Genetic Analysis of the Iron Uptake Region of the *Vibrio anguillarum* Plasmid pJM1: Molecular Cloning of Genetic Determinants Encoding a Novel *trans* Activator of Siderophore Biosynthesis†

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Clones carrying the iron uptake region of the *Vibrio anguillarum* plasmid pJM1 were subjected to insertion mutagenesis, using transposon Tn3::HoHo1 which carries a promoterless *lacZ* gene and can thus generate *lacZ* transcriptional fusions if inserted downstream from an indigenous promoter. Four classes of insertion mutants were obtained based on the level of expression of components of the iron uptake system, and six genetic units were defined according to the phenotype of the mutants. Five of the six genetic units were crucial for biosynthesis of the siderophore anguibactin. Insertions in the remaining genetic unit led to an iron uptake-deficient phenotype and showed either reduced levels of the outer membrane protein OM2 as well as anguibactin activity or a complete shutoff of both OM2 and anguibactin biosyntheses. Analysis of β -galactosidase production by cells carrying the *lacZ* fusion derivatives identified iron-regulated and constitutive transcriptional units as well as their orientation in the genetic units. Molecular cloning of pJM1 plasmid DNA noncontiguous to the iron uptake region also identified genetic determinants for a *trans*-acting factor required for full expression of anguibactin activity. Evidence obtained from bioassays, spectrophotometric measurements, and the *lacZ* fusion mutants suggested that the *trans*-acting factor is a novel activator of siderophore biosynthesis at the transcriptional level.

Iron is essential for the growth of most bacteria, but in vertebrates this element is found complexed to high-affinity iron-binding proteins and is unavailable for bacterial use (6, 12, 18, 27). Pathogenic bacteria capable of causing disseminated infections have thus evolved specialized iron assimilation systems to overcome this first line of the host defense mechanisms. *Vibrio anguillarum* 775, the etiological agent of the devastating fish disease vibriosis (20), carries the 65-kilobase-pair (kbp) plasmid pJM1, which is essential for its virulence (9). Plasmid pJM1 encodes a very efficient iron uptake system that allows *V. anguillarum* 775 to compete for iron with the high-affinity iron-binding proteins present in the host fluids (5). The OM2 outer membrane protein, essential for the energy-dependent iron transport process, and the siderophore anguibactin, the actual iron scavenger, are the two main pJM1-encoded components of this novel iron uptake system (8, 26). High levels of free iron are sensed as a turnoff signal by the system genes that are turned on under conditions of iron limitation, such as those found in the host fluids and tissues (8, 26, 28). Recent experimental evidence indicates that the pJM1 iron transport system is indeed expressed *in vivo* and can influence the outcome of the infection (28). Recently, a 17.6-kbp pJM1 DNA region encoding this system was cloned in the low-copy-number vector pVK102, which permitted its transfer to and expression in *V. anguillarum* cells (24).

In this work we report the genetic analysis of the iron uptake region of pJM1. By using insertion mutagenesis and *lacZ* fusions, we identified six distinct genetic units which are essential for the iron uptake process. We also identified a region in plasmid pJM1, noncontiguous to the iron uptake sequences, that encodes a novel *trans* activator of siderophore biosynthesis.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Escherichia coli* C2110 *polA* and *V. anguillarum* strains 775(pJM1), H775-3 (plasmidless), H775-3A(pJHC-T7), H775-3B(pJHC-T2612), H775-3C(pJHC-T11), 775-Tn1-6(pJHC9-8), 775-Tn1-6A(pJHC9-8, pJHC-T7), and 775-Tn1-6B were described previously (5, 8, 24, 26, 28). Plasmids pHoHo1 and pSShe (22), which carry the Tn3::HoHo1 and the transposase genes, respectively, were provided by E. Nester and S. Stachel; plasmid pRK2013 (10) or pRK2073 (15) was used as a helper in conjugations.

Plasmid DNA analysis. Isolation of plasmid DNA and agarose gel electrophoresis were performed as described previously (24). Molecular cloning of pJM1 DNA regions was carried out by ligation of partially digested pJM1 DNA with *Bam*HI or totally digested pJM1 DNA with *Kpn*I into the *Bam*HI site of pBR325 (3) or the *Kpn*I site of pJHC-T00181 (25), respectively. Transformation of *E. coli* HB101 cells with the ligation mixtures was performed by the method of Cohen et al. (4).

Determination of components of the iron uptake system. Bioassays were carried out as described previously (26), using *V. anguillarum* H-775-3C(pJHC-T11) as the indicator strain for detection of siderophore activity. Iron transport

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activity in mutants deficient in the production of the siderophore anguibactin was detected by applying 10 μ l of sterile *V. anguillarum* 775 pJM1 culture supernatant containing anguibactin to sterile filter disks placed on M9 minimal medium agar plates containing the iron chelator ethylenediamine-di(*o*-hydroxyphenyl)acetic acid (EDDA) at a 10 μ M concentration and seeded with the anguibactin-deficient mutant strain to be tested (1). Outer membrane proteins were isolated and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by immunoblot analysis as described previously (2, 23). Purification of siderophore from culture supernatants was carried out as described by Actis et al. (1, 2).

Transposition mutagenesis. Insertions into recombinant plasmids JHC-T7 and pJHC-T2612, which include the pJM1 iron uptake region, were generated by using Tn3::HoHo1, a derivative of Tn3 containing a promoterless *lacZ* gene and lacking a functional transposase (22). Tn3::HoHo1 is contained in plasmid pHoHo1 and can transpose in the presence of plasmid pSShe, which contains the transposase gene. To isolate the Tn3::HoHo1 insertions, plasmid DNA (pJHC-T7 or pJHC-T2612) was used to transform *E. coli* HB101 already harboring plasmids pHoHo1 and pSShe. Tn3::HoHo1 insertions on pJHC-T7 or pJHC-T2612 were isolated by mobilization to *E. coli* C2110 *polA* Nal^r by using as mobilizing plasmid either pRK2013 or pRK2073 from *E. coli* HB101. Selection for the final recipient harboring the insertion derivative was carried out in the presence of the antibiotics tetracycline, ampicillin, and nalidixic acid. Transfer of the Tn3::HoHo1 insertion derivatives to *V. anguillarum* was also carried out by three-strain crosses. The final recipient for this cross was a *V. anguillarum* strain already harboring plasmid pJHC9-8 as a source of *trans*-acting factor (Taf) (24). Conjugations between the donor *E. coli* C2110 carrying the Tn3::HoHo1 insertion, the *V. anguillarum* final recipient, and helper *E. coli* harboring either pRK2013 or pRK2073 were carried out as described previously (24). β -Galactosidase activity was assayed in *V. anguillarum* strains containing plasmids with a Tn3::HoHo1 insertion

essentially as described previously (16). Because Tn3::HoHo1 insertions resulted in iron uptake-deficient mutants, identification of pJM1 iron-regulated promoters fused to the *lacZ* genes was accomplished by using mild iron-limiting conditions, i.e., a one-tenth reduction (to 1 μ M) of the concentration of the iron chelator EDDA in M9 minimal medium. Iron-rich conditions were achieved by using 40 μ M FeCl₃.

RESULTS

Analysis of insertion mutants in the pJM1 iron uptake region. Recombinant plasmids pJHC-T7 and pJHC-T2612 (Fig. 1), which carry the pJM1 iron uptake region (24), are mobilizable by conjugation to *V. anguillarum*. Using these plasmids, we initiated an insertional mutagenesis analysis with the transposition sequence Tn3::HoHo1 (22), which carries a promoterless *lacZ* gene. After localization of the insertion site and orientation of the transposon by restriction endonuclease mapping (Fig. 1), each derivative was transferred by conjugation to *V. anguillarum* 775::Tn1-6, which harbors the pJM1 derivative pJHC9-8. This plasmid provided the Taf activity needed for full expression of the iron uptake system in *V. anguillarum* (see below). The transconjugant *V. anguillarum* strains were then analyzed for their ability to grow under iron-limiting conditions (minimal medium with 10 μ M EDDA) which fully induce the pJM1 iron uptake system. This analysis showed that most of the strains harboring insertion mutant plasmids were unable to grow under those conditions; that is, they showed an iron uptake-deficient phenotype (Table 1). The location of the Tn3::HoHo1 insertions generating iron uptake-deficient mutants is indicated in Fig. 1 by short stalks. However, some transconjugant strains were able to grow under these conditions, indicating that the mutation did not affect expression of the pJM1-mediated iron uptake system (Fig. 1, solid vertical bars; Table 1), identifying nonessential regions in between essential sequences for iron uptake. These results suggested the presence of several genetic units (I to VI) in

FIG. 1. Genetic and physical map of Tn3::HoHo1 insertion mutants in pJHC-T7 and pJHC-T2612. The physical map of pJHC-T7 and pJHC-T2612 for *Xho*I (X) and *Eco*RI (E) restriction endonucleases is represented by the bar. Vertical solid bars indicate transpositions that did not affect expression of the iron uptake system, while the short stalks correspond to iron uptake deficiency mutations. Insertions in which the *lacZ* gene is oriented from left to right are above the bar, and those with the *lacZ* gene oriented from right to left are shown below the bar. Large arrows indicate the orientation of *lacZ* transcription. β -Galactosidase was assayed as described by Miller (16), using M9 minimal medium with the addition of either 1 μ M EDDA or 40 μ M FeCl₃ in *V. anguillarum* strains harboring pJHC9-8 in addition to the pJHC-T7 or pJHC-T2612 derivatives carrying the Tn3::HoHo1 insertion. Transposon insertions with open circles correspond to those with no β -galactosidase activity (<30 U); closed circles indicate transposition derivatives that produced β -galactosidase constitutively (>30 U in the presence of 1 μ M EDDA or 40 μ M Fe³⁺). Transposition derivatives with closed squares are those that produced iron-regulated β -galactosidase activity (specific activity when grown in 1 μ M EDDA was at least threefold higher than that when grown in 40 μ M Fe³⁺). Numerals I to VI as well as the distinctive shading inside the bar symbolize the genetic units. The genetic characteristics for each transposition mutant are listed in Table 1.

TABLE 1. Properties of *V. anguillarum* harboring Tn3::HoHo1 insertion mutants in the pJM1 iron uptake region

Genetic unit	Recombinant source of iron uptake region	Insertion mutant no.	Iron uptake phenotype ^a	Anguibactin activity	Iron transport	OM2
I	pJHC-T2612	140	D	—	+	+
	pJHC-T7	63	D	—	+	+
	pJHC-T2612	16	D	—	+	+
	pJHC-T7	30	D	—	+	+
	pJHC-T7	62	D	—	+	+
Non-essential	pJHC-T2612	106	P	+	+	+
	pJHC-T2612	45	P	+	+	+
II	pJHC-T7	26	D	—	—	—
	pJHC-T7	20	D	L ^b	—	L
	pJHC-T7	28	D	L	—	L
	pJHC-T7	17	D	L	—	L
	pJHC-T7	15	D	L	—	L
	pJHC-T7	42	D	—	—	—
	pJHC-T7	14	D	L	—	—
III	pJHC-T7	1	D	—	+	+
	pJHC-T7	40	D	—	+	+
	pJHC-T2612	4	D	—	+	+
Non-essential	pJHC-T7	55	P	+	+	+
IV	pJHC-T7	43	D	—	+	+
	pJHC-T7	12	D	—	+	+
	pJHC-T7	22	D	—	+	+
	pJHC-T2612	6	D	—	+	+
	pJHC-T7	68	D	—	+	+
	pJHC-T2612	120	D	—	+	+
Non-essential	pJHC-T7	60	P	+	+	+
	pJHC-T2612	5	P	+	+	+
V	pJHC-T7	24		—	+	+
Non-essential	pJHC-T7	64	P	+	+	+
	pJHC-T7	23	P	—	—	—
VI	pJHC-T7	19	D	—	+	+
	pJHC-T7	2	D	—	+	+
	pJHC-T7	53	D	—	+	+
	pJHC-T7	29	D	—	+	+
	pJHC-T7	61	D	—	+	+
	pJHC-T7	38	D	—	+	+
	pJHC-T7	52	D	—	+	+
	pJHC-T7	57	D	—	+	+
Non-essential	pJHC-T7	44	P	+	+	+
	pJHC-T7	56	P	+	+	+

^a P, Proficient; D, deficient.^b L, Low.

the pJM1 iron uptake region. Further analysis of this region was carried out by testing for anguibactin production, iron transport activity, and OM2 biosynthesis. Table 1 and Figure 1 show that iron uptake-deficient mutants carrying derivatives with insertions in regions I (coordinates 3.7 to 5.9 kbp), III (coordinates 12.5 to 15.7 kbp), IV (coordinates 15.8 to 20.9 kbp), V (coordinates 21.3 to 21.7 kbp), and VI (coordinates 22 to 25.3 kbp) produced no anguibactin activity but were unaffected in iron transport activity and OM2 biosynthesis. Conversely, *V. anguillarum* strains carrying mutants with insertions in region II (coordinates 6.4 to 12.5 kbp) showed no iron transport activity. Table 1 shows that insertions in this genetic unit were divided into three classes. Insertions 26 and 42 resulted in complete shutoff of both

anguibactin and OM2 production. Insertions 20, 28, 17, and 15 had as a phenotype low but detectable production of both anguibactin and OM2. Insertion 14 abolished OM2 biosynthesis but resulted in a low but detectable level of anguibactin activity. None of the mutations in genetic unit II mapped within the structural gene for OM2, although they affected both anguibactin and OM2 biosyntheses. It is thus likely that the unit II genes affected by those insertions play regulatory roles in the biosynthesis of these two components (L. Waldbeser, L. Actis, and J. H. Crosa, manuscript in preparation).

Since Tn3::HoHo1 contains a promoterless *lacZ* gene, the direction of transcription of the genes where it had inserted could be determined by measuring the β -galactosidase activ-

ity produced by cells carrying the insertion derivatives. Significant levels of β -galactosidase activity are dependent on transcriptional fusions with the gene into which the element is inserted (22). Thus, expression of the *lacZ* gene should be under the control of *V. anguillarum* plasmid pJM1 promoters. Expression will only occur when the reading frame of *lacZ* is in the same orientation as the direction of transcription of the pJM1 gene. If these promoters could sense the iron status of the cell, then β -galactosidase production by the fusions would in turn become regulated by iron. Results of the analysis of production of β -galactosidase by *V. anguillarum* strains carrying the insertion derivatives in addition to pJHC9-8 are shown in Fig. 1. Mutant 63 in genetic region I produced iron-regulated β -galactosidase activity, while cells carrying insertions 140 and 16, which are in opposite orientation to insertion 63, showed β -galactosidase production that was not regulated by the iron status of the cells. These mutants were obtained by Tn3::HoHo1 insertion into pJHC-T2612 which is a partial *XhoI* digest of pJM1 DNA cloned in the *XhoI* site of pVK102. Since cloning of DNA in this site abolishes expression of the kanamycin resistance gene, it was conceivable that β -galactosidase gene transcription in fusions 140 and 16 initiated at the kanamycin resistance gene promoter. However, this possibility was ruled out because of the cloning orientation, which showed that transcription of the kanamycin resistance gene must occur in opposite orientation to that of these *lacZ* fusions (22). Therefore, region I contained at least two transcriptional units affecting anguibactin biosynthesis: one iron regulated and the other transcribed in the opposite direction and not regulated by iron. We next examined β -galactosidase production by insertion mutants in genetic unit II which were iron transport deficient and showed three different phenotypes depending on the levels of anguibactin or OM2 produced. Insertions 20, 17, 15, and 14, which resulted in low but detectable levels of anguibactin production, were characterized by high levels of iron-regulated β -galactosidase activity. The direction of transcription deduced from these insertions was opposite to that found with insertion 63 in genetic unit I. The rest of the insertions in this region resulted in no β -galactosidase activity (Fig. 1). Genetic units III to VI were characterized by insertions resulting in no anguibactin production. Insertions 1 and 4 in region III showed production of iron-regulated β -galactosidase. The direction of transcription for these fusions was the same as those leading to production of iron-regulated β -galactosidase in genetic unit II. Two insertions located in the same orientation in genetic unit IV led to production of β -galactosidase, although only one of them (insertion 6) was iron regulated. Insertion 24 located in genetic unit V did not show any β -galactosidase activity. No iron-regulated fusion was found in genetic unit VI. However, insertions 19, 2, and 29 in this unit resulted in the constitutive production of β -galactosidase. The direction of transcription in this unit was the same as that in genetic unit II. In summary, transcription as measured by β -galactosidase production independently of whether it is iron regulated or constitutive occurs in the same orientation in genetic units II, III, and VI. Genetic unit I shows constitutive transcription in the same orientation but iron-regulated transcription in the opposite direction, while we could not assess the direction of transcription in genetic unit V. Both iron-regulated and constitutive transcription were in genetic unit IV, in opposite orientation to that described for genetic unit II.

Molecular cloning of the Taf genes. *V. anguillarum* strains harboring recombinant clones containing the pJM1 iron

uptake region produced normal levels of the siderophore anguibactin under iron-limiting conditions only if they carried another pJM1 derivative such as pJHC9-8, in which the iron uptake region had been deleted (24). Therefore, a pJM1 sequence other than the iron uptake region must carry genetic determinants encoding a *trans*-acting factor (Taf) necessary for full expression of anguibactin biosynthesis. To further characterize this Taf, we cloned the Taf genes from the original pJM1 plasmid. pJM1 DNA was partially digested with *Bam*HI or completely digested with *Kpn*I. The DNA fragments were ligated into the *Bam*HI site of pBR325 or the *Kpn*I site of pJHC-T00181 and transformed into *E. coli* HB101 competent cells. The recombinant plasmids (Fig. 2) were then transferred to a *V. anguillarum* strain already harboring the cloned pJM1 iron uptake region contained in recombinant plasmid pJHC-T7. The transconjugant strains were finally tested for their ability to grow in M9 minimal medium agar plates containing EDDA. Only transconjugants carrying the Taf determinants should grow under these conditions. Figure 2 shows that plasmids pJHC-W44, pJHC-T6.14, and pJHC-T28.6 were the only recombinant derivatives carrying the genetic determinants for this *trans*-acting activity. It is of interest that some of these recombinant clones, such as pJHC-T28.6, showed a strong tendency to generate deletion derivatives of pJM1. All of these derivatives had also lost the ability to produce Taf activity. This type of instability was also found during our attempts to generate transposition insertion mutants in this region (data not shown).

Mechanism of action of the Taf. The Taf may play a role as either a regulator of anguibactin biosynthesis or a biosynthetic enzyme required to convert a precursor of anguibactin with low siderophore activity into anguibactin. To understand the mechanism of action of this Taf, we first quantitated the activity of anguibactin from cells carrying the iron uptake region clone pJHC-T7 either alone or with pJHC-T6.14, which carries the cloned Taf genes. Culture superna-

FIG. 2. Genetic and physical map of pJM1 and recombinant clones. The diagram shows the sites for restriction enzymes *Bam*HI (H), *Sal* (S), and *Kpn*I (K). A thin dashed line shows the iron uptake region in pJM1. Solid lines show pJM1 DNA fragments cloned in either pBR325 or pJHC-T00181. The plus or minus sign beside the recombinant clone designation indicates presence or absence of the Taf gene(s) as determined by the ability of *V. anguillarum* carrying pJHC-T7 in addition to the recombinant clone to grow in M9 minimal medium containing 24 μ M EDDA. The thick dashed line with arrowheads shows the deduced location of the Taf gene(s).

FIG. 3. Bioassay to detect anguibactin activity in the presence or absence of the Taf genes. Iron-depleted minimal medium agar was seeded with *V. anguillarum* H775-3C which harbors pJHC-T11, a recombinant clone encoding receptor activity (9). Samples of the purified product made by *V. anguillarum* (pJHC-T7, pJHC-T6.14) (A) and *V. anguillarum* (pJHC-T7) (B) were added to a filter disk and placed on top of the agar. After purification of the siderophore from both strains, the products were dissolved in the same volume of ethanol and 1 and 10 μ l were spotted in disks A and B, respectively. Controls included 10 μ l of culture supernatants of *V. anguillarum* carrying plasmid pJHC9-8 (C) or pJHC-T6.14 (D) only as a source of Taf and the plasmidless H775-3 (E). Positive control in disk F is 10 μ l of a 10 mM FeCl_3 solution.

tants from these strains were subjected to the procedure for anguibactin purification. The material thus obtained was used in bioassays to detect anguibactin activity. Figure 3 shows that the levels of anguibactin activity were 20-fold lower when the Taf genetic determinants were not present. As expected, culture supernatants of *V. anguillarum* carrying either pJHC9-8 or pJHC-T6.14 only produced no anguibactin activity.

The UV-visible absorption spectrum of the purified material from *V. anguillarum* (pJHC-T7) supernatant was identical to that from *V. anguillarum* (pJHC-T7, pJHC-T6.14), although it was present in lower amounts (data not shown). These results suggested that Taf may be enhancing the production of anguibactin rather than modifying a preformed precursor of this siderophore.

Additional support for a regulatory role for Taf was obtained by analysis of β -galactosidase production in cells carrying selected Tn^3 ::HoH₀₁ insertion derivatives in the presence or absence of pJHC-9-8 as a source of Taf. We selected insertions in genetic units II, which affected both anguibactin biosynthesis and iron transport, and III, which led to the impairment of anguibactin biosynthesis. The latter was chosen as a representative of the rest of the genetic units since they share the same phenotype. Table 2 shows that insertions 20, 17, 15, and 14 (genetic unit II) and 1 and 4 (genetic unit III) resulted in enhanced production of β -galactosidase only in the presence of Taf. Furthermore, specific DNA probes from genetic units II and III showed hybridization with steady-state *V. anguillarum* mRNA only under conditions of iron limitation (data not shown). These

TABLE 2. Taf enhancement of iron-regulated β -galactosidase activity in Tn3::HoHo1 insertions

Insertion mutant no.	β -Galactosidase activity (U) ^a	
	-Taf	+Taf
20	43	310
17	0	42
15	0	179
14	0	77
1	0	40
4	0	30

^a β -Galactosidase was assayed in *V. anguillarum* cells carrying the insertion mutant derivative alone or in addition to pJHC9-8, which encodes the Taf activity. Assays were carried out with cultures grown in M9 minimal medium containing 1 μ M EDDA; the units were calculated as described by Miller (16).

results suggest that Taf must thus act as a diffusible transcriptional activator of anguibactin biosynthetic genes.

DISCUSSION

Iron is an environmental signal that plays an important role as a regulator of the expression of virulence-associated factors such as the iron uptake system mediated by plasmid pJM1 in *V. anguillarum* (5, 7-9, 24, 26, 28). In this work, the effect of insertion mutations in the cloned pJM1 iron uptake region was studied to investigate their influence on the expression of these genes. The insertion mutants defined six distinct genetic units in the pJM1 iron uptake region. Insertions in genetic units I, III, IV, V, and VI affected only the production of anguibactin, suggesting that these units must encode enzymes of the biosynthetic pathway for anguibactin. In contrast, insertions in genetic unit II affected both iron transport and anguibactin activity. Three classes of mutants were identified in this region: those which resulted in a low but detectable level of both anguibactin and OM2 production, another class which led to a complete shutoff of anguibactin and OM2 biosynthesis, and a third type characterized as producing low anguibactin activity and no detectable OM2. However, the insertions in genetic unit II were mapped outside of the structural gene for the OM2 protein at both upstream and downstream positions (manuscript in preparation). It is thus likely that genetic unit II, in addition to encoding components of the iron transport system such as the outer membrane protein OM2, plays a role in regulation of the expression of the iron uptake system as a whole. Alternatively, it is possible that the insertions caused polar mutations that affected downstream genes such as the OM2 gene. β -Galactosidase assays of the *lacZ* fusions caused by these insertions led to identification of iron-regulatable and constitutive promoters within these genetic units. It was remarkable that within genetic unit I there were overlapping iron-regulated and constitutive transcriptional units that were transcribed in opposite directions. The extent of overlapping of these transcriptional units is not known, but due to the close proximity of insertions 140, 16, and 63, it is likely that one stretch of DNA in this pJM1 region must contain at least two genes for anguibactin biosynthesis running side by side in opposite strands. Analysis of the β -galactosidase produced by the *lacZ* fusions caused by insertions in genetic unit II demonstrated the existence of at least one iron-regulated transcriptional unit. Therefore, it appears that the organization of the pJM1-mediated iron uptake system is that of a global regulon with several transcriptional units regulated by the iron status of the cell. The genetic organi-

zation of this system also shows that regions encoding iron transport components as well as regulatory signals are interspersed between anguibactin biosynthetic genes.

Results presented in this work also demonstrated that a pJM1 DNA region other than the iron uptake sequences encoded a Taf required for full expression of the pJM1 iron uptake system. By cloning specific regions of pJM1, we were able to obtain recombinant plasmids carrying the genetic determinants for the Taf. Quantitation of anguibactin production by bioassays and analysis by spectrophotometric procedures supported the idea that the Taf functions as a *trans* activator for siderophore biosynthesis. This hypothesis was strengthened by the fact that β -galactosidase production by bacteria harboring the insertional *lacZ* fusions occurred only if a plasmid providing the Taf was also present. Since this effect was observed in several iron-regulated fusions affecting anguibactin production, it appears that Taf is a class-specific *trans* activator for anguibactin biosynthetic genes. To our knowledge this is the first report of a *trans* activator for procaryotic iron uptake via stimulation of the expression of the siderophore biosynthetic genes. It is of interest that many mammalian DNA viruses and yeasts also encode gene products necessary for the activation of specific promoters. In many cases the *trans*-acting product is a protein that might directly interact with a specific DNA sequence, as is the case for the α gene of herpesvirus (14) and Epstein-Barr virus (19), the GAL4 protein of yeasts (11), or the E2 gene product of bovine papillomavirus (21). Recent evidence demonstrated that the E2 gene product of this virus binds to the region of the viral enhancer sequences (17). In other cases such as adenovirus, the *trans*-activating protein appears to turn on the viral promoter by indirect mechanisms without direct interaction with enhancer or promoter sequences (13).

Due to the nature of the *lacZ* fusions caused by insertion of Tn3::HoHo1 in the plasmid pJM1 iron uptake region (22), it is likely that regulation of anguibactin gene expression by the *trans* activator in *V. anguillarum* is indeed at the transcriptional level. Further characterization of this system will permit us to unravel the molecular nature and precise site of action of this novel *trans* activator of siderophore biosynthesis.

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