

Comparative Analysis of *hmuO* Function and Expression in *Corynebacterium* Species[∇]

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We have constructed defined deletions in the *hmuO* gene from *Corynebacterium diphtheriae* and *Corynebacterium ulcerans* and show that the *C. ulcerans hmuO* mutation results in a significant reduction in hemoglobin-iron utilization, whereas in *C. diphtheriae* strains, deletion of *hmuO* caused no or only partial reduction in the utilization of heme as an iron source. We also show that expression from the *C. ulcerans hmuO* promoter exhibits minimal regulation by iron and heme whereas transcription from the *C. diphtheriae hmuO* promoter shows both significant iron repression and heme-dependent activation. These findings indicate that variability in HmuO function and expression exists among *Corynebacterium* species.

Iron is an essential nutrient for almost all bacteria, and the ability to acquire sufficient amounts of this element is important for many virulent organisms to cause disease in a mammalian host (6, 7). *Corynebacterium diphtheriae*, the etiological agent of diphtheria, uses various compounds as iron sources, including heme and hemoglobin (10, 18, 21, 22). *Corynebacterium* proteins involved in the uptake and utilization of heme as an iron source include an ABC-type heme transporter encoded by the *hmuTUV* genes (10, 22) and HmuO, a heme oxygenase that has been shown in vitro to oxidatively cleave the heme macrocycle, which results in the release of heme-bound iron (18, 31). In a previous study, it was shown that point mutations in the *hmuO* gene in the HC1 strain of *C. diphtheriae* C7 resulted in a diminished ability to use heme-iron (9, 18). This earlier study utilized a qualitative assay that measured the ability of *C. diphtheriae* HC1 mutants to use heme compounds as a sole iron source and used chemical mutagenesis to create the mutations (18). Due to the limitations of the method used to identify and screen for the heme utilization mutants in this earlier study, wild-type *C. diphtheriae* C7 could not be used for the isolation of *hmuO* mutations (18). Moreover, while the *C. diphtheriae* HC1 parent strain was useful for the identification of *hmuO* mutants, it was not an ideal background for the analysis of these mutants, since the strain is severely defective in iron uptake and likely contains multiple mutations in genes involved in iron transport (13, 16). Chemical mutagenesis was also used to obtain heme-iron utilization mutants of *Corynebacterium ulcerans* 712 (CU712), and two distinct groups of mutants were identified: one group was complemented by the *C. diphtheriae hmuTUV* transport genes, while the second group was complemented by the cloned *hmuO* gene (10, 18). The specific mutations in these *C. ulcerans* heme utilization mutants were not, however, specifically identified or further characterized.

Although *C. diphtheriae* HmuO was the first bacterial heme oxygenase to be identified, heme oxygenases that share sequence or structural similarities to HmuO have been found in both gram-negative and gram-positive organisms (17, 27, 33). A novel group of proteins that possess a heme-degrading activity, but have no amino acid sequence similarity to HmuO, have recently been described for several bacterial species, including *Staphylococcus aureus* (25), *Bacillus anthracis* (26), and *Bradyrhizobium japonicum* (15). Moreover, some of the genes that encode these novel heme degradation enzymes were shown to complement the defect in a *C. ulcerans* heme utilization mutant (15, 25), which suggests that these genes are associated with the utilization of heme as an iron source.

Expression of the *C. diphtheriae hmuO* gene has been previously examined, and its transcription was shown to be under a dual mode of regulation, which involves iron repression, mediated by DtxR (5, 19, 20), and heme-dependent activation that requires the ChrS-ChrA and HrrS-HrrA two-component signal transduction systems (3, 4). Previous studies showed that a DtxR binding site overlaps the *hmuO* promoter region and also demonstrated that a region upstream of the *hmuO* promoter is critical for the heme-dependent activation (20).

In this study, we characterized a defined deletion in the *hmuO* gene in the wild-type strain *C. diphtheriae* C7(–) (2) and also in Russian clinical isolates 1737 and 1716 (12, 14, 18). Additionally, the *hmuO* gene from CU712 was cloned and a *hmuO* deletion mutant of CU712 was constructed and characterized. We also show that iron and heme regulation of the *C. ulcerans hmuO* promoter is significantly different from that observed previously with the *C. diphtheriae hmuO* promoter.

Analysis of the CU712 *hmuO* gene and construction of *hmuO* deletion mutants. The genome sequence of *C. ulcerans* has not been determined; therefore, two oligonucleotide primers derived from *C. diphtheriae* sequences, primer hm1a (5'-GGCGGATCCGACAAGTAGTAGATGAGGTTGTTTTGGGGC-3') and primer thrC-1 (5'-CTGCATTGCCATGTCTTTAAATGC-3'), were used to amplify a 4-kb DNA fragment from CU712 that contained the *hmuO* gene, and this amplicon was subsequently cloned into pCRBluntII-TOPO vector (Invitrogen). The nucleotide sequence of the *C. ulcerans hmuO* gene was determined,

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	Cd-NCTC	Cd-C7	Cu	Cg	Cjk	HO-1
Cd-NCTC	X	97/97	67/77	58/68	43/58	34/54
Cd-C7	97/97	X	67/78	58/68	44/59	34/54
Cu	67/77	67/78	X	56/69	43/59	30/57
Cg	58/68	58/68	56/69	X	47/62	28/41
Cjk	43/58	44/59	43/59	47/62	X	38/56
HO-1	34/54	34/54	30/57	28/41	38/56	X

FIG. 1. Amino acid comparison between various *Corynebacterium* HmuO proteins and the human heme oxygenase HO-1. The numbers separated by a slash represent percent identity/similarity. Cd-NCTC, *C. diphtheriae* genome strain NCTC 13129 (8); Cd-C7, C7(-) strain; Cu, *C. ulcerans* 712; Cg, *Corynebacterium glutamicum*; Cjk, *Corynebacterium jeikeium*.

and the predicted product (24.0 kDa) was found to share 67% identity with and 77% similarity to HmuO from *C. diphtheriae*. A comparison of the amino acid sequences among HmuO proteins from various *Corynebacterium* species and the human heme oxygenase HO-1 is shown in Fig. 1. A BLAST search (1) of the recently completed genome sequence of *C. efficiens*, a species related to *C. glutamicum*, failed to identify a *hmuO* ortholog.

C. ulcerans strain CU29, a chemically derived mutant of CU712, was previously reported to have a reduced ability to use heme as an iron source, and it was shown that this defect is complemented by the presence of the cloned *C. diphtheriae hmuO* gene (18). The *hmuO* allele from CU29 was cloned and sequenced (as described above for the wild-type *hmuO* gene) and shown to contain a single point mutation that results in the amino acid change at position 86 from D to N, which is likely responsible for the heme-iron utilization defect observed in this mutant (data not shown). Examination of the recently solved crystal structure of *C. diphtheriae* HmuO reveals that D86 is located on the protein surface and is associated with a region involved in HmuO enzymatic activity (11).

The *hmuO* deletion mutant in *C. diphtheriae* C7 (C7 hmuOΔ) was previously described (4), and construction of an *hmuO* deletion in Russian clinical isolates 1737 (1737 hmuOΔ) and 1716 (1716 hmuOΔ) was done following the same procedure as described for C7. A *hmuO* deletion mutant was also constructed using CU712 (CU712 hmuOΔ) by allelic replacement as previously reported (4, 29). CU712 hmuOΔ contains a 305-bp internal deletion of *hmuO* and is predicted to generate an in-frame fusion between 84 amino acids at the N terminus and 26 residues from the C terminus. The mutation was confirmed by PCR and DNA sequence analysis (not shown). Regions essential for the catalytic activity of HmuO are deleted in both the *C. diphtheriae* and *C. ulcerans hmuO* deletion mutants.

Hemoglobin utilization assays. To assess the effect of the deletion of *hmuO* in *Corynebacterium* strains, a quantitative growth assay was developed to determine the capacity of wild-type and mutant bacteria to use hemoglobin as a sole iron source. Briefly, *Corynebacterium* strains were grown overnight (20 to 22 h at 37°C) in heart infusion broth (Becton Dickinson, Sparks, MD) with 0.2% Tween 80 (Aldrich, Milwaukee, WI) (HIBTW) and then inoculated to an optical density at 600 nm (OD₆₀₀) of 0.2 into fresh HIBTW that contained 12 μg/ml of

the iron chelator ethylenediamine di(*o*-hydroxyphenylacetic acid) (EDDA; Sigma, St. Louis, MO). Strains were grown for several hours at 37°C until log phase, at which time bacteria were recovered by centrifugation, resuspended in mPGT medium, a low-iron semidefined minimal medium (28), and then inoculated at an OD₆₀₀ of 0.03 into fresh mPGT medium that contained various supplements as indicated. After 20 to 22 h of growth at 37°C, the OD₆₀₀ of the cultures was determined. All strains grew well in the presence of FeSO₄ (+Fe; mPGT containing 1 μM FeSO₄), and all were inhibited for growth under low-iron conditions (-Fe; mPGT containing 3.6 μg/ml EDDA) (Table 1). All *Corynebacterium* strains grew to an OD₆₀₀ of 0.1 or less in mPGT medium that contained no added supplements (data not shown). EDDA was added to mPGT medium (-Fe) at 3.6 μg/ml to chelate trace iron that may have contaminated or subsequently dissociated from the heme and hemoglobin. Wild-type *Corynebacterium* strains were examined for their ability to use hemoglobin as an iron source over a range of concentrations. Wild-type CU712 and *C. diphtheriae* C7(-) utilized hemoglobin as an iron source at levels as low as 5 μg/ml, and hemoglobin levels higher than 50 μg/ml did not further enhance growth of any of the strains (Table 1 and data not shown). Heme also stimulated growth of both *C. diphtheriae* and CU712; however, as previously reported (4), heme was shown to be toxic to *C. diphtheriae*, but not to *C. ulcerans*, at concentrations higher than 5 μM (data not shown).

CU712 hmuOΔ, the *C. ulcerans hmuO* deletion mutant, showed minimal growth in the presence of hemoglobin, and CU29, the *hmuO* point mutant, showed results similar to those seen with CU712 hmuOΔ, which indicates that these strains

TABLE 1. Growth stimulation of *Corynebacterium* strains by iron and hemoglobin

Strain/plasmid	Growth (OD ₆₀₀) with various iron sources ^a					
	+Fe ^b	-Fe ^c	-Fe with hemoglobin (μg/ml)			
			Hb ^f (50)	Hb (25)	Hb (10)	Hb (5)
CU712 wild type	4.5	<0.1	4.8	2.8	0.5	0.3
CU712 hmuOΔ	4.0	<0.1	0.14	0.10	nd ^d	nd
CU712 hmuOΔ/pCUhmuO	3.8	<0.1	4.2	2.5	nd	nd
CU712 hmuOΔ/p37hmuO	4.2	<0.1	4.1	2.2	nd	nd
CU712 hmuOΔ/p1841	3.8	<0.1	0.13	0.08	nd	nd
C7(-) wild type	2.1	<0.1	4.3	2.7	0.7	0.4
C7 hmuOΔ	2.2	<0.1	1.1	0.7	nd	nd
C7 hmuOΔ/pCM2.6 (vector)	5.7 ^e	<0.1	1.9	0.9	nd	nd
C7 hmuOΔ/pCD293 (<i>hmuO</i> ⁺)	5.9	<0.1	5.5	2.6	nd	nd
C7 hmuOΔ-1841Δ	2.0	<0.1	0.9	0.5	nd	nd
1737 wild type	6.4	<0.1	5.8	3.5	nd	nd
1737 hmuOΔ	6.8	<0.1	6.6	3.3	nd	nd

^a OD₆₀₀ was determined for each culture after growth for 20 to 22 h in mPGT medium. Values shown are results from a representative experiment. All experiments were repeated at least three times, and the results of each assay varied by less than 20% from the mean.

^b +Fe medium is mPGT with 1 μM FeSO₄.

^c -Fe medium is mPGT with 3.6 μg/ml EDDA.

^d nd, not done.

^e C7 strains carrying pCM2.6 plasmids grow to higher densities than strains without plasmids.

^f Hb, hemoglobin.

are strongly defective in their ability to use hemoglobin as an iron source (Table 1 and data not shown). To confirm that HmuO was essential for *C. ulcerans* to utilize hemoglobin as an iron source, pCUhmuO, a pKN2.6-based vector (23) that contains a copy of the *C. ulcerans hmuO* gene, was introduced into CU712 hmuOΔ and shown to complement the heme-iron utilization defect observed in this mutant (Table 1). Similarly, the cloned *C. diphtheriae hmuO* gene on plasmid pCD293 (18) was also able to restore heme utilization to CU712 hmuOΔ (data not shown). All complementing clones used in this study were derived from low-copy-number (1 to 2 copies per cell), pNG2-based, *Corynebacterium-Escherichia coli* shuttle vectors (23).

The *C. diphtheriae* C7(−) *hmuO* deletion mutant C7 hmuOΔ showed a marked decrease relative to the wild-type strain in its ability to use hemoglobin as an iron source for growth; however, this defect was not as severe as that observed for CU712 hmuOΔ, as C7 hmuOΔ could maintain growth in the presence of 25 to 50 μg/ml hemoglobin (Table 1). The hemoglobin utilization defect in C7 hmuOΔ was complemented by the introduction of the cloned *C. diphtheriae hmuO* gene on plasmid pCD293 (Table 1), which confirms that a defect in HmuO is responsible for the diminished ability of C7 hmuOΔ to use hemoglobin as an iron source. C7 hmuOΔ was also complemented by the presence of the cloned CU712 *hmuO* gene on plasmid pCUhmuO (data not shown).

Surprisingly, deletion of the *hmuO* gene in the Russian clinical isolate 1737 hmuOΔ had no effect on its ability to use hemoglobin as an iron source, since growth of the mutant was comparable to that of the wild-type strain (Table 1). The HmuO protein from 1737 differs from C7 HmuO at four residues; however, these differences in sequence do not appear to impair HmuO function, since the cloned 1737 *hmuO* gene on pKN2.6 (p37hmuO) complemented the hemoglobin-iron utilization defect in CU712 hmuOΔ (Table 1). To further investigate HmuO function in *C. diphtheriae* strains, an *hmuO* deletion was constructed in another Russian clinical isolate, 1716 (1716 hmuOΔ), and characterization of this strain indicated a hemoglobin-iron utilization defect similar to that observed in the C7 strain (data not shown). Previous studies have indicated that *C. diphtheriae* strains exhibit significant genetic diversity in their iron utilization systems (12, 13), and the findings reported here suggest that *C. diphtheriae* strains C7 and 1716 require HmuO and an additional factor(s) to acquire heme-iron, while in strain 1737, HmuO is not essential for the utilization of heme as an iron source. PCR analysis using three different primer pairs, whose sequences correspond to internal regions of the *hmuO* gene that are conserved between strains C7, 1737, and CU712, failed to identify a PCR product when 1737 hmuOΔ chromosomal DNA was used as a template for PCR (M. P. Schmitt, unpublished data). These findings suggest that a second or similar copy of the *hmuO* gene is not encoded on the chromosome of 1737.

Analysis of *dip1841*. Bacterial proteins with heme-degrading activity that are distinct from previously described heme oxygenases have recently been reported in studies of *S. aureus* (IsdG and IsdI) (25), *B. anthracis* (IsdG and IsdI) (26), and *B. japonicum* (HmuQ and HmuD) (15). In addition, the *isdG* and *isdI* genes from *S. aureus* and *hmuQ* and *hmuD* from *B. japonicum* were shown to complement the heme-iron utilization defect in *C. ulcerans* CU29 (15, 25). A BLAST search of

the recently completed genome sequence of *C. diphtheriae* NCTC 13129 (8) has identified a putative ortholog of the IsdG/HmuQ proteins (designated *dip1841*) that is predicted to encode a 101-amino-acid product that has 25% and 28% identity and 46% and 44% similarity to IsdG and HmuQ, respectively. Studies based on the crystal structure of IsdG have identified residues important for enzymatic activity (32), some of which are conserved in Dip1841; moreover, Pfam analysis (<http://pfam.wustl.edu/>) indicates that Dip1841 is a member of the ABM family of monooxygenases, which also includes HmuQ and IsdG (15). Analysis of the *C. diphtheriae* genome suggests that *dip1841* is not linked to a heme or iron transport locus and is the distal gene in a two-gene operon (*dip1842-dip1841*). To determine whether Dip1841 can complement an *hmuO* mutant, both the *dip1841* gene alone and the complete *dip1841-dip1842* operon were cloned onto plasmid vector pKN2.6 to generate p1841 and p1841-42, respectively, and these constructs were tested for their ability to correct the hemoglobin-iron utilization defect in CU712 hmuOΔ. Neither p1841 nor p1841-42 was able to restore heme-iron utilization to CU712 hmuOΔ or CU29 (Table 1 and not shown). To determine whether Dip1841 has a role in the use of heme as an iron source in *C. diphtheriae*, a *dip1841* nonpolar in-frame deletion was constructed using wild-type and *hmuO* mutant strains of C7 (C7-1841Δ and C7 hmuOΔ-1841Δ) and 1737 (1737-1841Δ and 1737 hmuOΔ-1841Δ) using previously described methods (4, 29). The *dip1841* mutation was confirmed by PCR and is predicted to encode a product that contains four amino acids from the N terminus of Dip1841 fused in frame to two amino acids at the C terminus. The *dip1841* deletion in the wild-type C7 and 1737 strains or in 1737 hmuOΔ did not result in a decreased ability to use hemoglobin as an iron source, and the C7 double mutant, C7 hmuOΔ-1841Δ, exhibited a phenotype similar to that seen in the single *hmuO* mutant strain C7 hmuOΔ (Table 1 and data not shown). These findings suggest that *dip1841* is not involved in the utilization of hemoglobin as an iron source in *C. diphtheriae* and indicate the need for additional studies to identify the factor(s) involved in the extraction of heme-iron in the 1737 and C7 hmuOΔ strains.

Hemoglobin and iron regulation at the *C. ulcerans hmuO* promoter. Previous studies demonstrated that transcription from the *C. diphtheriae hmuO* promoter was repressed under high-iron conditions (with the repression mediated by DtxR) and activated by the ChrA-ChrS and HrrA-HrrS two-component systems in the presence of a heme source (3, 4, 19, 20). A DtxR binding site overlaps the *C. diphtheriae hmuO* promoter, and a 50-bp region upstream of the promoter is required for full heme-dependent expression (20); however, specific DNA sequences required for activation have not been identified. A comparison of the *hmuO* promoter regions between *C. diphtheriae* and *C. ulcerans* shows that critical residues in the −10 and −35 regions are relatively well conserved (Fig. 2A); however, the DtxR binding site in *C. ulcerans* has less homology to the 19-bp consensus binding sequence than that observed in *C. diphtheriae*. Specifically, *C. ulcerans* matches 13 of 19 bases whereas *C. diphtheriae* matches 15 of 19 bases with the consensus DtxR binding site, and 14 of 19 bases of the two species match (Fig. 2A and B). The region upstream of the promoter is poorly conserved between the species except for a 9-bp

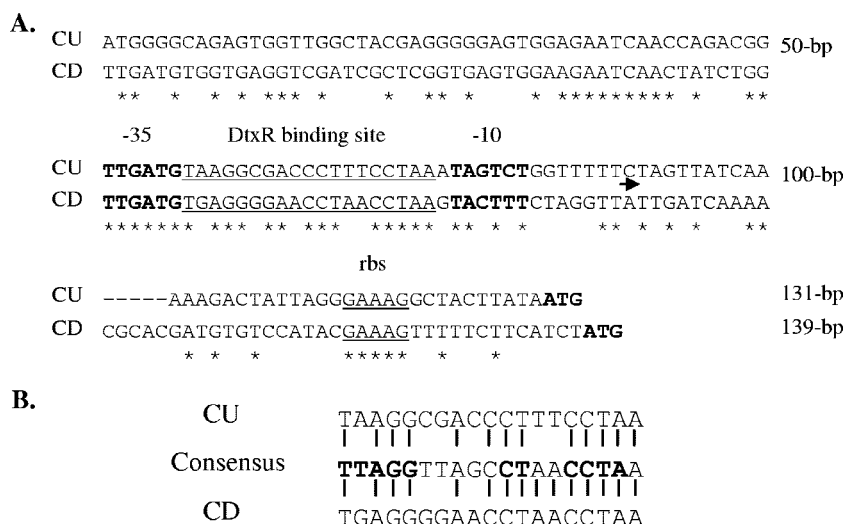


FIG. 2. A. Alignment of the nucleotide sequences in the *hmuO* promoter region between *C. ulcerans* (CU) and *C. diphtheriae* C7 (CD). DtxR binding sites are underlined. rbs, putative ribosome binding site; ATG, start codon for *hmuO*. The arrow indicates the start of transcription in *C. diphtheriae* (19). B. Alignment of the 19-bp DtxR binding sites from CU712 and *C. diphtheriae* C7 with the DtxR consensus binding sequence. Residues represented by bold characters indicate the most highly conserved nucleotides.

contiguous sequence located within 20 bp of the -35 region (Fig. 2A).

To compare expression between the *hmuO* promoters of *C. diphtheriae* and *C. ulcerans*, a *C. ulcerans hmuO-lacZ* promoter construct (pPO712), which contains a 315-bp DNA sequence from CU712 that extends from the 5' portion of the *hmuO* coding region to 60 bp upstream of the -35 sequence, was constructed in the low-copy-number promoter probe vector pCM502 (19). Plasmids pPO712 and pCPO-1, a pCM502-based plasmid that contains a *C. diphtheriae hmuO* promoter-*lacZ* fusion (20), were subsequently introduced into various *Corynebacterium* strains, and promoter activity was determined after overnight growth at 37°C in HIBTW medium containing various supplements. As shown in Table 2, and as reported previously (4, 19, 20), the *C. diphtheriae hmuO* promoter on pCPO-1 was strongly repressed by iron (+Fe) and showed only minimal expression in low-iron conditions ($-Fe$); however, in the presence of hemoglobin, expression of *hmuO* was enhanced more

than 30-fold. In contrast, transcription from the CU712 *hmuO* promoter was weakly repressed by iron, and expression in the presence of hemoglobin was enhanced only two-fold; however, overall promoter activity was more robust in *C. ulcerans* than in *C. diphtheriae* (Table 2). CU712 *hmuO*Δ/pPO712 showed significantly enhanced levels of *hmuO* expression in the presence of hemoglobin relative to wild-type results, and since this mutant showed a reduced ability to degrade heme, excess intracellular heme may function to further activate expression. Expression of pPO712 in *C. diphtheriae* C7 indicates that the *C. ulcerans hmuO* promoter is more strongly expressed under all conditions relative to the *C. diphtheriae hmuO* promoter (pCPO-1), and the lack of hemoglobin-dependent activation from the *C. ulcerans hmuO* promoter in a C7 mutant that is defective for both ChrA-ChrS and HrrA-HrrS suggests that the *C. ulcerans hmuO* gene is regulated by similar two-component systems in *C. ulcerans*. Additional studies will be required to determine whether *C. ulcerans* harbors these signal transduction systems or other putative activators that may be involved in the expression of *hmuO*.

Conclusions. The results from this study indicate that expression of *hmuO* in *C. ulcerans* is only weakly influenced by the presence of iron and heme and that the level of transcription of *C. ulcerans hmuO* is relatively high under all conditions examined, which is in contrast to what was observed with *C. diphtheriae*, for which significant levels of *hmuO* transcription are only observed in the presence of a heme source (Table 2 and reference 4). These observations suggest that in *C. ulcerans*, HmuO function is needed regardless of the iron or heme levels, whereas in *C. diphtheriae*, HmuO activity is primarily required in the presence of an external heme source. One possible explanation for the overall higher *hmuO* expression levels in *C. ulcerans* is that CU712 may have higher endogenous intracellular heme levels than *C. diphtheriae*. This observation would be consistent with what is observed in CU712

TABLE 2. *hmuO* promoter activity in *Corynebacterium* strains

Strain/plasmid ^a	LacZ activity ^d in:		
	+Fe ^b	-Fe ^c	-Fe/Hb ^d
C7 wt/pCP01 (C7/ <i>hmuO-lacZ</i>)	<0.5	3.0 (0.7)	112.0 (3.0)
CU712 wt/pPO712 (CU/ <i>hmuO-lacZ</i>)	46.7 (2.3)	129.7 (11.1)	256.6 (20.0)
CU712 <i>hmuO</i> Δ/pPO712	121.3 (15.5)	196.0 (38.0)	922.0 (120.0)
C7 wt/pPO712	10.9 (3.0)	15.5 (3.4)	155.3 (24.5)
C7chrASΔ-hrrASΔ/pPO712	1.7 (0.3)	2.6 (0.1)	2.5 (0.3)

^a LacZ activity was determined as described previously (24). Values represent the means (\pm standard deviations) of the results of three experiments.

^b +Fe, HIBTW medium.

^c -Fe, HIBTW medium with 12.5 μ g/ml EDDA.

^d -Fe HIBTW medium with Hb (hemoglobin) at 140 μ g/ml.

^e wt, wild type.

hmuOΔ, where the absence of HmuO results in higher levels of *hmuO* expression, which could be caused by higher intracellular heme levels.

In higher organisms, heme oxygenases are involved in both the acquisition of heme-iron and heme homeostasis (30). It is not known whether the *Corynebacterium* HmuO proteins are involved in heme homeostasis or other heme-associated metabolic activities. It is clear, however, from the findings in this study that HmuO is involved in the acquisition of heme-iron in *C. ulcerans* and in some *C. diphtheriae* strains. It is possible that in *C. ulcerans*, HmuO is involved in heme homeostasis or other related heme metabolic activities in both the presence and absence of environmental heme sources, while in *C. diphtheriae*, proteins other than HmuO may perform these functions in the absence of externally supplemented heme. This hypothesis may explain why an *hmuO* mutation in *C. ulcerans* fully abolishes heme utilization whereas a *C. diphtheriae* *hmuO* mutant is only partially defective in heme-iron acquisition (or has no defect, as seen with strain 1737). This reasoning assumes that in *C. diphtheriae* there are one or more factors in addition to HmuO that are involved in heme-iron utilization and possibly required for additional heme-associated activities, while it predicts that in *C. ulcerans*, HmuO is the only protein involved in the extraction of iron from heme. Whether there are additional proteins involved in heme homeostasis in *C. ulcerans* remains to be determined. Clearly, further studies are required to fully understand the mechanism and factors involved in the acquisition of iron from heme in pathogenic *Corynebacterium* species.

Nucleotide sequence accession number. The nucleotide sequence of a 777-bp region containing the *C. ulcerans* *hmuO* gene and flanking sequences has been assigned GenBank accession number EF186829.

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