Role of Catechol Siderophore Synthesis in *Vibrio vulnificus* Virulence

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We isolated a *Vibrio vulnificus* *TnphoA* mutant that was unable to produce catechol siderophores or to acquire iron from transferrin. This mutant showed reduced virulence in an infant mouse model. The *TnphoA* insertion was in an open reading frame designated *venB*. The *venB* gene cloned on a plasmid restored catechol production to the mutant. The deduced amino acid sequence of *venB* is 41% identical to the enzyme isochorismatase of *Escherichia coli* (*EntB*), an enzyme involved in the biosynthesis of the catechol siderophore enterobactin.

Vibrio vulnificus is a halophilic marine bacterium that has been associated with primary septicemia and serious wound infections (3, 14, 15). Primary septicemia is acquired by eating raw oysters or shellfish containing the organism, and wound infections are associated with exposure of wounds to seawater (12, 26). Primary septicemia is often associated with diseases which result in iron overload, such as cirrhosis, hemochromatosis, and thalassemia (11).

Iron seems to be particularly important in the pathogenesis of *V. vulnificus* infections. Wright et al. (27) directly correlated virulence with iron availability. They reported that the injection of iron into mice lowered the 50% lethal dose (LD_{50}) of a virulent strain of *V. vulnificus*. Injection of Desferal, a siderophore that *V. vulnificus* is able to use, also dramatically lowered the LD_{50} . In a study of the virulence characteristics of clinical and environmental isolates of *V. vulnificus*, Stelma et al. (25) found that virulent *V. vulnificus* isolates produced high titers of hemolysin, were resistant to inactivation by serum complement, produced a phenolate (catechol) siderophore, and utilized transferrin-bound iron. Avirulent isolates were unable either to produce significant amounts of phenolate siderophore or to utilize transferrin-bound iron. Their results suggested that the phenolate siderophore enabled the virulent isolates to acquire iron from highly saturated transferrin. Recently the structure of the catechol siderophore of *V. vulnificus* has been characterized and named vulnibactin (18).

We used streptonigrin enrichment following *TnphoA* mutagenesis to isolate a transferrin iron utilization mutant of *V. vulnificus*. The *V. vulnificus* transferrin iron utilization mutant showed altered virulence in an infant mouse model and the inability to produce catechol siderophores. We cloned a gene which restored the ability of the mutant to produce catechol siderophores when moved into the mutant strain *in trans*. We report here the entire sequence of this gene in *V. vulnificus*, which shows significant homology to the *entB* gene of *Escherichia coli*, and have designated this gene *venB* (*V. vulnificus entB* homolog).

Bacterial strains and plasmids. The characteristics of the *V. vulnificus* and *E. coli* strains and plasmids used in this study are described in Table 1. MO6-24 and CML44 were opaque

strains, indicating encapsulation. Plasmids pCML31, pTWR2, pTWR3, and pTWR10 are also depicted in Fig. 1.

Media. Strains were routinely grown in Luria broth (LB). Two types of low-iron media were used: LB medium with or without the addition of the iron chelator 2,2-dipyridyl (Sigma Chemical Co., St. Louis, Mo.) to a final concentration of 0.2 mM and LB medium made iron deficient by the addition of 75 μ g of ethylenediamine-di(*o*-hydroxyphenyl)acetic acid (EDDA) per ml, deferrated by the method of Rogers (19).

Isolation of a transferrin utilization mutant. We isolated a mutant defective in transferrin iron utilization by mutagenesis with *TnphoA* and treating the mutants with streptonigrin. The transposon vector *TnphoA* was used to obtain random insertions into the chromosome of *V. vulnificus* MO6-24 as previously described (28). We treated the *TnphoA* mutants with streptonigrin by a modification of a procedure previously described for isolation of transferrin uptake mutants of *Neisseria gonorrhoeae* (4). In brief, *V. vulnificus* MO6-24 *TnphoA* mutants (approximately 200,000 colonies) were suspended in 25 ml of LB chelated with 2,2-dipyridyl and incubated overnight at 37°C. The culture was resuspended in 50 ml of LB containing 75 μ g of EDDA per ml and 26 μ M iron-saturated transferrin and then was incubated at 37°C for 4 h. Streptonigrin (Sigma) was added to the culture at a 0.5- μ g/ml final concentration, and the incubation was continued for 0.5 h. The cells were washed and spread on LB-kanamycin plates (45 μ g/ml). One thousand of the surviving colonies were scored for the ability to grow with transferrin by transferring the colonies to LB agar and LB agar chelated with 75 μ g of EDDA per ml with 26 μ M Fe-saturated transferrin added. Mutants unable to use transferrin were tested for the ability to use hemoglobin, $FeSO_4$, hemin, and 100% Fe-saturated transferrin by plate assay.

Thirteen of the 1,000 tested survivors were unable to grow on LB-EDDA agar supplemented with 26 μ M iron-saturated transferrin. The alkaline phosphatase activities of the mutants were determined as described previously (13). All 13 mutants were negative for alkaline phosphatase activity after growth under low- or high-iron conditions (data not shown), indicating that the *TnphoA* fusions lacked signals for the secretion of the alkaline phosphatase of *TnphoA*. Southern blot analysis was performed on the 13 *V. vulnificus* mutants. Chromosomal DNA was digested with *SalI*, which cuts once within *TnphoA*. A Southern blot of the chromosomal DNA digests probed with a labeled *phoA* probe revealed a hybridizing 6.5-kbp band and a 13.5-kbp band in all 13 mutants, indicating that they were

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TABLE 1. Strains used in this study

Strain or plasmid	Relevant characteristics ^a	Reference or source
Strains		
<i>V. vulnificus</i>		
MO6-24	Poly ^r , opaque	27
CML44	<i>venB::TnphoA</i> ; Poly ^r Km ^r ; opaque	This study
<i>E. coli</i> DH5 α	F ⁻ <i>endA1 hsdR17 supE44 thi-1 recA1 gyrA96 relA1</i> Δ (<i>argF-lacZYA</i>)U169 (Φ 80 Δ <i>lacZ</i> M15) λ^-	10
Plasmids		
pBluescript SK-	Phagemid derived from pUC19; Ap ^r	Stratagene
pLAFR3	Cloning vector; Tc ^r	24
pCML31	7.5-kbp <i>SalI</i> chromosomal <i>TnphoA</i> gene fusion fragment from CML44 clone in pUC19; Ap ^r Km ^r	This study
pTWR2	Positive clone from <i>V. vulnificus</i> MO6-24 genomic library; 8.3-kbp chromosomal fragment in pBluescript SK-; Ap ^r	This study
pTWR3	3.0-kbp <i>EcoRI-HindIII</i> <i>V. vulnificus</i> subclone of pTWR3 in pLAFR3; <i>venB</i> and <i>viuB</i> clone; Tc ^r	This study
pTWR10	1.9-kbp <i>EcoRI-EcoRV</i> <i>V. vulnificus</i> subclone of pTWR3 in pLAFR3; <i>venB</i> clone; Tc ^r	This study

^a Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Poly^r, polymyxin B resistance; Tc^r, tetracycline resistance.

identical clones (data not shown). One mutant, designated CML44, was chosen for further analysis.

Iron utilization assay. CML44 was analyzed for the ability to utilize hemoglobin, hemin, FeSO₄, and iron-saturated transferrin by the procedure of Simpson and Oliver (23). CML44 was unable to use iron from 100% iron-saturated transferrin (Table 2). CML44 was able to use hemoglobin, hemin, and FeSO₄ as an iron source. All strains were able to use FeSO₄, hemoglobin, and hemin as iron sources.

Arnow assay and bioassay for catechols. The extraction of phenolic-type (catechol) siderophores and hydroxamate siderophores was carried out as previously described (22). Con-

centrated filtrate from the wild type, MO6-24, gave a positive result in the Arnow test (2) for catechols and a weakly positive result in the Csaky test (6) for hydroxamates. Concentrated filtrate from mutant CML44 gave negative results for catechol siderophores in the Arnow test and weakly positive results in the Csaky test. Vulnibactin extracted from the culture supernatant of MO6-24 by the procedure of Griffiths et al. (9) stimulated the growth of all strains (Table 2).

Production of siderophores was also determined by bioassay as previously described (22). Organisms (10⁵ cells per ml) of indicator bacterial strains were solidified in iron-depleted medium (LB agar and 75 μ g of EDDA per ml). The ability of the

FIG. 1. Restriction maps of pCML31, pTWR2, pTWR3, and pTWR10. The *phoA* insertion in CML44 chromosomal DNA interrupting the *venB* gene is shown. Kanamycin resistance (Km) on *TnphoA* is also represented. The cloned chromosomal DNA restriction fragments in plasmids pTWR2, pTWR3, and pTWR10 are depicted by thick solid lines. The positions of the *V. vulnificus* *venB* and *viuB* genes are shown, with arrows indicating the direction of transcription immediately below. The short arrows at the bottom indicate the start points and directions of sequencing experiments; the asterisks indicate synthetic oligonucleotides used as primers for sequencing, as opposed to the universal primer M13.

TABLE 2. Stimulation of growth of *V. vulnificus* strains by producer strains and various iron sources

Producer strain or iron compound	Diameter of zone of growth (mm) of indicator strain ^a			
	MO6-24	CML44	CML44 (pTWR3)	CML44 (pTWR10)
Transferrin (2.6 mM)	25	0	24	22
Fe (10 mM)	30	16	28	25
Hemoglobin (1 mM)	30	20	28	26
Hemin (8 mM)	28	20	28	30
Vulnibactin (2 mM)	22	15	20	22
MO6-24	28	16	26	24
CML44	10	9	13	12
CML44 (pTWR3)	20	16	17	18
CML44 (pTWR10)	23	18	22	23

^a The indicator strains were seeded into low-iron agar under conditions such that no growth occurred in the absence of usable exogenous siderophore or iron-containing compound.

producer strains to produce siderophores or the indicator strains to use siderophores was determined by measuring the growth of the indicator strains around 10- μ l spots of stationary-phase bacterial cultures (producer strains) after incubation at 37°C for 18 h. MO6-24 as a producer strain could provide usable siderophores to both the *V. vulnificus* wild type, MO6-24, and CML44. CML44 as a producer strain could provide a usable siderophore to MO6-24 and itself, although there was only a small amount of growth compared with that obtained with MO6-24 as the producer strain. This indicates that MO6-24 can use the hydroxamate siderophore from CML44.

Cloning of the *venB* gene and complementation of CML44 with pTWR3 and pTWR10. The gene fusion in strain CML44 between the gene designated *venB* and the *phoA* gene on *TnphoA* was cloned into pUC19 (pCML31). The entire *venB* gene was cloned by screening a recombinant λ ZAPII phage genomic library of *V. vulnificus* MO6-24 constructed by standard methods (using a kit from Stratagene, La Jolla, Calif.) with the 1.0-kbp *Sall-EcoRI* fragment upstream of the *TnphoA* fusion from pCML31. A hybridizing clone in pBluescript (pTWR2) was obtained and a restriction map was developed (Fig. 1). A 3.0-kbp *EcoRI-HindIII* fragment was subcloned into pLAFR3 (pTWR3), and a 1.9-kbp *EcoRI-EcoRV* fragment was subcloned into pLAFR3 (pTWR10). When pTWR3 was introduced into strain CML44, production of a catechol siderophore was restored as determined by both the Arnov test and the biologic siderophore assay (Table 2). Subclone pTWR10 (Fig. 1) also restored normal production of catechols.

DNA sequence. We determined the DNA sequence from both strands of DNA from *EcoRI* to 2.3 kbp downstream (Fig. 2) by the dideoxy-chain termination method of Sanger et al. (20) on double-stranded DNA template by using a Sequenase kit from United States Biochemical Corporation, Cleveland, Ohio. We also determined the DNA sequence from pCML31, upstream of the *TnphoA* fusion, and found this sequence to be identical to the corresponding portion of the *EcoRI-HindIII* fragment. The nucleotide sequence contained three open reading frames (ORF). The first ORF extends from upstream of the *EcoRI* site and ends at bp 351. The second ORF is 885 bp beginning 457 bp downstream from the *EcoRI* site and ending at bp 1341. A putative Shine-Dalgarno sequence is located just upstream from the initiating methionine. An 813-bp ORF was also identified downstream of *venB* starting at base 1408 and ending at 2220. A putative Shine-Dalgarno sequence was iden-

tified just upstream from the initiating methionine of the third ORF. A perfect inverted repeat, suggestive of a bidirectional transcription terminator, is indicated just beyond the termination codon. No sequences homologous to *E. coli* -10 boxes or -35 boxes indicative of promoter regions were identified in the sequence. We were also not able to identify dyad sequences homologous to an *E. coli* Fur box.

Homology of *V. vulnificus* *venB* to *E. coli* *EntB*. The deduced amino acid sequence of the second ORF in Fig. 2 was analyzed for homologous proteins with the BLAST algorithm (1, 8). The protein sequence was found to be homologous to *E. coli* enzyme 2,3 dihydro-2,3 dihydroxybenzoate synthase (isochorismatase), *EntB*, one of the enzymes responsible for the synthesis of the catechol (phenolate) siderophore enterobactin (17). Alignment of the 295 deduced amino acids of *venB* revealed 41% identity to the 288 amino acids of *EntB*.

Homology of the deduced amino acid sequence of the third ORF to *V. cholerae* *ViuB*. The deduced amino acid sequence of the third ORF in Fig. 2 was also analyzed for homologous proteins as described above. The best match was to the *V. cholerae* gene *viuB*, a gene required for ferric vibriobactin utilization by *V. cholerae* (5). Optimal alignment of the 271-amino-acid sequence encoded by the *V. cholerae* *viuB* gene with the 271 amino acids in the deduced sequence of the *V. vulnificus* ORF showed 80% identical residues between these proteins.

No sequences showing homology to -10 to -35 boxes were identified upstream of either *venB* or *viuB* of *V. vulnificus*, suggesting that these genes may be part of a polycistronic operon similar to the *E. coli* enterobactin operon *entCEBA*. The structure of this region in *V. vulnificus* differs from that in *V. cholerae*. In *V. cholerae* the gene for the vibriobactin receptor precursor (*viuA*) is immediately upstream of *viuB* (5). Unlike *venB* and *viuB* of *V. vulnificus*, both *viuB* and *viuA* of *V. cholerae* are monocistronic genes that contain potential Fur boxes within their promoters.

Virulence assay. LD₅₀ assays were performed by intragastric inoculation of 5-day-old suckling mice (CD-1; Charles River) with serial 10-fold dilutions of the bacterial suspensions of the wild type, MO6-24, and mutant CML44 grown in LB medium and suspended in 0.15 M NaHCO₃ (pH 8.15). Results were examined by probit analysis to estimate the LD₅₀ and determine whether the dose-response functions of MO6-24 and CML44 were significantly different (7, 21). The LD₅₀ of the mutant strain CML44 at 20 h was 10⁷. This was a significant increase compared with an LD₅₀ of 6.3 $\times$ 10⁴ for the wild type, MO6-24, at 20 h (P = 0.015). At 24 h the LD₅₀ of CML44 was 2.6 $\times$ 10⁶ and still significantly different from the wild-type LD₅₀ of 7.4 $\times$ 10⁴ (P = 0.048). No additional mice died at the 36-h point. This increase in the LD₅₀ of the mutant compared with the wild-type strain clearly suggests that the ability to produce a catechol siderophore is important for virulence in this animal model.

Conclusions. An association between virulence and the ability of *V. vulnificus* to acquire iron from transferrin has been noted in a number of studies (16, 25). Isolation of a transferrin utilization mutant of *V. vulnificus* allowed us to analyze the mechanism of transferrin uptake and helped to clarify the importance of iron acquisition in *V. vulnificus* pathogenesis. While our data seem to confirm the importance of the catechol siderophore in acquisition of iron from transferrin, it is also possible that the *venB* mutation affects the regulation of other genes involved in iron acquisition. Our observations are consistent with that of Stelma et al. (25), who found that the ability of *V. vulnificus* strains to use transferrin iron was associated with catechol siderophore production. This mechanism of

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E F N R K K E I T V A S L K K P E A C E S L N D I R L G I D T L D K E
G A A T T C A A T C G A A G A A A T A C C G T G C A T C G T T G A A G A A C C A G A G C G T G T G A A A G C C T A A T G A T T C G A C T G G G A A T C G A C A C T C T T G A T A A G 100
EcoRI
I V H I L S Q R M G Y V K A A A Q F K P D E K S I P A P E R V A S
A G A T T T G C A T A T C C T C T C T C A G A G A A T G G G A T A C G T G A A A G C T G C C G C A C A A T T T A A C C C G A T G A G A A A G C A T A C C T G C C C T G A G C G A G T G G C G T C 200
M L E E R R R H W A N E Q G L S E E Y V E A L F D N I I Q W Y I S Q
C A T G C T G A A G A A C G C G G C A T T G G G C A A T G A A C A G G G T T A T C A G A A G A G T A T G T G A A G A C A C T T T T T G A C A C A T T A T C C A A T G G T A C A T C A G T C A G 300
Q I Q H W R S Q K G L A P V S Q D
C A G A T C C A G C A T T G G C G A G C A A A A G G C C T T G C A C C A G T G A G T C A A G A T T A G C T T A T C A C T C G T A A C A C A C G C T A A G C G T C A T G C T C G T C T G T A 400
M G I P K I A G Y P L P T P A
G T G C C G C A T A T C G A A C C G C C A C C A T C A T G A C A A A C A A A T G A A A G G A T T A T C A C A T G G G A A T C C C A A A A T T G C T G G C T A T C C G T T G C C A A C C C C G C 500
SD
E F P D N R T G W T I D P D Q A V L L I H D M Q E Y F V N Y V Q P
T G A G T T T C C G G A T A C A G A A C G G C T T G A C A A T C G A T C C C G A C C A G G C G T A C T T T T G A T C C A C G A T A T G C A A G A A T T T C G T C A A C T A T T A T C A G C C A 600
C l s I S s p I
D S S P V V D I I Q H I Q R L K A A A K K A G I P V I Y T A Q P A N
G A T T C A T C C G A G T T G T G A C A T C A T T C A A C G C T T A A A G C C G A G C G A A A A A G G G C A T T C C C G T A T C A T A C G C C A G C C T C T A 700
Q H P T D R A L L T D F W G P G L N G D H V P I V E A L S P E E D
A C C A A C A T C C G A C A G A C C G A G C G T G C T C A C T G A T T T C T G G G G C C G G C C T G A A T G G C G A T C A T G A C T A T C G T T G A A G C A C T A G C C C A G A A G A G A 800
↑ T n p h o A
D I E Y V K W R Y S A P K K T P L L E F M R A Q G K S Q L I I S G
T G A C A T C G A A T C G T C A A T G C C G T T A C A G C C A T T T A A G A A C C C C A T T G C T C G A G T T T A T C C C G C C A G G G A A A G C C A G C T C A T C A T T T C T G G 900
I Y G H I G I L S T T L D A F M L D I Q P P V I G D A I A D F T R E
A T T T A T G C C A T A T C G G C A T T T C T G A C C A G T T A G A C C C T T C A T G C T G G A C A T T C A G C G T T T G T A T T G T G A T G C C A T C C G G A T T T A C T C G A G 1000
D H L R T L Q Y V A S R S G S V K R L D E A L D E I R S Q K P L T
A A G A T C A T T T A C G A C C T T G C A T A T T G G C C A G C G T A G T G A G C T A A A G C G T C T G A T G A G C A T T A G A A A C C G T T C C C A A A G C C C T A A C 1100
L E Q I Q Q D V A T S L G I Q P D E V D L D E D L M F V G L D S M
G C T G G A A C A G A T C C A G C A G A C G T T G C G A C G A C T T A G G A A T A C A C C A G A C G A G T T G A T C T T G A T G A G G A T T A A T G T T C G T T G G C T T G G A C T C G A T G 1200
R A M V L V E K W H Q Q G E N I S F G Q L M E A A S L R E W W L V I
C G G G C G A T G G T G T T G G A A A A T G C A T C A C A G G G T G A G A C A T T T C G T T T G T G C A A T T G A T G G A A G C G C T T C G C T T A G A G A G T G T G G C T G T G A 1300
E Q A R N E E Q T M A V A . . .
T T G A A C A A G G A A A T G A A G A C A A A C C A T G C C G T C C C T A A G T G T G A A G G T C A A A G A T A A C A A C G T C A G C T A G C A A A A A T A C A A A A A G A T T A G G 1400
SD
M S D S P E R V Y P M L L D F V R K E T I S K N L L R V T L T
T G A A A G A A T G A G T G A C G C C A G A G C G C T C A T C C A A T G T T G C T G G A T T T T G T A C G C A A A G A A C C A T C T C A A A A A C T T A C T C C G A T A A C G T T A A C 1500
G E D L I G F P E D Q N G S H I K V F P P N Q A S G I L Q L P V R E
G G A G A G A T T T A A T C G C C T T C C G G A A G A C A A A T G T T C G C A C A T C A A A G T G T T T T C C C A C C A A G C A A G T G C A T T C T C A G T T G C C G T G C G A G 1600
G D N V I W P E H K P V P R A Y S V R Q Y R A A V N E L D I D F V
A A G G G C A A C A C G T C A T T T G C C T G A A C A A A C C G T T C G A G A G C T T A C T C G G T C A G A C A A T A T C G T C G C G G G T G A A T G A G C T T G A C A T C G A T T T G T 1700
T H G E E T P G G G W A L K A D I G S Q I G L I G P A G P D P L I
G A C C G A T G G G A G A A C C C C G G C G T G T T G G C A C T A A A G C A G A T A T A G C T C G C A A A T T G G T T A A T T G G G C C A G C A G G C C T G A T C C G C T A A T T 1800
E P A D W H I I A G D L S A V P A I S A I L E K L F S D A K G Y V F
G A A C C C G G A T T G C A T A T A A T C G A G G T G A T C T C T C G G C G G T G C C T G C G A T T A G T G C C A T C T T G G A A A G T T G C C C A G T G A C C C A A A G G C T A T G T G T 1900
I E V D E I E D I H D L V H P Q K M A I N W L M P N P H D A E P A
T T A T T G A G T G A T G A A T T G A A T T C A C G A T C T T G T T C A C A A A A A T G G C C A T C A A T T G G T G A T G C C G A A T C C T A T G A T C G C G A G C C G C 2000
EcoRV
L A K A I K Q L P A P E A T S L S A F I A G E N H S V I N C R K
A T T A G C T A A G C G A T T A A G A A C T A C C C G C C C G A A A G C G A C C T C G C T G C T G T T T A T C G C G G T G A A A A C C A T A G T G T G A T C A A C T G C C C T A A G 2100
I L R S D Y Q I A R D K L Y A I P Y W K R G K T E E A Y H D E R H D
A T C T T G C G A G T A C A T C A G A T C G C G T G A C A A C T C T A C C G A T T C C T T A C T G G A A A G A G G T A A G A C G G A A G A C G T A T C A C G A T G A A C G T C A T G 2200
V M D A A Y . . .
A T G T G A T G G A T G C T G C G T A T T A G T C C A C T G C C G T T G T T C A T G A T A G C G C C T C G A A C A G A A A G C G A T T T C A T T G A A T C G C G T T T C T T T A T T C 2300

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FIG. 2. Nucleotide sequence from the *EcoRI* site to 2.3 kbp downstream. The locations of restriction enzyme sites are noted, as is the location of the *TnphoA* insertion in CML44 (↑). The deduced amino acid sequences encoded by the ORF are shown in one-letter code above the sequence. The Shine-Dalgarno sequences (SD) of *venB* and *viuB* are indicated below the line. The termination codons of *venB* and *viuB* (. . .) are shown. The termination codon of *ViuvB* is followed by an inverted repeat suggestive of a transcription terminator (arrows below the sequence).

transferrin utilization is also suggested by the observations of Simpson and Oliver (22), who found that an avirulent strain of *V. vulnificus* did not produce a catechol siderophore.

Morris et al. (16) found that there was no apparent correlation between production of specific siderophores or iron-regulated outer membrane proteins and the ability of a strain to utilize transferrin-bound iron. However, they did not specifically measure the ability of the strains to use siderophore iron. It is possible that the strains that produced a catechol siderophore but could not use transferrin iron lacked one or more enzymes or siderophore receptors to allow the organism to use siderophore iron.

Multiple factors are probably necessary for the virulence of *V. vulnificus*. Many studies have emphasized the importance of the ability of *V. vulnificus* to use host iron for the virulence of this organism (16, 22, 23, 25, 27). These previous observations were based on phenotypic analyses of various clinical and environmental isolates of *V. vulnificus*. In this report, we have

presented the first genetic analysis of a *V. vulnificus* mutant that lacked the ability to use transferrin iron; we found that the mutation occurred in a gene that is probably responsible for catechol synthesis. We further showed that this mutation conferred decreased virulence in *V. vulnificus* in an infant mouse model of infection, directly linking reduced virulence with a specific mutation. Further genetic studies of *V. vulnificus* iron uptake mutants should allow elucidation of the mechanisms by which *V. vulnificus* obtains host iron and the importance of these factors for the virulence of the organism.

Nucleotide sequence accession number. The GenBank accession number for the sequence presented in this article is U32676.

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