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Regulation of the pAD1-Encoded Sex Pheromone Response in *Enterococcus faecalis*: Nucleotide Sequence Analysis of *traA*

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The *Enterococcus faecalis* plasmid pAD1 conjugatively transfers in response to a sex pheromone, cAD1, excreted by potential recipient cells. A key determinant responsible for regulation of pAD1 transfer is *traA*, which encodes a negative regulator also believed to function in signal sensing. In this study, we analyzed the nucleotide sequence and transcription of *traA*. A protein of 319 amino acids with a molecular weight of 37,856 was inferred and found to exhibit limited homology with several DNA-binding proteins. Analysis of Tn*917-lac* insertions resulting in transcriptional *lacZ* fusions within the 3′ end of the *traA* transcript showed that it overlaps slightly with a convergently-transcribed C-region transcript. Insertional mutations affecting TraA repressor function and signal sensing functions were localized.

Conjugative transfer of a unique group of *Enterococcus faecalis* plasmids occurs in response to specific sex pheromones secreted by potential recipients (for a review, see reference 8). One of the best characterized of these systems is the 60-kb hemolysin/bacteriocin plasmid pAD1. Expression of pAD1 genes required for donor-recipient aggregation and plasmid transfer are specifically induced by the peptide pheromone, cAD1 (34). Changes occurring during the pheromone response include the production of novel surface proteins, formation of mating aggregates, and ability to transfer pAD1 DNA (5, 14, 16, 26). Donors exposed to pheromone self-aggregate in the absence of recipients, forming visible clumps in broth cultures (14) or dry fracturable colonies on solid media (47). Both self-aggregation and the dry-colony morphology have been correlated with production of pheromone-inducible surface proteins (15, 47). After receiving a copy of pAD1, cAD1 production by the recipient is shut down (49, 50), and expression of genes required for aggregation and transfer is repressed. In addition, a plasmid-encoded competitive inhibitor of cAD1, designated iAD1, is produced (6, 27).

Genetic analyses, including insertional mutagenesis with Tn*917* and Tn*917-lac*, have revealed a 6- to 7-kb segment of pAD1 responsible for regulation of the pheromone response; five distinct regions have been identified (6, 15, 26, 47) and are depicted in Fig. 1. *traB* and the C region are transcribed constitutively in the same direction. *traB* product is believed to play a role in pheromone shutdown (47, 50), whereas evidence suggests that the C-region product is involved in transduction of the pheromone signal or signal sensing and may also facilitate negative regulation of the pheromone response (47). All C-region mutants isolated to date show reduced sensitivity to induction by cAD1 (15, 47).

iad encodes the competitive inhibitor iAD1, which is able to mask low levels of cAD1 (i.e., which might be present when a potential recipient is far away) (27). Recently the position and sequence of *iad* were reported (6). The determinant is located between *traA* and the E region and is believed to encode a protein 22 amino acid residues in length; the carboxy-terminal 8 residues correspond to iAD1 (33).

The E region is located downstream of *iad*. It was found to be transcribed only when cAD1 was present or when TraB or TraA was dysfunctional (47, 49). Insertional mutagenesis of the E region resulted in loss of the plasmid's ability to express genes required for aggregation and plasmid transfer (15, 47). For this reason, the E region was hypothesized to produce one or more positive regulators of the pheromone response (47).

traA encodes a negative regulator of the pheromone response. Studies in which *traA* was insertionally mutagenized with Tn*917* or Tn*917-lac* resulted in mutants of two different types. The first class of mutants was derepressed in expression of cellular aggregation and plasmid transfer functions, indicating that repressor activity was affected. The second class exhibited only partial derepression of mating functions but was insensitive to further induction with cAD1 (26, 47). For this reason, it was hypothesized that the *traA* product plays a role in pheromone signal sensing as well as negative regulation (47). Studies showed that the *traA* transcript was produced under both inducing and noninducing conditions but was transcribed in the opposite direction of other regulatory determinants such as *traB* and those in the C and E regions (47).

In this study, we conducted a nucleotide sequence analysis in conjunction with characterization of pAD1::Tn*917-lac* derivatives to gain further insight into a key portion of the pAD1 transfer regulatory region. We report the sequence of *traA* and the localization of mutations within the determinant which result in the loss of repressor activity and those resulting in loss of signal-sensing function. Transcriptional data showing that *traA* and C-region transcripts overlap are presented. The potential significance of this observation as well as potential modes of action of TraA are discussed.

MATERIALS AND METHODS

Strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. pAD1::Tn*917* and Tn*917-lac* derivatives were generally characterized in an *E. faecalis* OG1X host background.

Media. Antibiotics were used in the following concentra-

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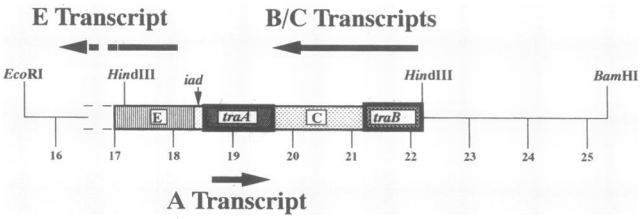

FIG. 1. Regulatory region of pAD1. The arrow above each region indicates the direction of transcription through the region as determined in previous studies (6, 47). The dashed lines around and over the E region indicate uncertainty as to the extent of sequences contained within the E region. The numbers refer to kilobase positions on the pAD1 map.

tions: ampicillin, 120 μg/ml; erythromycin, 10 μg/ml; chloramphenicol, 20 μg/ml; rifampin, 25 μg/ml; fusidic acid, 25 μg/ml; and streptomycin, 1,000 μg/ml. Media used were Luria broth (11) for culture of *Escherichia coli* and THB (Todd-Hewitt broth [Difco Laboratories, Detroit, Mich.]) or N2GT (nutrient broth no. 2 [Oxoid Ltd., London, England] supplemented with 0.2% glucose and 0.1 M Tris-HCl [pH 7.5]) for culture of *E. faecalis*. When solid medium was required, 1.5% agar (Difco) was added. X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside [Sigma Chemical Co., St. Louis, Mo.]) was used at a concentration of 100 or 200 μg/ml. Synthetic cAD1 was used at a concentration of 40 ng/ml.

Enzymes and reagents. The source of enzymes, reagents, and standard primers used for creation of nested deletions and sequence analysis has previously been reported (44). Restriction enzymes were obtained from Boehringer Mannheim (Indianapolis, Ind.). Unique synthetic primers were obtained from the DNA Core Facility (The University of Michigan, Ann Arbor).

DNA cloning; restriction and sequence analysis. *E. faecalis* (9) and *Escherichia coli* (32) plasmid DNA was isolated by isopycnic CsCl-ethidium bromide gradient centrifugation. The recombinant plasmid pAM7500 was created by first gel extracting a 5.0-kb *Hin*dIII fragment of pAD1 (2) and then cloning the fragment into the *lacZ* determinant in pBluescript SK (Stratagene, San Diego, Calif.), using standard cloning procedures (32). The exonuclease III/mung bean nuclease procedure described by Stratagene was used to create nested deletions of pAM7500 in both orientations. Sequencing was done by the dideoxy-chain termination method (38) directly from double-stranded plasmids (3), using previously described modifications (44). In most cases, sequence data were confirmed with reactions using the nucleotide analog dITP. In some cases, to confirm DNA sequence from nested deletions, unique synthetic primers were used.

Sequence analysis of pAD1, pAD1::Tn*917*, and pAD1::Tn*917-lac* derivatives was accomplished by first dialyzing the template against H_2O. Then 1 to 3 μg of the template was precipitated by addition of 10% 3 M sodium acetate and 2.5 volumes of 95% ethanol. After overnight incubation at −20°C, the sample was centrifuged for 10 min in an Eppendorf centrifuge. One milliliter of ice-cold 70% ethanol was used to rinse the pellet, and the sample was centrifuged again. After vacuum desiccation, the sample was used directly for a sequencing reaction (as described above). One primer (5′-CCTAAACACTTAAGAG-3′) within the right end of Tn*917* (40) was used to determine the right-end junction sequences of Tn*917* and Tn*917-lac* inserts in pAD1.

Sequences were stored and analyzed by using the DNA Inspector IIe program (Textco, Lebanon, N.H.). Additional sequence analysis was performed with the IBI MacVector program (GenBank data base release 61.0, 1990 [International Biotechnologies, Inc., New Haven, Conn.]). Protein structure determinations were made with the latter program.

TABLE 1. Strains and plasmids

Strains and plasmids	Relevant phenotype[a]	Reference or source
Strains		
Enterococcus faecalis		
OG1X	*str*	26
DS16	*erm* (contains pAD1)	46
FA2-2	*rif fus*	7
Escherichia coli DH5α	F^- φ80 *lacZΔM15 endA1 recA1 hsdR17* (rK^- mK^+) *supE44 thi-1 d*$^-$ *gyrA96* Δ(*lacZYA-argF*)*U169*	Promega
Plasmids		
pBluescript SK	*amp*	Stratagene
pAM727	pAD1 with a Tn*917* insert in *traA* (Tra^E)	26
pAM728	pAD1 with a Tn*917* insert in *traA* (Tra^E)	26
pAM964	pAD1 with a Tn*917* insert in the C region	15
pAM2100	pAD1 with a Tn*917-lac* insert in *traA* (the A10 insert, Agg^c, Tra^c, β-Gal^-, reduced, constitutive production of isp)	47
pAM2109	pAD1::Tn*917-lac* (β-Gal^c)	K. Weaver
pAM2116	pAD1 with a Tn*917-lac* insert in *traA* (Agg^c, β-Gal^c)	K. Weaver
pAM2120	pAD1 with a Tn*917-lac* insert in *traA* (the A120 insert, Agg^c, Tra^c, β-Gal^c)	47
pAM2160	pAD1 with a Tn*917-lac* insert in the C region ($Tra^{E,I}$, $Agg^{E,I}$, β-Gal^c)	47
pAM2180	pAD1 with a Tn*917-lac* insert in the C region (the PR18 insert, $Tra^{E,I}$, $Agg^{E,I}$, β-Gal^-)	47
pAM7500	pAD1 *Hin*dIII fragment containing the C region, *traA*, *iad*, and portions of the E region and *traB* inserted into pBluescript SK (see Materials and methods)	This study

[a] Agg, aggregation; Tra, transfer in 10-min broth matings; isp, cAD1-inducible surface proteins; β-gal, β-galactosidase production; C, under inducing and noninducing conditions; I, cAD1 inducible; E, elevated basal level.

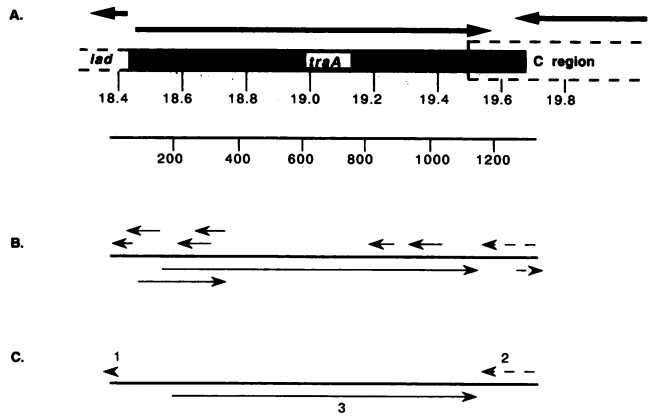

FIG. 2. ORF analysis of *traA*. (A) The approximate locations of the C region, *traA*, and *iad*, with arrows indicating the direction of transcription as determined in previous studies (6, 47). Numbers immediately below refer to kilobase positions on the pAD1 map; numbers farther below correspond to base positions in the sequence. (B) Locations and orientations (5′→3′) of ORFs, beginning with ATG start codons. The area of sequence corresponds to that shown in panel A. (C) Locations of ORFs preceded by strong ribosome binding sites (see text). A dashed line indicates that the entire sequence of the ORF is not known.

This program uses the methods of Chou and Fasman (4) and of Garnier et al. (19) to predict protein secondary structure.

Characterization of pAD1::Tn*917-lac* isolates. LacZ activity of Tn*917-lac* fusions was determined by streaking isolates on media containing X-Gal with or without the addition of cAD1. Colony color was observed after 36 h of incubation at 37°C. Colony morphology was determined as previously described (47). Isolates were streaked on media with or without cAD1. After incubation, colonies were viewed by oblique light microscopy to determine whether aggregation factors were expressed. A dull colony appearance was previously correlated with lack of expression of pheromone-inducible surface proteins, whereas a brighter, grainier colony appearance was correlated with expression of these proteins (47). In a previous study (26), the latter phenotype was referred to as dry because these bright, grainy colonies fracture when touched with a toothpick (dull colonies are soft [47]).

Assays for iAD1 activity were performed as previously described (27), using *E. faecalis* DS16 as the responder strain. *E. faecalis* FA2-2 was used as the host strain for production of test filtrate. Cultures were harvested during late exponential growth (a reading of 90 on a Klett-Summerson colorimeter [Klett Manufacturing Co., Long Island City, N.Y.] with a no. 54 filter). One unit of iAD1 activity is defined to be the amount required to reduce a given cAD1 titer by half (27).

Nucleotide sequence accession number. The GenBank accession number for the nucleotide sequence presented here is M84374.

RESULTS

Sequence analysis of *traA*. Previous Tn*917-lac* mutagenesis studies (47, 48) indicated that DNA corresponding to *traA* mapped within the fragment defined by *Hin*dIII sites at map positions 17.2 and 22.2 (Fig. 1). This *Hin*dIII fragment was cloned, and a portion of the fragment was sequenced (Fig. 2). Computer-generated open reading frame (ORF) analysis of the region revealed nine possible ORFs of 20 or more amino acids with an ATG start codon (Fig. 2B). The location of a 10th ORF, with unknown 5′ sequences, is also shown.

In *E. faecalis* and closely related bacteria, ORFs encoding protein products appear to be preceded by sequences with strong homology to the complement of a portion of the *E. coli* 16S RNA, GGAGG (18). Figure 2C shows those ORFs preceded within 20 bases of the predicted start by GGAGG (allowing for one mismatch). One ORF of substantial size (ORF 3) was identified within the region previously defined as *traA*. The sequence of this ORF and the deduced amino acid sequence are shown in Fig. 3. The ORF consists of 957 bases, which would encode a protein of 319 amino acids with a molecular weight of 37,856. A strong ribosome binding site, GGAG, was found to precede the putative start by 9 bases. An alternate upstream start site was not chosen because of the lack of a strong ribosome binding site. There are two adjacent ATGs 54 amino acid residues downstream from the chosen site, with a possible (weak) ribosome binding site preceding them. Translation initiation here would give rise to an approximately 32-kDa protein. However, analysis of TraA protein synthesized from an expression vector in an in vitro transcription/translation system revealed a 39- to 40-kDa protein consistent with primary usage of the start site shown in Fig. 3 (44a).

Potential transcription initiation signals were sought on the basis of homology with the *Escherichia coli* consensus promoter (σ^{70}) sequences (21) of TTGACA (−35) and TATAAT (−10). Strong transcription initiation signals were not found, although sequences with weak homology are indicated. (We note that signals other than those for a σ^{70}-like system are possible.) It is interesting to note that the putative −35 sequence of *traA* was found to map within 12 bases of the −35 sequences previously proposed to act as promoter elements for *iad*, which is divergently transcribed (6). A putative transcription termination signal for the *traA* transcript is also indicated in Fig. 3. This appears to be a weak factor-independent terminator consisting of 9-base perfect indirect repeats separated by 4 bases and followed by four T's (ΔG = −10.2 kcal [ca. −42.7 kJ] [45]).

The additional ORFs depicted in Fig. 2C are ORF 1, which corresponds to *iad* (6), and ORF 2, which may encode a C-region product (the complete coding region of this ORF extends beyond the nucleotide sequence data obtained). Interestingly, the stop codon of the C-region ORF is encoded by the complement of the 3 bases immediately 3′ of the *traA* stop codon. Both of these stop codons are found within the putative *traA* transcription termination signal.

Deduced amino acid sequence of TraA. The predicted product of *traA* has a pI of 5.8 and lacks a typical signal sequence for protein export (35). Additionally, the hydropathy profile of TraA does not indicate any regions of hydrophobicity extensive enough to allow the protein to span the cell membrane (data not shown).

The deduced protein of *traA* exhibited weak homology with several DNA-binding proteins: *Bacillus subtilis Bsu*RI restriction enzyme (17% conserved plus 18% identical residues [30] within the first 281 residues of TraA), *B. subtilis* DNA polymerase III (15% conserved plus 15% identical residues [39]), and T5 DNA polymerase (14% conserved plus 19% identical residues [31] within the first 227 residues of TraA). The homology detected to the latter two proteins was within regions of these proteins predicted to play a role in 3′→5′ exonuclease activity. Homology was also detected to *Bacillus anthracis cya*, which encodes an adenylate cyclase (16% conserved plus 15% identical residues [17]). The pro-

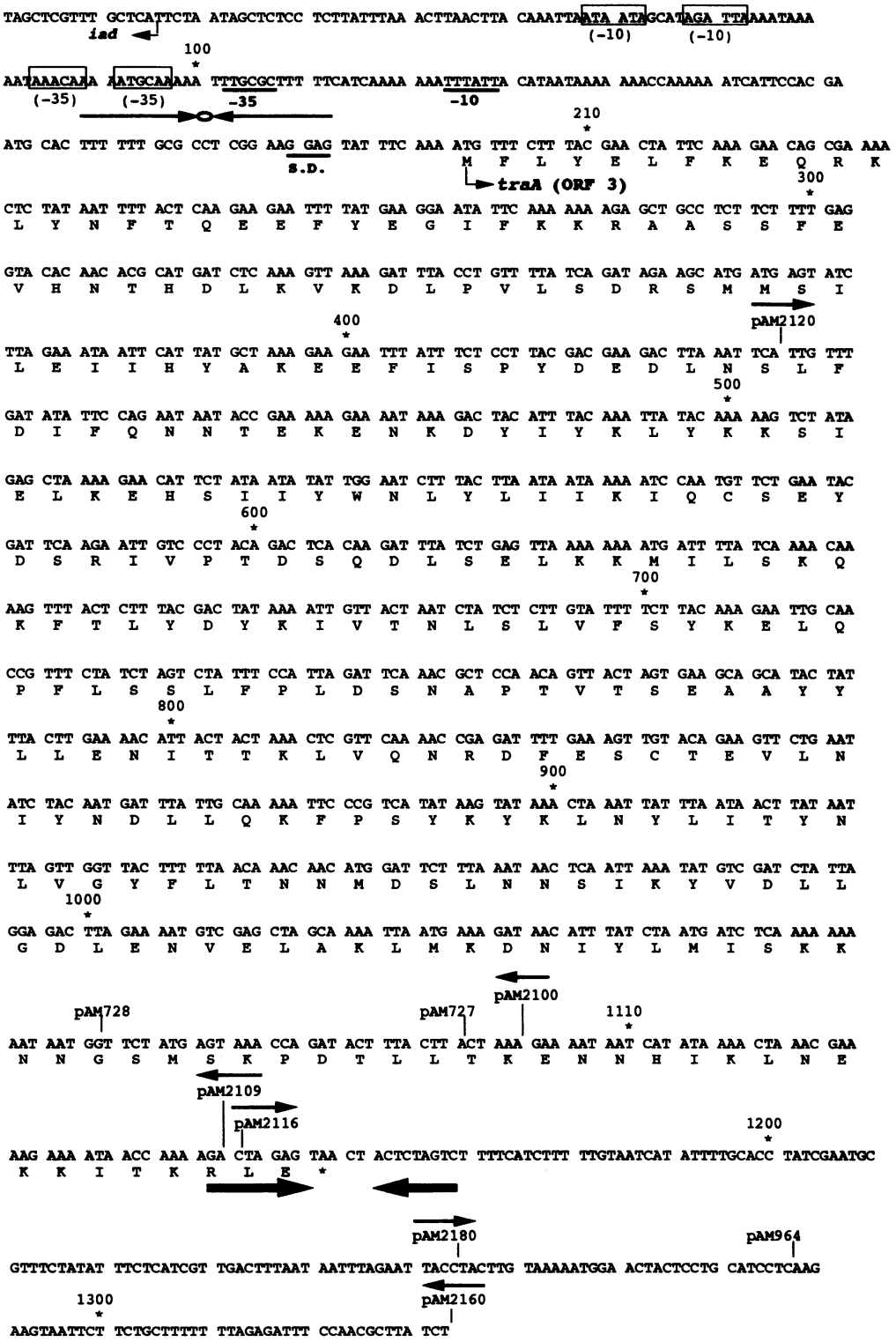

FIG. 3. Nucleotide and deduced amino acid sequences of *traA* (ORF 3; see Fig. 2). Potential transcription initiation signals are marked −35 and −10, and the potential ribosome binding site is indicated (S.D.). The complementary sequence of the 5′ end of *iad* (6) is also shown (note that *iad* is transcribed on the opposite strand). The complements of sequences proposed to serve as transcription initiation signals for the *iad* transcript (6) are boxed and labeled (−10) or (−35). The positions of Tn*917* and Tn*917-lac* inserts within the region are indicated by bars with the designation for the related plasmid bearing each insertion. In the case of Tn*917-lac* inserts, an arrow above the plasmid designation indicates the orientation of the *lacZ* determinant (5′→3′). Bold arrows indicate the position of the proposed transcription termination signal of the *traA* transcript. ⟜⟜ indicates a region of dyad symmetry.

TABLE 2. Characterization of plasmids with Tn*917* or Tn*917-lac* inserts mapping within or near *traA*

Plasmid	Position of transposon insert[a]	ORF interrupted[b]	Colony morphology[c]	Orientation[d]	LacZ activity[e]
pAM2120	435	3	Dry	R	C
pAM728	1070	3	Dry		
pAM727	1099	3	Soft		
pAM2100	1101	3	Dry	L	N
pAM2109	1149	3	Dry	L	C
pAM2116	1150	3	Dry	R	C
pAM2180	1254	2	S-D, responder	R	N
pAM964	1287	2	S-D, responder		
pAM2160	1333	2	S-D, responder	L	C

[a] Data correspond to positions shown in Fig. 2A and Fig. 3.

[b] ORFs indicated are those shown in Fig. 2C.

[c] Observations of colony morphology were made after streaking isolates on solid media with and without cAD1. Data confirm those reported in previous studies (see references for individual plasmids in Table 1) except in the case of pAM727 (see Results). Dry, constitutively dry colony morphology; soft, constitutively soft colony morphology; S-D, responder, semidry colony morphology under noninducing conditions and dry colony morphology under inducing conditions.

[d] Orientation of the *lacZ* determinant of Tn*917-lac* derivatives was determined by sequence analysis. R, 5′→3′ orientation; L, 3′→5′ orientation in relation to the sequence shown in Fig. 3.

[e] LacZ production was determined by streaking isolates containing pAD1::Tn*917-lac* derivatives on solid media containing X-Gal, with or without cAD1. Results confirm previously reported findings (see references for individual plasmids in Table 1). C, light blue colonies regardless of the presence of cAD1; N, white colonies regardless of the presence of cAD1.

posed nucleotide binding site of this protein was not conserved in the predicted product of *traA*. The carboxy-terminal 60 residues of TraA showed homology to several *B. subtilis* and *B. subtilis* bacteriophage sigma factors, including bacteriophage SPO1 σ^{gp28} (13% conserved plus 27% identical residues [10]) and *B. subtilis* σ^{29} (15% conserved plus 20% identical residues [43]). Homology with the sigma factors was within regions of those proteins believed to function in DNA binding (23).

Analysis of pAD1::Tn*917* and pAD1::Tn*917-lac traA* mutants. The positions of a number of previously constructed Tn*917* and Tn*917-lac* inserts within *traA*, determined by sequence analysis, are shown in Fig. 3. Cells containing pAD1::Tn*917* and Tn*917-lac* derivatives previously characterized as *traA* mutants (Table 1) mapped within ORF 3 (Table 2 and Fig. 3). Cells containing pAD1::Tn*917-lac* inserts having the colony morphology of C-region mutants (partially derepressed but further inducible) mapped downstream of the putative *traA*.

E. faecalis containing pAD1::Tn*917* or pAD1::Tn*917-lac* derivatives with inserts in *traA* exhibit one of two phenotypes. The majority display full derepression of aggregation and transfer potential, while others show partial derepression of these functions and cannot be further induced by cAD1 (26, 47). It was hypothesized that the inserts in the latter cases affected a signal-sensing or signal-transducing function of TraA (47). Previous studies identified three variants with *traA* inserts conferring this phenotype. These were pAM2100 (47), pAM727, and pAM728 (26). These inserts were found to cluster in a region 51 to 85 bases from the 3′ end of *traA*. Inserts causing a loss in repressor function were found to map 5′ and 3′ of this region (i.e., pAM2120 and pAM2116; Table 2 and Fig. 3).

The colony morphology of OG1X(pAM727) was previously reported to be dry (26). In the present study, the colony morphology of this strain was found to be soft. The reason for this discrepancy is not known. This isolate, as previously reported (26), was found to express iAD1 at levels 1 to 2 U higher than did other *traA* mutants.

Transcription within *traA*. In a previous study (47), Tn*917-lac* mutagenesis was performed to generate transcriptional *lacZ* fusions within *traA*. Transcription was found to occur at a low level in a rightward direction (in relation to the sequence shown in Fig. 3). The precise positions and orientations of some of these inserts were determined by sequence analysis. The position, orientation, and LacZ production of each insert are indicated in Table 2 and Fig. 3. The data show that transcription proceeds in a rightward direction, reflecting the strand encoding *traA*. Interestingly, transcription close to the 3′ end of *traA* was found to occur bidirectionally. LacZ activity from the fusion in pAM2109 showed transcription in a leftward direction, whereas activity data from the fusion in pAM2116, which is oriented in the opposite direction (mapping 1 base 3′ to the insert in pAM2109) showed transcription in a rightward direction. The data imply that transcription occurs in both orientations within this region. The lack of β-galactosidase production from inserts in pAM2100 and pAM2180 indicate that this bidirectional transcription does not extend more than 104 bases downstream of *traA* (toward the C region) or more than 45 bases further upstream into *traA*. It is important to note that the pAM2109 and pAM2116 inserts map within the putative transcription termination signal of *traA*. This termination signal has appropriately placed runs of T's on both strands and may act as a transcription termination signal for both *traA* and C-region transcripts.

DISCUSSION

A key portion of pAD1, responsible for regulation of the mating response, has been sequenced, and an ORF believed to correspond to TraA was identified. The inferred protein has a molecular weight of about 38,000. The apparent absence of a membrane-spanning region suggests that TraA is cytoplasmic. TraA may receive the inducing signal (cAD1) indirectly by interacting with a membrane-associated signal-transducing protein(s) or directly (if cAD1 enters the cell during the induction process). Tn*917*/Tn*917-lac* insertions in *traA* give rise to variants that are (i) fully derepressed for aggregation and plasmid transfer or (ii) partially repressed but with loss of sensitivity to pheromone. Since the latter variants involved insertions clustered near the 3′ end of the determinant, it is conceivable that the corresponding region on TraA interacts directly with a signal-transducing protein or even pheromone itself.

The function of TraA as both a signal sensor and repressor suggested that it might fall into the same class as other proteins involved in bacterial two-component regulator-sensor systems (41). At least one member of this class of proteins, *Bordetella pertussis* VirR, is thought to act as both a sensor and regulator (42). However, no homology between the deduced amino acid sequence of TraA and that of sensor or regulator proteins involved in two-component systems was detected. TraA did exhibit limited homology to several DNA-binding proteins. One class of DNA-binding proteins exhibit a helix-turn-helix motif in the region of the protein involved in DNA contact (22). Computer analysis (see Materials and Methods) did not predict such a configuration within TraA. Another class of DNA-binding proteins does not exhibit the helix-turn-helix motif; certain proteins which must interact with inducers (e.g., TrpR) or signal-transduc-

ing proteins (e.g., OmpR) (12) to be active are included in this group. TraA may be a member of this latter class of proteins.

A previous study indicated that *traA* is transcribed at a relatively low level (47). If TraA were also involved in negatively regulating itself, the *lacZ* expression detected in Tn*917-lac* derivatives may overestimate the actual level of expression that would occur under normal (uninduced) conditions. Conceivably the level of *traA* transcription may be regulated by or linked to the level of transcription occurring within *iad*, which maps in close proximity to *traA* and is divergently transcribed (1, 6). Transcriptional regulatory factors often bind in regions of dyad symmetry which overlap with promoter elements (24, 25). One potential operator region located between *iad* and *traA* is shown in Fig. 3. Further study will be required to determine whether *traA* and *iad* transcription levels are in fact linked.

LacZ activity data involving inserts at the 3′ end of *traA* indicate that *traA* and C-region transcripts overlap. (The C region has recently been found to determine a 60-kDa protein now designated TraC [44b].) It is possible that the level of transcription occurring in opposite orientations affects termination of *traA* transcription. This model is attractive because it would help to explain the partially derepressed phenotype (i.e., reduced TraA function?) of certain derivatives with insertions in the C region mapping near the 3′ end of *traA* (47). Derepression in these mutants may be due to an indirect effect on *traA*. Such an insert might, for example, decrease the level of transcription from the C region into *traA*, resulting in a higher proportion of longer, less stable *traA* transcripts. The transcript representing the *int* gene of bacteriophage λ is produced in two alternative forms. The longer message carries a degradation signal, making it less stable than the shorter message (20). The short *traA* complementary portion of the C-region transcript might also serve to stabilize *traA* message by pairing with and protecting the 3′ end of the message from degradation. Complementary pairing might prevent or slow *traA* message degradation in the same manner that intrastrand pairing has been proposed to function in message stabilization (29).

TraA may function by binding within the operator region of a promoter(s) much like the λ Cro or *Escherichia coli lac* repressor (28, 37). Indeed, recent results from DNA band shift and footprinting experiments (44a) have shown that the TraA protein binds between *iad* and *traA*. Studies on the E region (36) indicate that pheromone-inducible transcription of positive regulators encoded within this region may result from transcription antitermination events. If so, TraA may have the capacity to function as a termination factor as is the case with the *lac* repressor (13). Further study will be required to define the mode of action of TraA and to explore the relationship of the interaction of TraA and other regulatory factors controlling pAD1 transfer.

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REFERENCES

1. **Beck, C. F., and R. A. J. Warren.** 1988. Divergent promoters, a common form of gene organization. Microbiol. Rev. **52:**318–326.
2. **Benson, S. A.** 1984. A rapid procedure for isolation of DNA fragments from agarose gels. BioTechniques **2:**66–68.
3. **Chen, E. Y., and P. H. Seeburg.** 1985. Supercoiled sequencing: a fast and simple method for sequencing plasmid DNA. DNA **4:**165–170.
4. **Chou, P. Y., and G. D. Fasman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47:**45–148.
5. **Clewell, D. B., and B. L. Brown.** 1980. Sex pheromone cAD1 in *Streptococcus faecalis*: induction of a function related to plasmid transfer. J. Bacteriol. **143:**1063–1065.
6. **Clewell, D. B., L. T. Pontius, F. Y. An, Y. Ike, A. Suzuki, and J. Nakayama.** 1990. Nucleotide sequence of the sex pheromone inhibitor (iAD1) determinant of *Enterococcus faecalis* conjugative plasmid pAD1. Plasmid **24:**156–161.
7. **Clewell, D. B., P. K. Tomich, M. C. Gawron-Burke, A. E. Franke, Y. Yagi, and F. Y. An.** 1982. Mapping of *Streptococcus faecalis* plasmids pAD1 and pAD2 and studies relating to transposition of Tn*917*. J. Bacteriol. **152:**1220–1230.
8. **Clewell, D. B., and K. E. Weaver.** 1989. Sex pheromones and plasmid transfer in *Enterococcus faecalis*. Plasmid **21:**175–184.
9. **Clewell, D. B., Y. Yagi, G. M. Dunny, and S. K. Schultz.** 1974. Characterization of three plasmid deoxyribonucleic acid molecules in a strain of *Streptococcus faecalis*: identification of a plasmid determining erythromycin resistance. J. Bacteriol. **117:**283–289.
10. **Costanzo, M., and J. Pero.** 1983. Structure of a *Bacillus subtilis* bacteriophage SPO1 gene encoding a RNA polymerase σ factor. Proc. Natl. Acad. Sci. USA **80:**1236–1240.
11. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Dodd, I. B., and J. B. Egan.** 1990. Improved detection of helix-turn-helix DNA-binding motifs in protein sequences. Nucleic Acids Res. **18:**5019–5025.
13. **Deuschle, U., R. Gantz, and H. Bijard.** 1986. *lac* repressor blocks transcribing RNA polymerase and terminates transcription. Proc. Natl. Acad. Sci. USA **83:**4134–4137.
14. **Dunny, G. M., R. A. Craig, R. Carron, and D. B. Clewell.** 1979. Plasmid transfer in *Streptococcus faecalis*: production of multiple sex pheromones by recipients. Plasmid **2:**454–465.
15. **Ehrenfeld, E. E., and D. B. Clewell.** 1987. Transfer functions of the *Streptococcus faecalis* plasmid pAD1: organization of plasmid DNA encoding response to sex pheromone. J. Bacteriol. **169:**3473–3481.
16. **Ehrenfeld, E. E., R. E. Kessler, and D. B. Clewell.** 1986. Identification of pheromone-induced surface proteins in *Streptococcus faecalis* and evidence of a role for lipoteichoic acid in formation of mating aggregates. J. Bacteriol. **168:**6–12.
17. **Escuyer, V., E. Duflot, O. Sezer, A. Danchin, and M. Mock.** 1988. Structural homology between virulence-associated bacterial adenylate cyclases. Gene **71:**293–298.
18. **Ferretti, J., and R. Curtiss III.** 1987. Compilation of nucleotide sequences that signal the initiation of transcription and translation in Streptococcus, p. 263. *In* J. Ferretti and R. Curtiss III (ed.), Streptococcal genetics. American Society for Microbiology, Washington, D.C.
19. **Garnier, J., D. J. Osguthorpe, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120:**97–120.
20. **Gaurneros, G., C. Montanez, T. Hernandez, and D. Court.** 1982. Posttranscriptional control of bacteriophage λ *int* gene expression from a site distal to the gene. Proc. Natl. Acad. Sci. USA **79:**238–242.
21. **Harley, C. B., and R. P. Reynolds.** 1987. Analysis of *E. coli* promoter sequences. Nucleic Acids Res. **15:**2343–2361.
22. **Harrison, S. C., and A. K. Aggarwal.** 1990. DNA recognition by proteins with the helix-turn-helix motiff. Annu. Rev. Biochem. **59:**933–969.
23. **Helman, J. D., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57:**839–872.
24. **Himeno, T., T. Imanaka, and S. Aiba.** 1986. Nucleotide se-

quence of the penicillinase repressor gene *penI* of *Bacillus licheniformis* and regulation of *penP* and *penI* by the repressor. J. Bacteriol. **168:**1128–1132.
25. **Hoopes, B. C., and W. R. McClure.** 1987. Strategies in regulation of transcription initiation, p. 1231–1240. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
26. **Ike, Y., and D. B. Clewell.** 1984. Genetic analysis of the pAD1 pheromone response in *Streptococcus faecalis*, using transposon Tn*917* as an insertional mutagen. J. Bacteriol. **158:**777–783.
27. **Ike, Y., R. A. Craig, B. A. White, Y. Yagi, and D. B. Clewell.** 1983. Modification of *Streptococcus faecalis* sex pheromones after acquisition of plasmid DNA. Proc. Natl. Acad. Sci. USA **80:**5369–5373.
28. **Johnson, A. D., B. J. Meyer, and M. Ptashne.** 1979. Interactions between DNA bound repressors govern regulation by the λ phage repressor. Proc. Natl. Acad. Sci. USA **76:**5061–5065.
29. **King, T. C., and D. Schlessinger.** 1987. Processing of RNA transcripts, p. 703–718. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
30. **Kiss, A., G. Posfai, C. C. Keller, P. Venetianer, and R. J. Roberts.** 1985. Nucleotide sequence of the *Bsu*RI restriction-modification system. Nucleic Acids Res. **13:**6403–6421.
31. **Leavitt, M. C., and J. Ito.** 1989. T5 DNA polymerase: structural-functional relationships to other DNA polymerases. Proc. Natl. Acad. Sci. USA **86:**4465–4469.
32. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
33. **Mori, M., A. Isogai, Y. Sakagami, M. Fujino, C. Kitada, D. B. Clewell, and A. Suzuki.** 1986. Isolation and structure of the *Streptococcus faecalis* sex pheromone inhibitor, iAD1, that is excreted by the donor strain harboring plasmid pAD1. Agric. Biol. Chem. **50:**539–541.
34. **Mori, M., Y. Sakagami, M. Narita, A. Isogai, M. Fujino, C. Kitada, R. Craig, D. B. Clewell, and A. Suzuki.** 1984. Isolation and structure of the bacterial sex peromone, cAD1, that induces plasmid transfer in *Streptococcus faecalis*. FEBS Lett. **178:**97–100.
35. **Oliver, D. B.** 1987. Periplasm and protein export, p. 56–69. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
36. **Pontius, L. T., and D. B. Clewell.** Unpublished data.
37. **Riggs, A. R., and S. Bourgeois.** 1968. On the assay, isolation and characterization of the *lac* repressor. J. Mol. Biol. **34:**361–364.
38. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
39. **Sanjanwala, B., and A. T. Ganesan.** 1989. DNA polymerase III gene of *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **86:**4421–4424.
40. **Shaw, J. H., and D. B. Clewell.** 1985. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B-resistance transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **164:**782–796.
41. **Stock, J. B., N. J. Alexander, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53:**450–490.
42. **Stibitz, S., W. Aaronson, D. Monack, and S. Falkow.** 1989. Phase variation in *Bordetella pertussis* by frameshift mutation in a gene for a novel two-component system. Nature (London) **338:**266–269.
43. **Straiger, P., J. Bouvier, C. Bonamy, and J. Szulmajster.** 1984. A developmental gene product of *Bacillus subtilis* homologous to the sigma factor of *Escherichia coli*. Nature (London) **312:**376–378.
44. **Su, Y., M. C. Sulavik, P. He, K. K. Makinen, P. Makinen, S. Fielder, R. Wirth, and D. B. Clewell.** 1991. Nucleotide sequence of the gelatinase gene (*gelE*) from *Enterococcus faecalis* subsp. *liquefaciens*. Infect. Immun. **59:**415–420.
44a. **Tanimoto, K.** Personal communication.
44b. **Tanimoto, K., and F. An.** Personal communication.
45. **Tinoco, I., P. N. Borer, B. Dengler, and M. D. Levine.** 1973. Improved estimation of secondary structure in ribonucleic acids. Nature (London) **246:**40–41.
46. **Tomich, P. K., F. Y. An, S. P. Damle, and D. B. Clewell.** 1979. Plasmid-related transmissibility and multiple drug resistance in *Streptococcus faecalis* subspecies *zymogenes* strain DS16. Antimicrob. Agents Chemother. **15:**828–830.
47. **Weaver, K. E., and D. B. Clewell.** 1988. Regulation of the pAD1 sex pheromone response in *Enterococcus faecalis*: construction and characterization of *lacZ* transcriptional fusions in a key control region of the plasmid. J. Bacteriol. **170:**4343–4352.
48. **Weaver, K. E., and D. B. Clewell.** 1989. Construction of *Enterococcus faecalis* pAD1 miniplasmids: identification of a minimal pheromone response regulatory region and evaluation of a novel pheromone-dependent growth inhibition. Plasmid **22:**106–119.
49. **Weaver, K. E., and D. B. Clewell.** 1990. Regulation of the pAD1 sex pheromone response in *Enterococcus faecalis*: effects of host strain and *traA*, *traB*, and C region mutants on expression of an E region pheromone-inducible *lacZ* fusion. J. Bacteriol. **172:**2633–2641.
50. **Weaver, K. E., and D. B. Clewell.** 1991. Control of *Enterococcus faecalis* sex pheromone cAD1 elaboration: effects of culture aeration and pAD1 plasmid-encoded determinants. Plasmid **25:**177–189.