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# QseA Directly Activates Transcription of *LEE1* in Enterohemorrhagic *Escherichia coli*[▽]

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**Quorum sensing (QS) in enterohemorrhagic *Escherichia coli* (EHEC) regulates the expression of the locus of enterocyte effacement (LEE). The LEE contains five major operons named *LEE1* through *LEE5*. QseA was previously shown to be activated through QS and to activate the transcription of *LEE1*. The *LEE1* operon encodes Ler, the transcription activator of all other LEE genes, and has two promoters: a distal promoter (P1) and a proximal promoter (P2). We have previously reported that QseA acts on P1 and not P2. To identify the minimal region of *LEE1* that is necessary for QseA-mediated activation, a series of nested-deletion constructs of the *LEE1* promoter fused to a *lacZ* reporter were constructed in both the EHEC and *E. coli* K-12 backgrounds. In an EHEC background, QseA-dependent activation of *LEE1* can be observed for the entire regulatory region (beginning at nucleotide −393 and ending at nucleotide −123). In contrast to what occurred in EHEC, in K-12 there was no QseA-dependent activation of *LEE1* transcription between base pairs −393 and −343. These data indicate that a QseA-dependent EHEC-specific regulator is required for the activation of transcription in this region. We also observed QseA-dependent *LEE1* activation from nucleotides −218 to −123 in K-12, similar to results of the nested-deletion analysis performed with EHEC. Electrophoretic mobility shift assays established that QseA directly binds to the region of *LEE1* from bp −173 to −42 and not to the region from bp −393 to −343. These studies suggest that QseA activates the transcription of *LEE1* by directly binding upstream of its P1 promoter region.**

Enterohemorrhagic *Escherichia coli* (EHEC) colonizes the large intestine, where it forms attaching and effacing (A/E) lesions. A/E lesions are characterized by the destruction of the microvilli and the rearrangement of the cytoskeleton to form a pedestal-like structure that cups the bacterium (31, 40, 66). A/E lesions are characteristic of EHEC, enteropathogenic *E. coli* (EPEC), rabbit EPEC, and *Citrobacter rodentium* infections (28). The genes involved in the formation of A/E lesions are encoded within a chromosomal pathogenicity island named the *l*ocus of *e*nterocyte *e*ffacement (LEE) (36). The LEE region contains five major operons, *LEE1*, *LEE2*, *LEE3*, *LEE5*, and *LEE4* (8, 11, 38), that encode a type III secretion (TTS) system (24), an adhesin (intimin) (25), that adhesin's receptor (Tir) (29), and effector proteins (9, 27, 30, 37, 65). The LEE-encoded TTS system also translocates effector proteins encoded outside the LEE region (NleA, -B, -C, -D, -E, and -F and EspFu, among others); these other effectors are also important for virulence and pedestal formation (4, 6, 7, 14, 15, 19, 41, 53, 64).

EHEC senses three cell-to-cell signals to activate expression of the LEE genes: one is a bacterial autoinducer (AI-3) produced by the normal human gastrointestinal microbial flora, and the others are the host hormones epinephrine/norepinephrine (NE) produced by the host (61). AI-3 is a quorum-sensing (QS) signal produced by several species of bacteria, including commensal *E. coli* as well as several other intestinal bacterial species (62, 68). Both epinephrine and NE are present in the gastrointestinal tract. NE is synthesized by the adrenergic neurons within the enteric nervous system (13). Epinephrine is synthesized in the central nervous system and in the adrenal medulla; it acts in a systemic manner after being released into the bloodstream, thereby reaching the intestine (46). AI-3 and epinephrine/NE are agonistic signals, and responses to both signals can be blocked by adrenergic antagonists (5, 61, 69). These signals are sensed by sensor kinases in the membrane of EHEC that relay this information to a complex regulatory cascade, culminating in the activation of flagellum regulon, LEE, and Shiga toxin expression (5, 35). One of these sensors is QseC, which autophosphorylates in response to epinephrine, NE, and AI-3 (5). Further QS regulation of the LEE genes is complex and requires QseA (57, 58), which, in concert with several global regulators in EHEC, ensures the correct kinetics of LEE gene expression.

The LEE pathogenicity island undergoes complex regulation in EHEC and EPEC. Although the LEE is regulated in EHEC and EPEC in similar manners, there are some distinct differences in the ways that they regulate this pathogenicity island. EPEC contains three genes borne by a 70-kb virulence plasmid, *perA*, *perB*, and *perC* (plasmid-encoded regulator), with PerC being involved in the activation of expression of the LEE genes (3, 17, 38). GadX, a positive regulator of the glutamate decarboxylase genes in EPEC, plays a repressive role in the regulation of the transcription of *per* (54). Although the *per* locus is absent in EHEC, Iyoda and Watanabe (23) observed that EHEC encodes five PerC homologs, renamed PchA, -B, -C, -D, and -E, which positively regulate the expression of the LEE genes.

Transcription of the LEE genes is silenced by the nucleoid

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TABLE 1. Strains and plasmids used in this study

| Strain or plasmid | Relevant characteristic(s) | Source or reference |
|---|---|---|
| Strains | | |
| DH5α | Host *E. coli* strain for cloning experiments | Stratagene |
| TOP10 | Host *E. coli* strain for pCR Blunt II TOPO | Invitrogen |
| 86-24 | Stx2$^+$ EHEC strain (serotype O157:H7) | 18 |
| VS145 | 86-24 *qseA* mutant strain | 58 |
| VS151 | VS145 with plasmid pVS150 | 58 |
| MC4100 | *araD139* Δ(*araABC-leu*)*7679 galU galK* Δ(*lac*)*X74 rpsL thi* | 55 |
| FS02 | MC4100 *qseA* mutant strain | This study |
| FS76 | FS02 with plasmid pVS150 | This study |
| Plasmids | | |
| pRS551 | *lacZ* reporter gene fusion vector | 56 |
| pVS143 | *qseA*::*cat* cloned into pCVD442 | 58 |
| pBADMycHis | C-terminal Myc-His tag cloning vector | Invitrogen |
| pBlueScriptII | Cloning vector | Stratagene |
| pVS241 | *qseA* in pBADMycHisA | 57 |
| pVS150 | *qseA* in pACYC177 | 58 |
| pVS232Z | *LEE1*::*lacZ* in pRS551, base pairs −393 to +323 | 60 |
| pVS200 | *ler*::*lacZ* in pRS551, base pairs −393 to −42 | This study |
| pVS204 | *ler*::*lacZ* in pRS551, base pairs −343 to +86 | This study |
| pVS206 | *ler*::*lacZ* in pRS551, base pairs −218 to +86 | This study |
| pVS224 | *ler*::*lacZ* in pRS551, base pairs −173 to +86 | This study |
| pVS225 | *ler*::*lacZ* in pRS551, base pairs −123 to +86 | This study |

TABLE 2. Primers used in this study

| Primer | Oligonucleotide (5′–3′) | Reference or source |
|---|---|---|
| orf1 F | CGGAATTCATGTGCTGCGACTGCGTTCG | 60 |
| ler 2F | CGGAATTCCTGGGGATTCACTCGCTTGC | This study |
| ler 4F | CGGAATTCCGCTTAACTAAATGGAAATGC | This study |
| ler 5F | CGGAATTCAGATGATTTTCTTCCATTTAAT | This study |
| ler 6F | CGGAATTCGATTTTTTTGTTGAGACACAT | This study |
| ler R1 | CGGGATCCTCTATCAAATTAGGACACAT | This study |
| ler R2 | CGGGATCCGTATGGACTTGTTGTATGTG | This study |
| ler R3 | CGGGATCCGTCGGCCTACGCCCGACC | This study |
| ler −173F | CGGGATCCCGATGATTTTCTTCTATATCATTG | This study |
| ler −42R | CGGAATTCCGCGACCTTATCAGGAAGGACC | This study |
| ApR | CGGGATCCGGTGAGCAAAAACAGGAAGG | This study |
| ApF | GGAATTCGAAAGGGCCTCGTG ATA CGC | This study |

protein H-NS. The *LEE1* operon encodes Ler, the LEE-encoded regulator, which was shown to be required for the expression of other operons within the LEE by disrupting H-NS-mediated silencing of transcription (3, 10, 12, 20, 38, 50, 59). Ler was shown to activate the transcription of the *LEE2*, *LEE3*, *LEE4*, and *LEE5* operons (3, 10, 12, 20, 38, 50, 59), and there are conflicting reports on whether Ler is involved in the autoregulation of its own promoter (1, 2, 10, 38, 60). In EHEC, the *LEE1* operon contains two promoters: P1 and P2 (58). The P1 (distal) transcriptional start site (163 base pairs upstream of the translational start site) is common to EHEC and EPEC, while the P2 (proximal) transcriptional start site (32 base pairs upstream of the translational start site) is present only in EHEC (58, 60). In *Citrobacter rodentium*, a systematic mutagenesis approach was utilized to understand further the complexity of the LEE genes in this system (7). Mutants with deletions in the LEE genes were analyzed for TTS, LEE gene expression, changes in actin polymerization, and virulence in the mouse model. In this analysis, two important regulators were found, *orf10* and *orf11*. Orf10 was renamed GrlR, for global regulator of LEE repressor, while Orf11 was renamed GrlA, for global regulator of LEE activator. This study suggests that GrlA is involved in the activation of the transcription of *ler*, while GrlR represses the transcription of *ler* (7). Additionally, Ler activates the transcription of *grlRA* (1, 10). GrlR and GrlA form hetero- and homodimers in vitro, and recently, Iyoda and Watanabe observed that the ClpXP protease is involved in the positive regulation of the LEE, possibly through the control of the stability of GrlR (22). Further regulation of the LEE genes involves the RpoS alternative sigma factor (22, 60), the RcsCDB and EvgSA two-component systems (43, 63), and the Hha (52) and integration host factor (12) nucleoid proteins EtrA and EivF (71). Posttranscriptional regulation of the LEE genes has also been reported (47, 48).

QseA is a member of the LysR family of regulators and has been shown to activate the transcription of *LEE1* and, consequently, the other LEE operons (58). Members of the LysR family of regulators contain a characteristic helix-turn-helix DNA-binding domain at the amino terminus, typically within amino acid residues 1 to 65 (51). These proteins also regulate the expression of linked genes from divergent promoters, but this is not always the case, as many activate the expression of unlinked virulence genes (51). LysR proteins have been shown to bind the promoter in proximity to the bacterial RNA polymerase (RNAP) (51). Many members of the LysR family of regulators have also been identified as regulators of virulence factors in pathogenic bacteria. For example, PtxR positively regulates the production of exotoxin A in *Pseudomonas aeruginosa* (70). AphB, of *Vibrio cholerae*, is involved in the QS cascade and regulation of the ToxR regulon (32–34). Here we show that QseA activates the transcription of *LEE1* and, consequently, *ler* by two means: by directly binding upstream of the P1 promoter and indirectly binding through a yet-unidentified EHEC-specific factor.

## MATERIALS AND METHODS

**Bacterial strains and culture conditions.** The strains and plasmids used in this study are listed in Table 1. The wild-type (WT) EHEC (strain 86-24) (18), an isogenic *qseA* mutant (strain VS145), and complemented strain VS151 were previously described (58). The *E. coli* K-12 MC4100 *qseA* mutant (named FS02) was constructed by allelic exchange using the vector pVS143 (*qseA*::*cat* cloned into the R6K plasmid pCVD442), and the mutants were selected on media containing chloramphenicol and 5% sucrose as previously described (58). The *qseA* mutant (FS02) was complemented with plasmid pVS150 (58), generating strain FS76. All *E. coli* strains were grown aerobically in LB medium or Dulbecco modified Eagle medium at 37°C. Selective antibiotics were added at the following concentrations: 100 μg ml$^{-1}$ ampicillin, 50 μg ml$^{-1}$ kanamycin, 100 μg ml$^{-1}$ streptomycin, 30 μg ml$^{-1}$ tetracycline, and 30 μg ml$^{-1}$ chloramphenicol.

**Recombinant DNA techniques.** Standard methods were used to perform plasmid purification, PCR, ligation, restriction digestion, transformation, and DNA gel electrophoresis (49). All oligonucleotide primers are listed in Table 2.

**Construction of *LEE1* or *ler* deletion *lacZ* operon fusion constructs.** Transcriptional fusion constructs with the promoterless *lacZ* gene were made by amplifying regions of the *ler* promoter using *Pfx* DNA polymerase, using the primers listed in Table 2, and cloning them into the EcoRI-BamHI restriction sites of plasmid pRS551, which contains a promoterless *lacZ* cassette (56). This generated plasmids pVS232Z, pVS204, pVS206, pVS224, pVS225, and pVS200, listed in Table 1 (see also Fig. 3). Plasmid pVS232Z was constructed by amplifying the regulatory region of *LEE1* from bp −393 to +323 using primers orf1 F and ler R3 and has been described previously (60). Plasmid pVS204 was constructed by amplifying the regulatory region of *LEE1* from bp −343 to +86 using primers ler 2F and ler R2. Plasmid pVS206 was constructed by amplifying the regulatory region of *LEE1* from bp −218 to +86 using primers ler 4F and ler R2. Plasmid pVS224 was constructed by amplifying the regulatory region of *LEE1* from bp −173 to +86 using primers ler 5F and ler R2. Plasmid pVS225 was constructed by amplifying the regulatory region of *LEE1* from bp −123 to +86 using primers

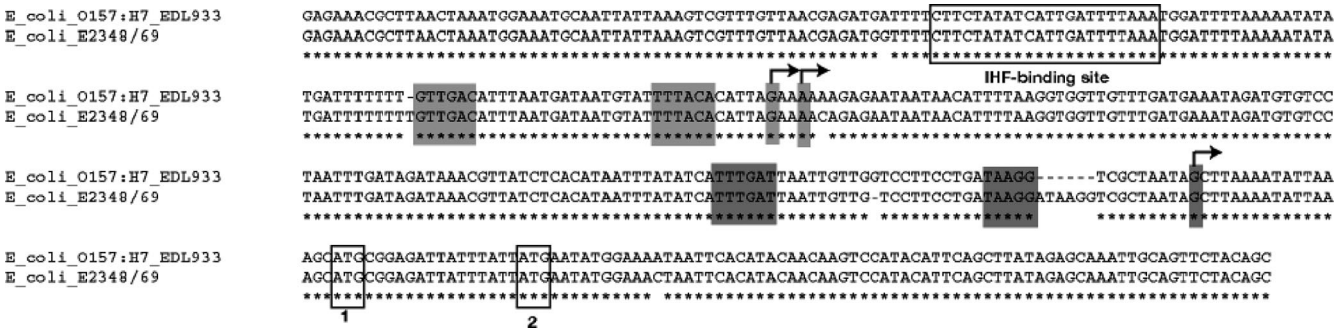


FIG. 1. Alignment of the sequences of the *LEE1* regulatory region of EHEC and EPEC. Lightly shaded areas correspond to the distal promoter (P1); darkly shaded areas correspond to the EHEC-specific proximal promoter (P2). Unshaded box 1 corresponds to the assigned ATG based on a longer reading frame; unshaded box 2 corresponds to the ATG that is associated with a putative ribosome binding sequence.

ler 6F and ler R2. Plasmid pVS200 was constructed by amplifying the regulatory region of *LEE1* from bp −393 to −42 using primers orf1 F and ler R1 (see Fig. 3A).

These transcriptional-fusion amplicons were each electroporated into 86-24 (WT EHEC), VS145 (*qseA* isogenic mutant in 86-24), and VS151 (VS145 with pVS150) for the EHEC deletion analysis. The transcriptional-fusion amplicons were separately transformed into MC4100 (WT K-12), FS02 (*qseA* isogenic mutant of MC4100), and FS76 (FS02 with pVS150) for the *E. coli* K-12 deletion analysis (Table 1).

**β-Galactosidase activity assay.** The strains containing the transcriptional *lacZ* fusions were grown in LB in the appropriate selective antibiotic at 37°C to an optical density at 600 nm of 1.0. These cultures were diluted 1:10 in Z buffer (60 mM $Na_2HP04 \cdot 7H_2O$, 40 mM $NaH_2PO4 \cdot H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, 50 mM β-mercaptoethanol) and assayed for β-galactosidase activity by using *o*-nitrophenyl-β-D-galactopyranoside as a substrate as previously described (39).

**Purification of QseA.** Plasmid pVS241 was constructed by amplifying the *qseA* gene with *Pfx* polymerase (Invitrogen) and cloning this amplicon into pBADMycHisA (Invitrogen) digested with XhoI/HindIII (57). To purify the QseA-Myc-His protein, the *E. coli* strain containing pVS241 was grown at 37°C in LB to an optical density at 600 nm of 0.7, at which point the expression of the protein was induced with 0.2% arabinose for 3 h at 37°C, and subsequently the protein was purified using nickel affinity chromatography under native conditions according to the manufacturer's instructions (QIAGEN).

**EMSAs.** In order to study the direct binding of QseA to the promoter of *ler*, electrophoretic mobility shift assays (EMSAs) were performed using the purified QseA-Myc-His protein and PCR-amplified DNA probes. *Taq* DNA polymerase was used to amplify the *ler* promoter base pairs −393 to −42, −173 to −42, and −393 to −300 for a DNA probe from EHEC using primers orf1 F/ler R1, Ler promoter −173F/Ler promoter −42R, and orf1 F/ler R3, respectively. Additionally, the *bla* region, which served as the negative control, was amplified from pBR322 using primers ApR and ApF. DNA probes were then end labeled using [γ-$^{32}$P]ATP and T4 polynucleotide kinase (Invitrogen). End-labeled probes were run on a 6% polyacrylamide gel, excised, and purified using the QIAGEN PCR purification kit.

EMSAs were performed by adding purified QseA-Myc-His protein (0 to 5 μg) to end-labeled probes (10 ng) at increasing concentrations equivalent to 2 to 15 kcpm per reaction with 5× band shift buffer [5× transcription buffer (60 mM HEPES, pH 7.5, 5 mM EDTA, 3 mM dithiothreitol, 300 mM KCl, 25 mM $MgCl_2$), 50 ng/μl poly(dI-dC), 500 μg/ml bovine serum albumin (NEB)] and water for 20 min at 4°C. A 5× Ficoll loading buffer (5% Ficoll, 0.1% bromphenol blue) was added to reaction mixtures and immediately loaded onto a 5% polyacrylamide gel that was prerun for 1 h at 50 V and 4°C. The gels were electrophoresed, dried, and exposed within a phosphorimage cassette.

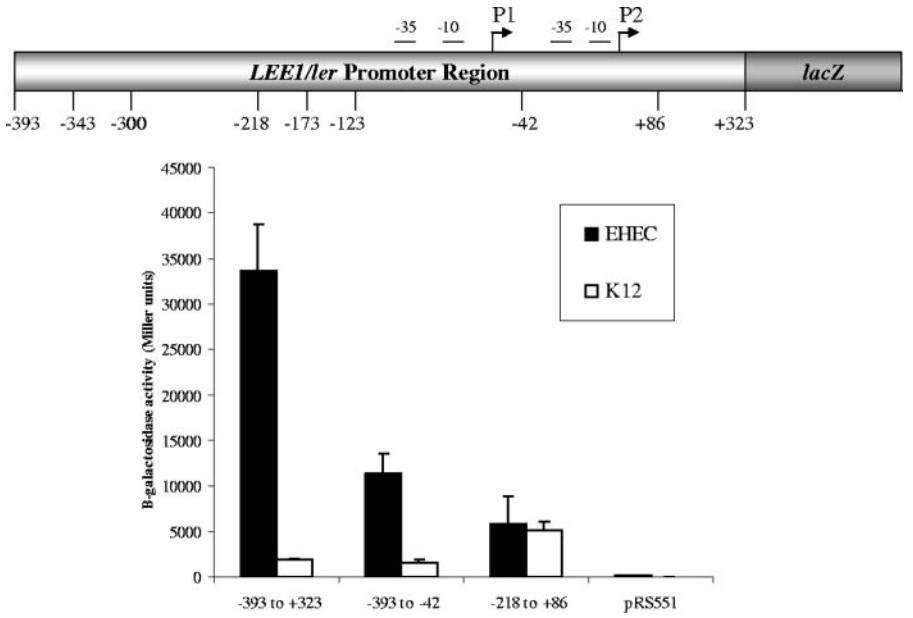


FIG. 2. Transcription of *LEE1*::*lacZ* reporter fusion constructs within an EHEC and an *E. coli* K-12 background. β-Galactosidase activities are depicted in Miller units, and the numbering of base pairs is in relation to the P2 (proximal) *LEE1* promoter transcriptional start site.

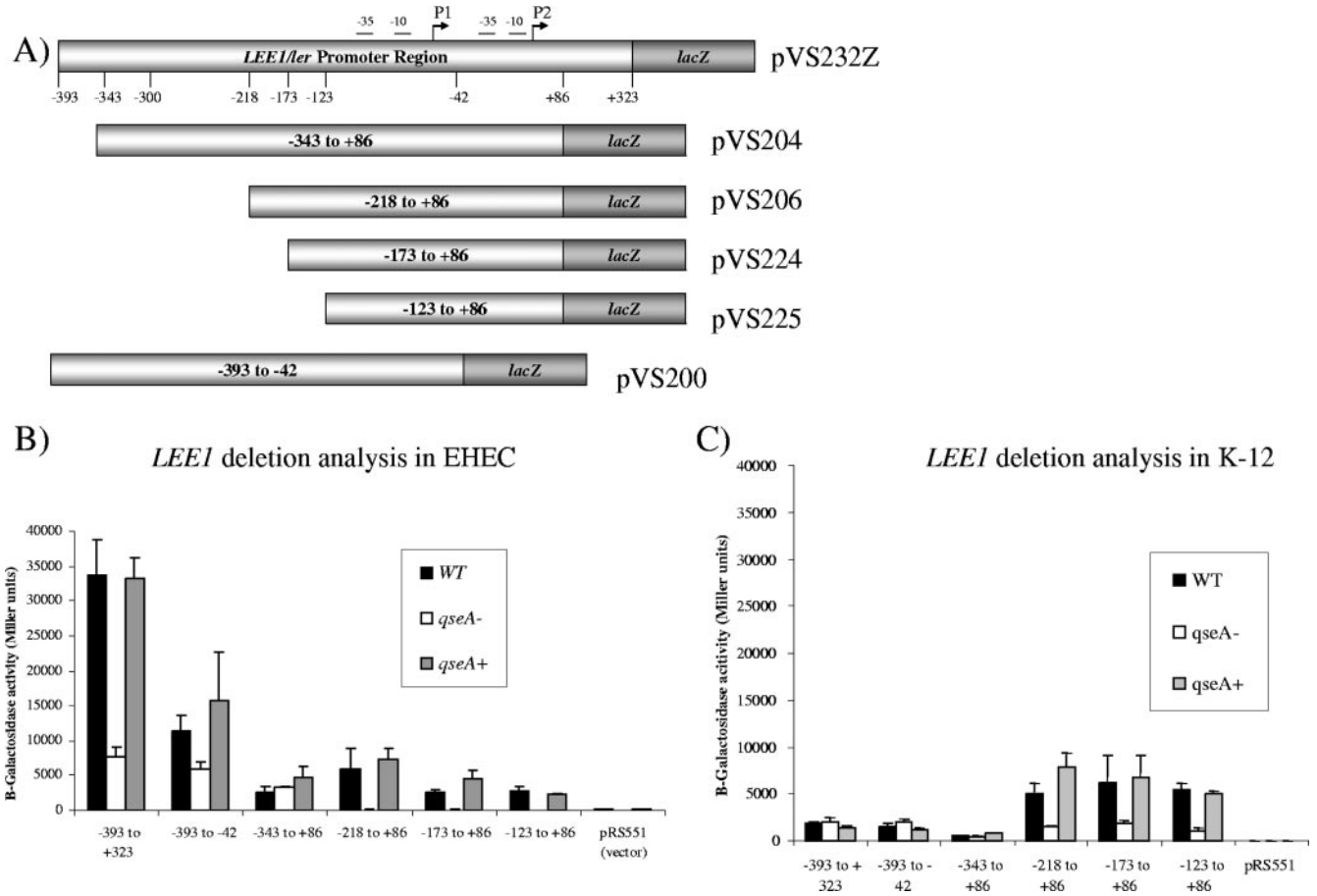


FIG. 3. (A) Schematic of nested-deletion analysis. The numbering of the bases (−393 to +323) is based on the P2 transcriptional start site. (B) Deletion analysis of the *LEE1* promoter in EHEC. Fragments of the *LEE1* promoter were electroporated into 86-24 (WT), VS145 (*qseA* mutant), and VS151 (*qseA* complemented) and assessed for β-galactosidase activity. Error bars indicate standard deviations. (C) Deletion analysis of the *LEE1* promoter in *E. coli* K-12. Fragments of the *LEE1* promoter were transformed into MC4100 (WT), FS02 (*qseA* mutant), and FS76 (*qseA* complemented) and assessed for β-galactosidase activity. Error bars indicate standard deviations.

## RESULTS

**Deletion analysis of the *LEE1* operon.** The *LEE1* operon encodes Ler, which is the activator of the LEE genes (38). Consequently, environmental regulation of the LEE is thought to occur primarily with the transcriptional control of *LEE1*. We have previously reported using primer extension experiments to show that the EHEC *LEE1* operon has two transcriptional start sites and, consequently, two promoters. The distal P1 promoter is present in both EPEC and EHEC, while the proximal P2 promoter is present only in EHEC (58) (Fig. 1). To validate further these primer extension data, we constructed several *LEE1*::*lacZ* transcriptional fusion genes and assessed their levels of transcription within both an EHEC and an *E. coli* K-12 background. The full-length *LEE1* fusion construct from bp −393 to +323 (nucleotides were numbered in relation to the transcriptional start site of P2) yielded 35,000 Miller units of β-galactosidase activity within an EHEC background, but its transcription was highly repressed (by 17.5-fold) in *E. coli* K-12, yielding 1,989 Miller units (Fig. 2). There was a threefold decrease in the basal-level transcription of *LEE1* within an EHEC background with the fusion construct with bp −393 to −42, compared to the level obtained with the construct with bp −393 to +323 (the fragment from bp −393 to −42 lacks the P2 promoter of *LEE1*). These data corroborate our previous primer extension data (58). The transcription of the fusion construct from bp −393 to −42 is also repressed in *E. coli* K-12 compared to its transcription in EHEC (Fig. 2). This repression observed in K-12 is relieved only with the fusion construct with bp −218 to +86, suggesting that a repressor present in K-12 acts through the region from bp −393 to −218 of *LEE1*. Either this repressor is absent in EHEC or an EHEC-specific activator acting through the region from bp −393 to −218 counteracts this repression.

We have previously reported, using primer extensions, that QseA acts on P1 and not P2 (58). In order to identify the minimal regulatory region of *LEE1* that is necessary for QseA-mediated activation, a series of constructs with nested deletions in the *LEE1* promoter was generated (Fig. 3A). These deletion constructs were then fused to a promoterless *lacZ* cassette and used for nested-deletion analysis of the WT, *qseA* mutant, and complemented strains in both the EHEC and K-12 backgrounds (Fig. 3A).

In an EHEC background, the transcription of *LEE1*::*lacZ* is decreased in the *qseA* mutant, compared to levels of transcrip-

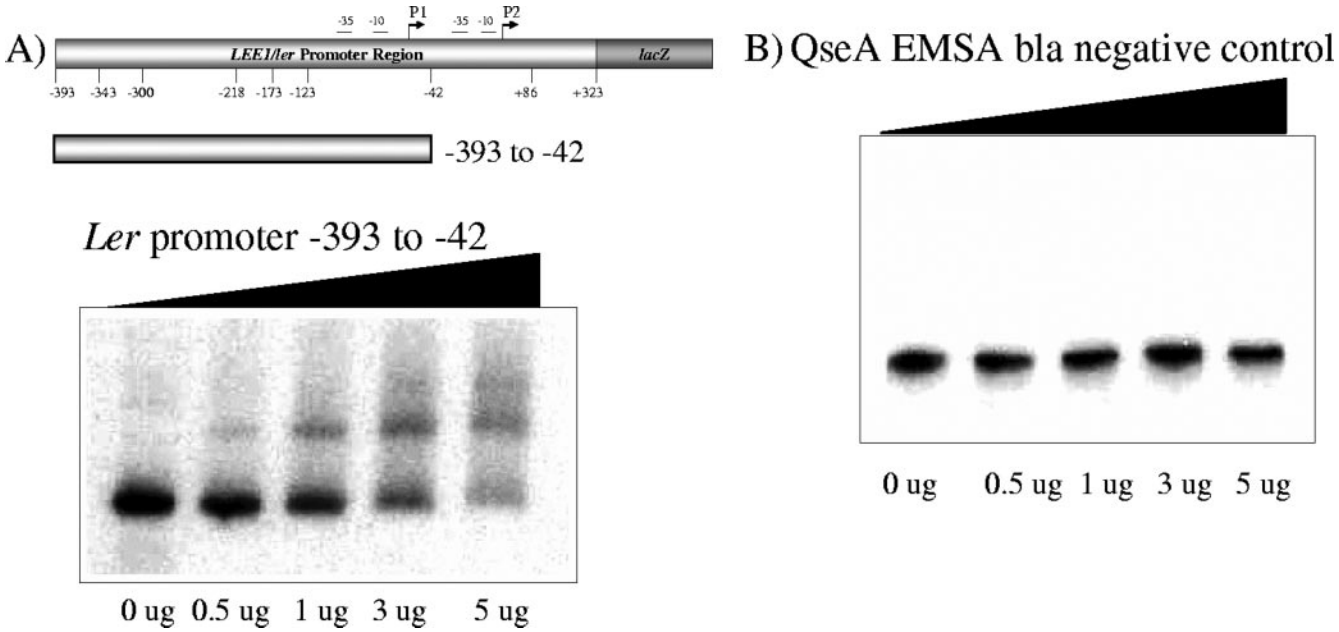


FIG. 4. (A) EMSA of the *LEE1* promoter fragment containing bp −393 to +42 with increasing amounts of QseA. (B) EMSA of the *bla* promoter fragment with QseA (negative control).

tion in the WT and complemented strains, in the promoter fusion constructs with base pairs −218 to +86, −173 to +86, and −123 to +86 (Fig. 3B). These data suggest that the transcription activation of the *LEE1* promoter between base pairs −218 and +86 is QseA dependent. Importantly, QseA-dependent activation of P1 can be observed up to nucleotide −123 (which corresponds to nucleotide −50 in P1) (Fig. 3B). This proximity to the promoter suggests that QseA may directly interact with RNAP in P1, which would be consistent with the mechanism of transcriptional regulation reported for other LysR transcriptional regulators (51). Additionally, we observed QseA-dependent regulation of the entire promoter region, as seen using the fusion construct with bp −393 to +323 (Fig. 3B). As mentioned above, there is a threefold decrease in the basal level of transcription of *LEE1* in the WT strain with the fusion gene with base pairs −393 to −42 compared to the level in the construct with base pairs −393 to +323, although activation of the fragment from base pairs −393 to −42 is still dependent on QseA. These data further suggest that QseA activates the transcription of the *LEE1* operon through the P1 promoter in EHEC (58).

Results of the nested-deletion analysis of *E. coli* K-12 are shown in Fig. 3C. Using the mutant *qseA* in K-12 and the complemented strain, we were able to determine whether the levels of regulation of the *LEE1* promoter are different between *E. coli* K-12 and EHEC. In contrast to what occurred in EHEC, QseA-dependent activation of *LEE1* transcription was not observed between base pairs −393 and −343 in *E. coli* K-12 (Fig. 3C). These data indicate that a QseA-dependent EHEC-specific regulator is required for the transcription activation of this region. Transcription of the gene with base pairs −343 to +86 was repressed and QseA independent in both the EHEC and K-12 backgrounds (Fig. 3B and C), suggesting that a conserved, common repressor acts on this region. Hence, the EHEC-specific transcription activator may act through the region at bp −393 to −343 to counteract repression by this common repressor. The transcription of *LEE1*::*lacZ* was restored to 5,000 Miller units (levels similar to the ones in EHEC) in the fusion construct with bp −218 to +86 (Fig. 3B and C). We also observed QseA-dependent *LEE1* activation from nucleotides −218 and −123 (Fig. 3C), similar to the results of the nested-deletion analysis performed with EHEC (Fig. 3B). These data suggest that QseA may directly bind to the *LEE1* promoter between base pairs −123 and −42.

**QseA directly binds to *LEE1*.** In order to assess whether QseA directly interacts with the *LEE1* regulatory region, we performed EMSAs with QseA purified under native conditions. The *qseA* gene was cloned into the pBADMycHis vector (Invitrogen) to generate a C-terminal Myc-His fusion construct under the control of the *araC* (pBAD) promoter. The C-terminal fusion was chosen because the helix-turn-helix DNA binding motif of QseA is in the N terminus. QseA was expressed from the resulting vector, pVS241, using 0.2% arabinose and purified using a nickel affinity column under native conditions (Fig. 4A). Plasmid pVS241 has previously been shown to complement QseA-dependent phenotypes in a *qseA* mutant (57), indicating that the QseA-Myc-His fusion protein is functional.

To determine whether QseA interacts directly with the *LEE1* promoter, we initially generated a probe harboring base pairs −393 to −42 of the *LEE1* promoter. This region was shown to be important in the QseA-dependent regulation of the *LEE1* promoter (Fig. 3). This probe was PCR amplified and end labeled using [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase (Invitrogen). The constitutive *bla* promoter fragment was used as a negative control. With the addition of increasing concentrations of the His-tagged QseA protein, a shift of *LEE1* promoter region base pairs −393 to −42 was observed

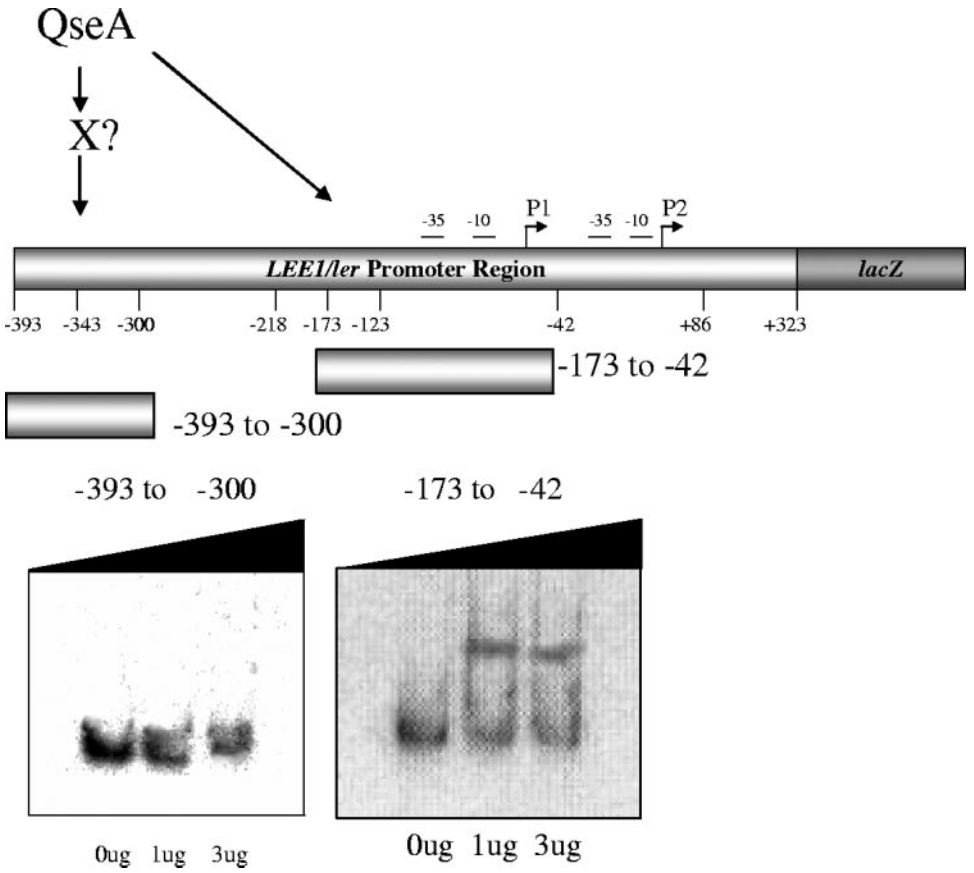


FIG. 5. EMSA of the *LEE1* promoter fragments containing bp −393 to −300 and −173 to −42 with increasing amounts of QseA.

(Fig. 4B). The negative-control *bla* gene did not shift with the addition of increasing concentrations of QseA protein (Fig. 4B), suggesting that QseA binding is specific to the *LEE1* promoter (Fig. 4B).

We also performed EMSAs using probes harboring base pairs −393 to −300 and −173 to −42 of *LEE1* (Fig. 5). These EMSAs established that QseA directly binds to the region from bp −173 to −42 of *LEE1* but not to the region from bp −393 to −300. The region from bp −393 to −300 has been shown to be activated by QseA in EHEC but not in K-12 (Fig. 3). Of note, we also observed that the transcription of *LEE1* in the WT EHEC strain with bp −393 to +323 decreased sevenfold relative to that with the fragment from bp −218 to +86 (Fig. 3B), further suggesting that there is another yet-unidentified transcriptional activator acting in the region between bp −393 and −300. These studies allowed us to conclude that QseA activates the transcription of *LEE1* by directly binding upstream of its P1 promoter region. In EHEC, QseA also activates the larger transcripts in the region between bp −393 to −300 indirectly, through an unknown activator that is present in EHEC and is absent in *E. coli* K-12.

## DISCUSSION

QseA is an intermediary transcription factor within the AI-3/epinephrine/NE signaling cascade, which controls virulence gene expression in EHEC (61). QseA was previously described as a transcriptional activator of the LEE through the activation of the *LEE1* promoter (58), which encodes the Ler regulator, which is essential for virulence (72). Consequently, an EHEC *qseA* mutant revealed a striking reduction in TTS compared to that in the WT (58). In addition, using in vivo-induced-antigen technology, John et al. (26) reported that QseA expression is induced during human infection, further underscoring the importance of QseA-mediated regulation for EHEC pathogenesis.

This study aimed to investigate the molecular mechanisms by which QseA activates the transcription of *LEE1*. Through nested-deletion analyses of the *LEE1* promoter within the EHEC and *E. coli* K-12 backgrounds, a region between base pairs −123 and +86 (numbered according to the P2 transcriptional start site) was shown to be essential for the QseA-dependent transcriptional activation of the *ler* promoter (Fig. 3). QseA has previously been shown (using primer extension) to control the transcription of *LEE1* through the distal (P1) promoter (58). Our nested-deletion analyses of EHEC and K-12 confirmed these previous observations. These studies also showed that transcription of the longer *LEE1* constructs is highly repressed within an *E. coli* K-12 background (Fig. 2 and 3), consequently masking the ability to observe the P1- or P2-dependent transcription of *LEE1* in this background (Fig. 2). However, within an EHEC background, we could readily observe a threefold decrease in the transcription of *LEE1* in the absence of P2 (Fig. 2), corroborating our previous primer extension data mapping both promoters in *LEE1* (58). The

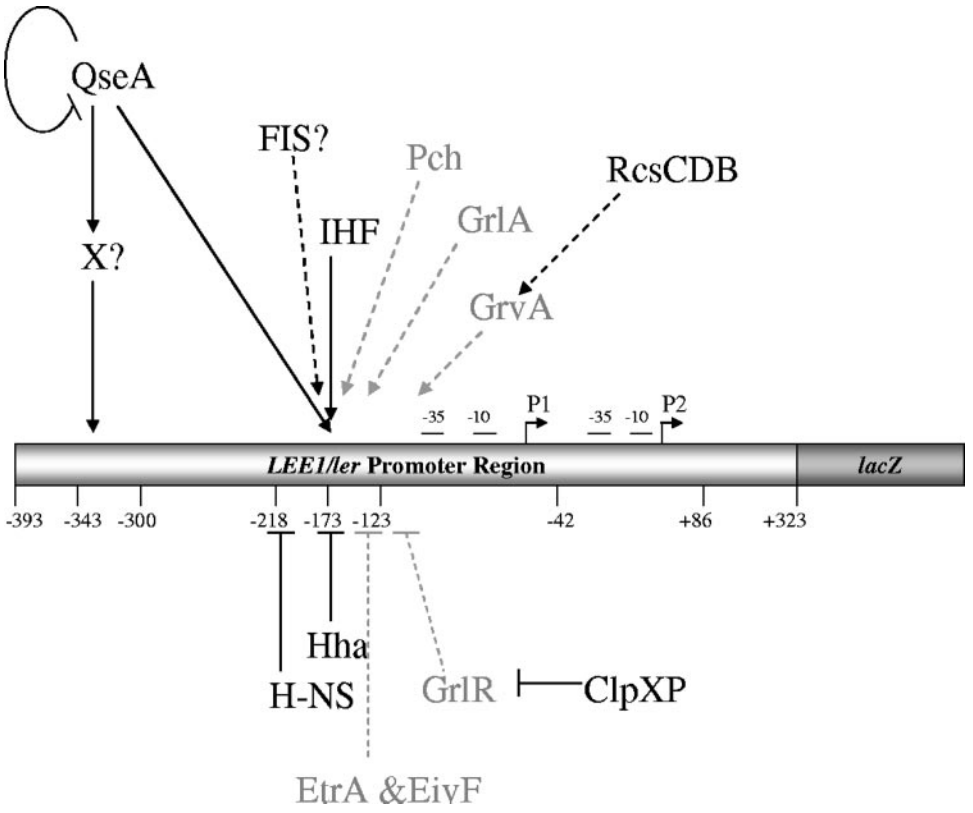


FIG. 6. Schematic depiction of LEE regulation. Factors shown in gray are present in both *E. coli* K-12 and EHEC (and also in EPEC), while regulators shown in black are specific to EHEC (some are shared with EPEC, e.g., GrlR, GrlA, and Ler). Solid lines represent regulators whose direct interactions with the target promoter have been biochemically defined, and dashed lines represent interactions which occur indirectly or have not yet been shown to bind to the target gene. IHF, integration host factor.

transcription repression of the longer *LEE1* fusion genes within *E. coli* K-12 may account for the contrasting results reported by Porter et al. (45) concerning transcription through the *LEE1* P2 promoter. Porter et al. used *LEE1*::*lacZ* transcription fusion constructs within an *E. coli* K-12 background starting 362 bp upstream from the *LEE1* translational start site, which corresponds to our fusion construct beginning at −343 bp, which is highly repressed in K-12 (Fig. 3). Of note, there is also conflicting information in the literature related to the *LEE1 (ler* is the first gene within *LEE1*) translational start site. Elliott et al. (10) assigned the translational start site to the first predicted methionine of *ler*. In contrast, Perna et al. (44), also taking into consideration the presence of a ribosome binding site consensus sequence upstream of the ATG, assigned the translational start site to the second predicted methionine of *ler*.

EMSA experiments demonstrated that QseA directly interacts with the region between base pairs −173 and −42 (Fig. 5). We also observed QseA-dependent transcriptional activation at base pairs as close as −123 to +86 of the *LEE1* promoter (Fig. 3). The −123 nucleotide corresponds to −50 in relation to the −35 region of the P1 promoter. These data suggest that QseA, a member of the LysR family of transcriptional regulators, binds in close proximity to the P1 promoter and may interact with the RNAP in a fashion similar to that of other LysR-like proteins (42, 51).

QseA was unable to bind in EMSA experiments in the region between base pairs −393 to −300 (Fig. 5), which is activated only in a QseA-dependent fashion in EHEC. These data suggest that QseA regulation of this region is indirect and involves another as-yet-unidentified transcriptional regulator that is absent in *E. coli* K-12. Given the transcriptional repression observed in the longer fusions (base pairs +393 to −232) in K-12 (Fig. 3C), one possibility is that this EHEC-specific transcription factor counteracts the action of a repressor. The transcriptional regulation of *LEE1* is very complex and involves factors shared between EHEC and *E. coli* K-12, as well as regulators specific to EHEC (some of these are also shared with EPEC and/or *C. rodentium*). Common regulators with *E. coli* K-12 include QseA (58), FIS (16), integration host factor (12, 67), H-NS (1, 67), Hha (52), ClpXP (22), and RcsCDB (63) (Fig. 6). There are several transcription factors in EHEC that are absent in *E. coli* K-12 (as mentioned above, some are shared with EPEC and *C. rodentium*). These include the Pch regulators (23, 45), GrlA and GrlR (1, 7, 21, 22), GrvA (63), and EtrA and EivF (71) (Fig. 6). However, only the Pch regulators and GrlA (encoded within the LEE region by the *grlRA* operon) have been shown to activate the transcription of *LEE1* (7, 21, 23). Gene array studies (M. Kendall and V. Sperandio, unpublished results) demonstrated that none of the *pch* genes are regulated by QseA. Using a *grlRA*::*lacZ* transcription fusion construct, we established that QseA activates the transcription of GrlR and GrlA (R. Russell and V. Sperandio,

unpublished studies). However, direct binding to *LEE1* by GrlA has not been demonstrated, and GrlA activates the transcription of *LEE1* through the −40 region of P1 (corresponding to the −123 region for P2 in our studies) (1). The EHEC-specific QseA-dependent factor that activates the transcription of *LEE1* acts through the region from bp −393 to −300 (Fig. 3); consequently, this factor is also not likely to be GrlA.

Studies thus far of the intricate regulation of QS in EHEC have shown that many factors are involved in the activation and repression of virulence genes. Here we showed that QseA activates the expression of *LEE1* (*ler*) by binding to a region of DNA in close proximity to the promoter P1, close to the binding site of RNAP. The P1 promoter of *LEE1* is present in both EHEC and EPEC. Hence, the observation that QseA activates expression through this promoter is consistent with previous studies indicating that QseA activates the expression of the LEE genes in both EHEC and EPEC (57, 58). This activation by QseA leads to the Ler activation of other genes in the LEE pathogenicity island, which are necessary for TTS and A/E lesion formation. The concerted action of QseA with a plethora of other transcription factors to modulate *LEE1* transcription may ensure the correct kinetics of LEE gene expression during infection.

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