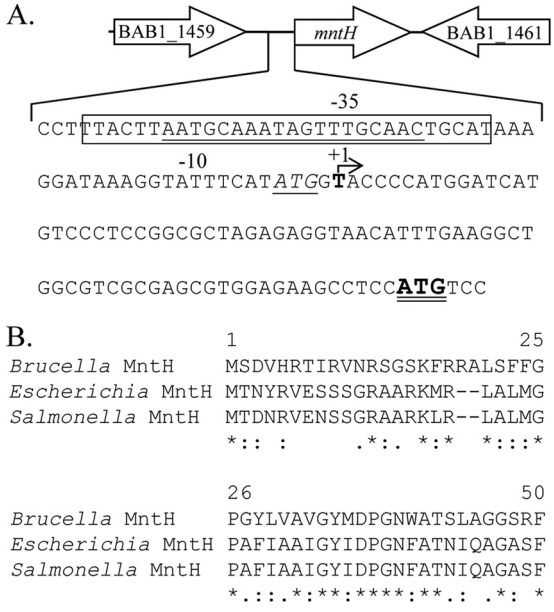

FIG 1 (A) Genetic organization of the *mntH* gene and surrounding genes in *B. abortus* 2308. The transcriptional start site for *mntH* identified by primer extension is indicated in boldface with an arrow and a +1 designation above it. The predicted Mur binding site (or MRS) is underlined, and the Mur binding region identified by DNase I footprint analysis in this study is enclosed in a box. The predicted *mntH* start codon annotated in the *B. abortus* 2308 genome sequence in GenBank is denoted in italics with a single underline, and the ATG that is proposed to be the authentic *mntH* start codon based on the experimental findings presented in this report is depicted in boldface with a double underline. (B) ClustalW alignment of the N-terminal amino acids of the *Brucella abortus* MntH with the corresponding amino acids of the MntH proteins of *Escherichia coli* (GenBank accession no. NP_416893.1) and *Salmonella enterica* serovar Typhimurium (GenBank accession no. NP_461349.1). The revised annotation of the *Brucella mntH* coding region described in the text was used for this analysis.

RESULTS

Mur directly binds to the *mntH* promoter in *B. abortus* 2308. A previous study showed that Mur is required for the manganese-responsive repression of *mntH* expression in *B. abortus* 2308 (1), but a direct interaction between Mur and the *mntH* promoter has not been demonstrated. A putative Mur recognition site (MRS) (8), 5′-AATGCAAATAGTTTGCAAT-3′, lies upstream of the *mntH* coding region in the *Brucella melitensis* 16M genome sequence (32), and this same genomic arrangement occurs in *B. abortus* 2308 (Fig. 1A). To evaluate the functionality of this MRS, primer extension analysis was first used to determine the transcriptional start site for *mntH* in *B. abortus* 2308 and define the promoter for this gene. As shown in Fig. 1A, the putative MRS overlaps the −35 region of the *mntH* promoter, which would be consistent with the predicted function of Mur as a repressor of *mntH* expression. Interestingly, the thymidine (T) residue identified as the transcriptional start site for *mntH* represents the middle nucleotide in the second codon of the *mntH* coding region annotated in the *B. abortus* 2308 and *B. melitensis* 16M genome sequences. The proposed start codon for the annotated *mntH* coding region is presented in italics and underlined in Fig. 1A. Because we were unable to detect a transcriptional start site for the *mntH* gene upstream of the T residue depicted in Fig. 1A, we propose that an ATG start codon 81 nucleotides downstream of the annotated start codon (shown in boldface with a double underline in Fig. 1A) is the authentic start codon for the *Brucella mntH* gene. This would produce a *Brucella* MntH protein that matches the N terminus of the *E. coli* and *Salmonella* MntH proteins better than those annotated in the *B. abortus* 2308 and *B. melitensis* 16M genome sequences (Fig. 1B).

The *Brucella* Mur binds in a specific manner to a 110-bp DNA fragment representing the *mntH* promoter region in an EMSA (Fig. 2A). The addition of the chelator EDTA alleviates the capacity of Mur to bind to the *mntH* promoter (Fig. 2B), and the inhibitory effect of EDTA can be overcome by adding increasing concentrations of $MnCl_2$ to the reaction mixtures. These experimental findings show that Mn^{2+} can modulate the DNA binding activity of the *Brucella* Mur and further support its proposed role as a manganese-responsive transcriptional repressor.

A DNase I footprint analysis determined that the Mur binding site in the *mntH* promoter is comprised of the 28-nucleotide sequence 5′-TACTTAATGCAAATAGTTTGCAACTGCA-3′ (Fig. 3), which overlaps the predicted MRS in the *mntH* promoter (Fig. 1A). In an attempt to identify specific subsets of nucleotides in the MRS that are required for Mur binding, five synthetic double-stranded DNA fragments representing the *mntH* promoter, each with a mutated version of a portion of the CAAATAGTTTG core region of the MRS, were evaluated for their capacity to bind to Mur in an EMSA. As shown in Fig. 4, the synthetic *mntH* promoter fragments with mutations affecting the CAAATAG residues

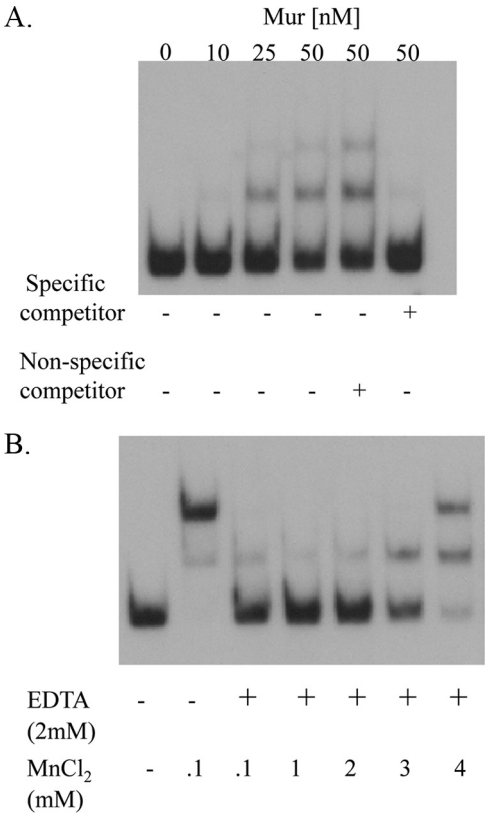

FIG 2 Mur binds to the *mntH* promoter region in *B. abortus* 2308 in a manganese-responsive fashion in an EMSA. (A) 0.25 nM labeled DNA probe was used in these reactions; the concentration of recombinant Mur added to the reaction mixtures is shown above the lanes, and the plus-or-minus symbols below the lanes indicate whether the reaction mixtures contain a 100× concentration of an unlabeled DNA fragment representing the *mntH* promoter region (specific competitor) or an unlabeled DNA fragment representing sequences upstream of the *Brucella recA* gene (nonspecific competitor). (B) 0.1 pM labeled DNA probe was incubated with 50 nM recombinant Mur in these reaction mixtures. The concentration of $MnCl_2$ present in the reaction mixture is shown below the lanes, and the plus symbols indicate that the reaction mixtures contain 2 mM EDTA.

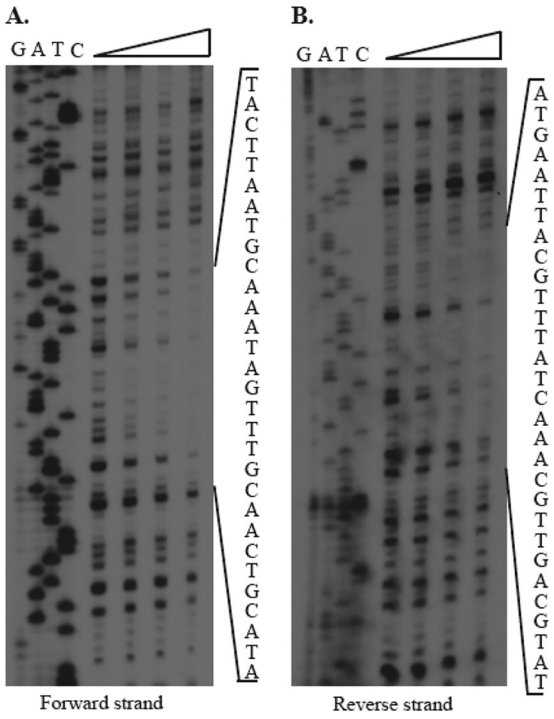

FIG 3 DNase I footprinting of the *B. abortus mntH* promoter with recombinant *Brucella* Mur. The triangle above the lanes indicates that the corresponding reaction mixtures contain increasing concentrations (0, 1, 5, and 10 μg) of recombinant *Brucella* Mur.

in the core region of the MRS (e.g., fragments 1, 2, 4, and 5) did not show the same altered mobility patterns in response to increasing concentrations of Mur in an EMSA, as did a fragment containing the native core region of the MRS or one in which only the TTTG component had been mutated (fragment 3). These experimental findings indicate that the CAAATAG region of the MRS plays an important role in the specificity of Mur binding to the *mntH* promoter in *B. abortus* 2308.

DISCUSSION

The gene designated BAB1_1668 in the *B. abortus* 2308 genome sequence is predicted to encode a homolog of the ferric uptake regulator Fur. This gene was originally cloned during an attempt to identify the iron-responsive regulator of the siderophore biosynthesis genes in *B. abortus* 2308 by genetic complementation (27). A plasmid-borne copy of BAB1_1668 restores the iron-responsive repression of the *fiu-lacZ* fusion in the *E. coli fur* mutant H1780 (15). However, a derivative of *B. abortus* 2308 from which BAB1_1668 has been deleted exhibits wild-type regulation of its siderophore biosynthesis genes in response to environmental iron levels (31). Moreover, a recombinant version of this *Brucella* Fur homolog does not bind to the promoter regions of the iron-responsive *dhbC* (3) or *bhuA* genes (26) in an EMSA (E. Menscher, unpublished data). These findings suggest that BAB1_1668 does not encode an iron-responsive transcriptional regulator in *B. abortus* 2308. Instead, data presented in this and a previous report (1) indicate that the BAB1_1668 gene product is serving as a manganese uptake regulator or a Mur.

Although Mur proteins belong to the Fur superfamily of bacterial metalloregulators (20), their activity as transcriptional repressors is modulated *in vivo* by manganese instead of iron. Mur proteins have been described exclusively in the alphaproteobacteria, where Mur regulates the expression of manganese acquisition genes in *Rhizobium leguminosarum* (7) and *Sinorhizobium meliloti* (5, 28). Recent studies have also shown that proteins currently designated Fur are functioning as manganese-responsive, instead of iron-responsive, regulators in *Bradyrhizobium japonicum* (16, 17) and *Agrobacterium tumefaciens* (19), and thus they appear to be functional Mur homologs as well.

As demonstrated in this report, Mur binds to the *mntH* promoter region in *B. abortus* 2308 and directly regulates the expression of this gene in a manganese-responsive manner. The 30-nucleotide sequence in the *mntH* promoter protected by Mur in the DNase I protection studies contains a motif (5′-AATGCAAATAGTTGCAA-3′) that matches well with the consensus Mur recognition sequence (MRS) or Mur box defined by Rodionov et al. (30) and MRSs identified upstream of the *mntH* gene of *B. japonicum* (16), the *sitA* gene of *R. leguminosarum* (8), and the *mntA* and *sitA* genes of *S. meliloti* (5, 28) (Fig. 5). Like the *Brucella mntH* gene, all of these genes are involved in manganese

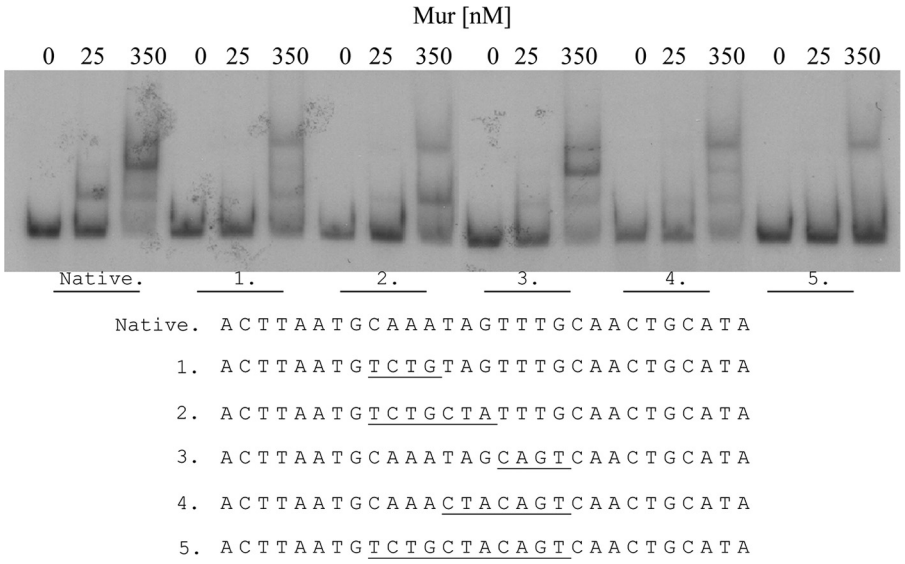

FIG 4 Mapping of the subsets of the nucleotides present in the MRS of the *B. abortus* 2308 *mntH* promoter that are required for its interaction with Mur in an EMSA. The concentration of Mur added to the reaction mixtures is indicated above the lanes; the specific synthetic oligonucleotides (labeled native, 1, 2, 3, 4, and 5) used in the EMSA reactions are indicated below the lanes, and the nature of the nucleotide substitution in each synthetic oligonucleotide used in these reactions is shown at the bottom of the figure.

transport and have been shown to be regulated by Mur. The location of the region of the *mntH* promoter protected by Mur in *B. abortus* 2308 (−55 to −24 with respect the transcriptional start site) is very similar to the location of the region protected by Mur in the *B. japonicum* promoter (−56 to −22 with respect to the transcriptional start site) (16). The selective regional mutagenesis of the MRS in the *Brucella mntH* promoter sequence identified a 7-nucleotide motif in this region that appears to be critical for the specificity of Mur binding. These findings are similar to those obtained with Fur in *E. coli* (9) and *Caulobacter* (6), where groups of nucleotides rather than individual nucleotides have been shown to be responsible for the specific binding of Fur to Fur boxes.

Manganese is an essential micronutrient for *Brucella* strains (10, 35), and the importance of MntH for the efficient acquisition of this divalent cation *in vitro* and *in vivo* has been documented experimentally (1). Like most other biologically beneficial metals, manganese can be toxic if accumulated beyond the levels needed by the bacterial cell, although the potential toxicity of this metal appears to be considerably less than that of other beneficial metals, such as iron, zinc, nickel, or copper (25, 39). A direct role for Mur in preventing manganese toxicity in *B. abortus* 2308 by repressing *mntH* expression has not been observed *in vitro*, however, since both the parent strain and the *mur* mutant exhibit equivalent growth characteristics during cultivation in laboratory media containing up to 10 mM $MnCl_2$. At higher concentrations, both strains exhibit equivalent levels of growth restriction (E. Menscher, unpublished).

The bioinformatics-based study performed by Rodionov et al. (30) suggests that *mntH* and a gene designated *irr1* by this group (BMEI1563 and BAB1_0393 in the *B. melitensis* 16M and *B. abortus* 2308 genome sequences, respectively) are the only *Brucella* genes directly regulated by Mur. It is important to note, however, that these genes do not encode the *Brucella* iron-responsive regulator Irr that has been characterized by Martínez et al. (21, 22). Instead, the genes encoding Irr are designated BAB1_2175 and BMEI1955 in the *B. abortus* 2308 and *B. melitensis* 16M genome sequences, respectively. This distinction is important because Mur regulates *irr* expression and cellular manganese levels modulate Irr activity in *B. japonicum* (17, 29), and evidence for indirect effects of manganese on the expression of iron metabolism genes has been reported for *A. tumefaciens fur* (19) and *S. meliloti mur* mutants (5).

During their residence in host macrophages, the brucellae must deal with both endogenous reactive oxygen species (ROS) generated by their respiratory metabolism as well as exogenous ROS generated by the NADPH activity of the host phagocyte (32). Efficient manganese transport mediated by MntH has been linked to ROS resistance in *B. abortus* 2308 (1), and Irr is required for the optimal expression of the siderophore biosynthesis (22) and heme

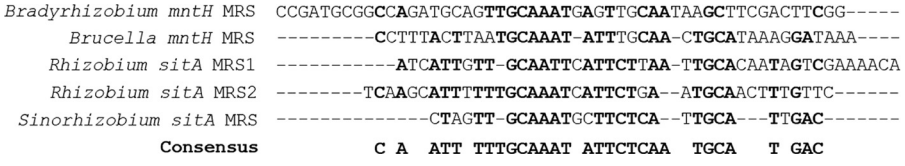

FIG 5 Comparison of the experimentally documented Mur binding sites present in the promoter regions of the *mntH* and *sitA* genes of *Brucella abortus*, *Bradyrhizobium japonicum* (16), *Rhizobium leguminosarum* (8), and *Sinorhizobium meliloti* (28).

transport genes (2) in this bacterium. Having the capacity to modulate *irr* expression or Irr activity such that iron acquisition genes are only maximally expressed in *Brucella* strains when these bacteria possess protective levels of manganese would be beneficial in their intracellular niche in the host, because it could reduce the risk for ROS toxicity due to iron-mediated Fenton chemistry. Thus, to better understand the importance of Mur in the physiology of *Brucella* strains, it will be important to determine whether or not Mur directly regulates the expression of *irr* or whether cellular manganese levels influence Irr activity. Likewise, it will be important to define the biological function of Irr1 and determine if the corresponding gene is regulated by Mur.

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