

The Two-Component System GrvRS (EtaRS) Regulates *ace* Expression in *Enterococcus faecalis* OG1RF

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Expression of *ace* (adhesin to collagen of *Enterococcus faecalis*), encoding a virulence factor in endocarditis and urinary tract infection models, has been shown to increase under certain conditions, such as in the presence of serum, bile salts, urine, and collagen and at 46°C. However, the mechanism of *ace*/Ace regulation under different conditions is still unknown. In this study, we identified a two-component regulatory system GrvRS as the main regulator of *ace* expression under these stress conditions. Using Northern hybridization and β -galactosidase assays of an *ace* promoter-*lacZ* fusion, we found transcription of *ace* to be virtually absent in a *grvR* deletion mutant under the conditions that increase *ace* expression in wild-type OG1RF and in the complemented strain. Moreover, a *grvR* mutant revealed decreased collagen binding and biofilm formation as well as attenuation in a murine urinary tract infection model. Here we show that GrvR plays a major role in control of *ace* expression and *E. faecalis* virulence.

Enterococcus faecalis is a Gram-positive commensal bacterium of the gastrointestinal tract and also a common cause of hospital-acquired infections and endocarditis (1). During experimental infections, the adhesin to collagen of *E. faecalis* (Ace) plays an important role which has been attributed to mediating binding of *E. faecalis* to human extracellular matrix (ECM) proteins such as collagen (types I and IV), laminin, and dentin (2–4). Different infection models have shown attenuation of *ace* deletion mutants (5–7) and specific anti-recombinant Ace (rAce) antibodies were shown to confer protection against *E. faecalis* infection in an experimental endocarditis model (6). Therefore, Ace is considered a virulence factor in *E. faecalis* infection.

In order to better understand the role of Ace, it is important to study the regulation mechanisms of *ace* expression and surface display. Previous works have identified several environmental factors regulating *ace* expression; e.g., transcription of *ace* was increased when *E. faecalis* was grown at 46°C and grown in the presence of 40% horse serum, urine, and bile salts (5, 8, 9). In addition, levels of Ace on the cell surface are dependent on the *E. faecalis* strain and growth phase (10–12). With *E. faecalis* OG1RF, Ace is increased in the early exponential phase but reduced in the stationary phase; however, with *E. faecalis* JH2-2, it is maintained in later growth phases (10–12). The decrease in stationary-phase Ace in strain OG1RF was shown to be dependent on a functional *fsr* quorum-sensing system controlling the expression of gelatinase (GelE), which cleaves Ace from the OG1RF cell surface in late-phase cultures (11). Strains such as JH2-2 (lacking a complete *fsr* system operon) as well as *fsr* and *gelE* mutants of OG1RF do not cleave Ace from the surface (11), since they do not produce gelatinase. In other words, the amount of Ace on OG1RF strains is regulated in part at the posttranslational level. At the transcriptional level, the enterococcal regulator of survival (Ers) was previously reported as a repressor of *ace* expression in *E. faecalis* JH2-2 (5). In *E. faecalis* OG1RF, this regulator does not seem to play a role, as deletion of *ers* did not affect *ace* expression under the

various tested conditions (13). Deletion of *ccpA* encoding the transcriptional regulator CcpA (catabolite control protein A) from OG1RF resulted in significantly decreased levels of Ace surface expression in the early growth phase and an impaired ability to adhere to collagen in comparison to the wild-type (12). However, transcriptional levels of *ace* were similar in both OG1RF and the *ccpA* mutant, indicating that CcpA is not directly involved in regulating *ace* transcription.

Therefore, a transcriptional regulator that controls *ace* expression in OG1RF under various environmental conditions has not yet been identified. In this study, we identified the two-component regulatory system (TCS) GrvRS (global regulator of virulence; formerly EtaRS [14]) as a positive regulator of *ace* transcription in *E. faecalis* OG1RF under various environmental conditions.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Bacterial strains and plasmids used in this study are described in Table 1. *E. faecalis* and *Escherichia coli* were grown normally in brain heart infusion (BHI) and Luria Bertani (LB) media at 37°C, respectively. *E. faecalis* strains were also grown in BHI

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TABLE 1 Strains and plasmids used in this study

Strain or plasmid	Description ^a	Reference or source
<i>E. faecalis</i> strains		
CK111	OG1Sp <i>upp4::P23 repA4</i> ; provides RepA for replication of pHOU1	17
OG1RF	Fus ^r Rif ^r	41
TX10275	TCS disruption mutant; OG1RF_12536::pBluescript SK(-) with <i>aph(3')-IIIa</i> Km ^r	14
TX10276	TCS disruption mutant; OG1RF_11414::pBluescript SK(-) with <i>aph(3')-IIIa</i> Km ^r	14
TX10292	TCS disruption mutant; OG1RF_11029::pBluescript SK(-) with <i>aph(3')-IIIa</i> Km ^r	14
TX37200	TCS disruption mutant; OG1RF_10259::pBluescript SK(-) with <i>aph(3')-IIIa</i> Km ^r	14
TX10293	TCS disruption mutant; <i>grvR</i> ::pBluescript SK(-) with <i>aph(3')-IIIa</i> Km ^r	14
TX5733	Δ <i>grvR</i> ; nonpolar in-frame deletion of <i>grvR</i> from OG1RF	This study
TX5735	<i>grvR</i> *; reconstituted <i>grvR</i> of TX5733 with a silent nucleotide change for differentiation from OG1RF	This study
TX5652	Δ <i>ace</i> ; nonpolar in-frame deletion of <i>ace</i> from OG1RF	This study
<i>E. coli</i> strains		
EC1000	<i>E. coli</i> host strain; provides RepA; Km ^r	42
DH5 α	<i>E. coli</i> host strain used for routine cloning	Stratagene
Plasmids		
pHOU1	Plasmid for mutagenesis; Gm ^r	16
pCR-TOPO	Plasmid for PCR cloning; Km ^r	Invitrogen
pTEX6075b	<i>ace</i> promoter:: <i>lacZ</i> fusion in pKAF7; Em ^r	13
pTX5735a	pCR-TOPO containing <i>grvR</i> for complementation	This study
pTX5735b	pTX5735a containing a point mutation in Leu-109 codon of <i>grvR</i>	This study
pTX5735c	pHOU1 containing <i>grvR</i> * from pTX5735b	This study
pTX5733	pHOU1 containing Δ <i>grvR</i> construct	This study

^a Em, erythromycin; Fus, fusidic acid; Gm, gentamicin; Km, kanamycin; Rif, rifampin.

supplemented with 40% horse serum (BHIS) and in BHI with bile salts (0.02 and 0.04%) at 37°C as well as in BHI at 46°C (13). For the biofilm assay, *E. faecalis* was grown in tryptic soy broth supplemented with 0.25% glucose (TSBG) (15). Bile esculin azide (BEA) agar was used to quantitate enterococcal CFU from mouse tissue. Growth curves were determined as follows: *E. faecalis* strains were grown overnight and reinoculated (optical density at 600 nm [OD₆₀₀] = 0.05) into BHI or BHIS and the OD₆₀₀ was measured every hour until stationary phase. Antibiotics used for *E. faecalis* were erythromycin (10 µg/ml), fusidic acid (25 µg/ml), rifampin (100 µg/ml), and gentamicin (150 µg/ml); for *E. coli*, erythromycin (250 µg/ml), gentamicin (25 µg/ml), and kanamycin (50 µg/ml).

Construction of deletion mutants and complementation. A *grvR* nonpolar in-frame deletion mutant was created using the pHOU1 plasmid (16). DNA fragments upstream (664 bp) and downstream (795 bp) of the *grvR* gene were amplified with primer pairs UpF-BamHI plus UpR and DownF plus DownR-SphI, respectively. The open reading frame (ORF) of *grvR* is 687 bp, and the deletion was designed so that the internal 621 bp of the gene were in-frame deleted, leaving 6 bp of the N end (including the start codon) and 60 bp of the C end (including the termination codon) of *grvR* in the Δ *grvR* strain. Primers used in this study are listed in Table S1 in the supplemental material. Amplified fragments were joined by overlapping PCR, digested with BamHI and SphI, and then ligated into pHOU1 digested with the same restriction enzymes. The construct, designated pTEX5733, was electroporated into *E. faecalis* CK111 (17), which was then conjugated with *E. faecalis* OG1RF. The first recombination event was selected on BHI agar containing fusidic acid, gentamicin, and X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) (200 µg/ml). Blue colonies showing fusidic acid and gentamicin resistance were further characterized to verify recombination into the *grvR* region using outside primer pairs of outsideF plus DownR-SphI and UpF-BamHI plus outsideR. The second recombination event was obtained by spreading the first recombinants on MM9YEG supplemented with 10 mM p-Cl-Phe as described previously (16). For complementation (reconstitution) of Δ *grvR*, the *grvR* region was amplified with the primers UpF-BamHI plus DownR-SphI and then subcloned into pCR-TOPO plasmid (Invitrogen,

Carlsbad, CA). A silent mutation (CTA) was introduced into the leucine codon TTA corresponding to amino acid position 109 using the primers pmF and pmR (see Table S1 in the supplemental material), resulting in *grvR**. Leucine is the most frequent amino acid (28 out of 228 amino acids) in GrvR. Among 28 leucine codons, codon usage was as follows: 16 TTA, 5 CTT, 4 TTG, and 3 CTA. Therefore, the complemented strain contains the same Leu-109 but with a silent nucleotide mutation to distinguish the complemented strain from wild-type OG1RF. Deletion (TX5733 [Δ *grvR*]) and complemented (TX5735 [*grvR**]) strains were confirmed by DNA sequencing and by pulsed-field gel electrophoresis patterns. The *ace* nonpolar markerless deletion mutant of OG1RF with a deletion from the *ace* start codon to the termination codon was also constructed with pHOU1 plasmid using the primer pairs AceA plus AceB (to amplify the upstream fragment) and AceC plus AceD (to amplify downstream fragment).

β -Galactosidase activity assay. Five previously described OG1RF TCS mutants (14) were electroporated with pTEX6075b containing an *ace*_{OG1RF} promoter-*lacZ* fusion (13) and selected on BHI plates containing X-Gal and erythromycin. For β -galactosidase assays, overnight cultures of *E. faecalis* were reinoculated to an initial OD₆₀₀ of 0.05 into prewarmed BHI or BHIS containing erythromycin. At mid-exponential phase (OD₆₀₀ = 0.5 \pm 0.05), 1 ml of samples was centrifuged, suspended in 1 ml Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄, 50 mM β -mercaptoethanol [pH 7.0]), and disrupted using a mini-BeadBeater (BioSpec Products, Bartlesville, OK). After centrifugation, supernatants were used for the β -galactosidase activity as described previously (18).

RNA isolation and Northern blot analysis. Total RNA isolation and Northern hybridizations were performed as described previously (13). An overnight culture was reinoculated into 20 ml BHI broth or BHIS to a starting OD₆₀₀ of 0.05. At the mid-exponential phase (OD₆₀₀ = 0.5 \pm 0.5), 5 ml of the culture was mixed with 10 ml RNAprotect reagent (Qiagen, Hilden, Germany) and collected for RNA isolation. The pellet was suspended in 1 ml RNawiz (Ambion, Austin, TX) and subjected to bead beating for 1 min using a mini-BeadBeater. RNA was extracted according

to the manufacturer's protocol and cleaned with the RNeasy minikit (Qiagen, Hilden, Germany). Primers used for amplification of *ace* and *ers* internal hybridization probes were previously described (13). Primers, del probeF, and del probeR were used for amplification of the *grvR* hybridization probe (see Table S1 in the supplemental material).

Mutanolysin cell wall extracts and Western blot analysis. Surface expression of Ace was detected by Western blot analysis after mutanolysin treatment. Mutanolysin treatment, SDS-PAGE, and Western hybridization with anti-Ace monoclonal antibody 70 were performed as previously described (11, 13).

Adherence assay. *E. faecalis* strains were grown for 16 h in BHI at 46°C, harvested, washed three times in phosphate-buffered saline (PBS; pH 7.4), and adjusted to an OD₆₀₀ of 1.0. Ten micrograms of collagen IV in 100 µl of PBS was used to coat 96-well 4HBX plates (Thermo Scientific, Rochester, NY) overnight at 4°C. Bovine serum albumin (BSA) was used as a control. After decanting, each well was blocked with 200 µl of 2% BSA for 2 h at 4°C and washed three times with PBS. A total volume of 100 µl of bacteria (OD₆₀₀ = 1.0) was added to each well and incubated at room temperature for 2 h under static conditions. The wells were washed three times with PBS, and cells were fixed with Bouin's fixation solution (Sigma-Aldrich Co., St. Louis, MO) for 20 min at room temperature. Each well was washed three times with PBS and stained with 1% (wt/vol) crystal violet for 10 min at room temperature. After three washes with PBS, an ethanol-acetone (8:2 ratio) solution was used to elute the stained dye. The absorbance of each well was measured at 570 nm using a 96-well plate reader (Thermo Labsystems, Vantaa, Finland).

Biofilm assay. For *in vitro* biofilm formation assays (15), *E. faecalis* was grown overnight at 37°C in TSBG, diluted to an initial OD₆₀₀ of 0.05 in 200 µl TSBG in 96 well 4HBX plates, and incubated for additional 24 h. As described for the adherence assay, *E. faecalis* was fixed with Bouin's solution for 30 min, stained with 1% crystal violet for 30 min, and solubilized in ethanol-acetone (8:2 ratio) solution to determine the OD₅₇₀ using the 96-well plate reader.

Mouse urinary tract infection model. Preparation of mice, *E. faecalis* OG1RF, and the Δ *grvR* and *grvR*^{*} strains and all other stages of the mixed-infection competition experiments were performed as previously described (19). In brief, wild-type OG1RF versus the Δ *grvR* strain and the *grvR*^{*} strain versus the Δ *grvR* strain were tested in a mixed-infection competition assay by inoculating a bacterial mix (estimated as approximately 1:1 by OD, with subsequent CFU determination to determine the actual ratio) intraurethrally via an inserted catheter, and the catheter was removed soon after the inoculation. Animals were sacrificed 48 h after infection, and bacteria were recovered by plating tissue homogenates of kidney pairs and urinary bladders. After 24 to 48 h, all colonies that grew from tissue homogenates (up to 47/mouse and two controls in a 96-well microtiter plate) were picked into wells containing BHI, grown overnight, and then used to prepare DNA lysates from colonies on nylon membranes that were hybridized under high-stringency conditions, using intragenic DNA probes of *ace* and *grvR* to generate the percentage of wild type and mutant in the bacteria recovered from kidneys and bladders (15). The animal experimental procedures were preapproved and were carried out in accordance with the institutional policies and the guidelines stipulated by the animal welfare committee, University of Texas Health Science Center at Houston.

Statistical analysis. Differences in biofilm between *E. faecalis* strains were compared by the Mann-Whitney test. The percentage of the Δ *grvR* strain in the inoculum versus the percentage of the Δ *grvR* strain in the kidneys and bladders of individual mice coinfecting with the Δ *grvR* strain and either OG1RF (or the *grvR*^{*} strain) in the competition assay were analyzed by the paired *t* test using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA).

RESULTS

An *ace* promoter-*lacZ* fusion indicates that GrvRS regulates *ace* expression. Previously, we generated a series of knockout mu-

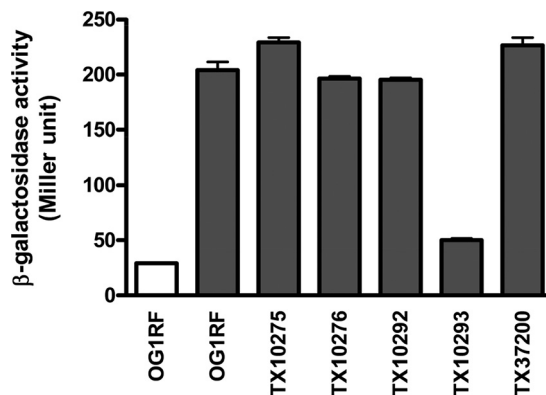


FIG 1 *ace* promoter activity in TCS mutants of *E. faecalis* OG1RF. β -Galactosidase activities of OG1RF and its TCS mutants containing pTEX6075b were determined. Measurements were carried out in duplicate in two strains grown in BHI (white bar) or BHIS (gray bars).

tants of *E. faecalis* OG1RF TCS (14). To investigate *ace* expression in these mutants, we introduced an *ace* promoter-*lacZ* fusion (13) into OG1RF and five of its TCS mutants and determined the β -galactosidase activity after growth in BHIS and, for OG1RF, also in BHI (Fig. 1). As expected (8, 13), promoter activity of *ace* in OG1RF was induced by the presence of serum (Fig. 1), and we found that expression of *ace* was significantly reduced (about 4-fold) in one of the TCS mutants, TX10293 (Fig. 1). TX10293 contains a polar insertion of a kanamycin resistance transposon that disrupts *grvR* (14). To confirm the involvement of the GrvRS system in *ace* expression, we constructed a nonpolar markerless in-frame deletion of *grvR* (Δ *grvR*) in *E. faecalis* OG1RF. To avoid a possible overdose effect of putting the transcriptional regulator *grvR* in *trans*, we complemented (restored) the Δ *grvR* strain by reconstituting the chromosomal deletion rather than cloning the gene into a plasmid. In this manner, we also avoided the use of antibiotics to maintain the plasmid. To distinguish the complemented (*grvR*^{*}) strain from wild-type OG1RF, a silent tagging mutation was introduced into the most abundant amino acid in GrvR, leucine (12.3% of GrvR protein). OG1RF and the Δ *grvR* and *grvR*^{*} strains showed similar growth rates in BHI and in BHIS at 37°C (see Fig. S1 in the supplemental material). Compared to OG1RF and the *grvR*^{*} strain, the Δ *grvR* strain was heat resistant and acid sensitive (see Fig. S2 in the supplemental material), in agreement with previous results with the disruption mutant TX10293 (14).

Northern hybridization indicates that *ace* transcripts are significantly decreased in the Δ *grvR* mutant. Expression of *ace* in OG1RF and its Δ *ace*, Δ *grvR*, and *grvR*^{*} mutants was determined by Northern hybridization under different stress conditions previously shown to increase *ace* expression (8, 13), with strains grown to mid-exponential phase at 46°C (Fig. 2A). Results showed that *ace* mRNA was present in OG1RF and the complemented *grvR*^{*} strain but absent in the Δ *ace* strain and barely detectable in the Δ *grvR* strain, confirming that GrvR regulates *ace* expression (Fig. 2A). The *ers* gene (13) was used as an RNA quality and quantity control.

We then investigated expression of *ace* in OG1RF and the Δ *grvR* mutant grown to mid-exponential phase in the presence of serum (BHIS) and in BHI with bile salts (0, 0.02, and 0.04%) at 37°C and at 46°C in BHI (Fig. 2B). Results showed that *ace* mRNA

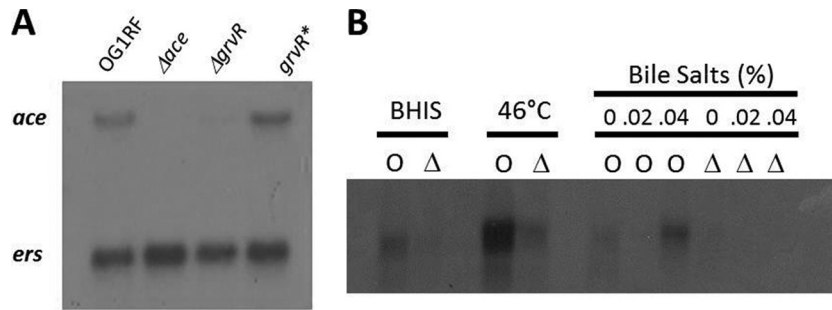


FIG 2 Northern hybridization of *ace* in *E. faecalis* strains. Northern blot analysis was performed with total RNA (10 μg per lane) and hybridized with internal probes of *ace* (A and B) and *ers* (A). The *ers* gene was used as an RNA quality and quantity control. (A) Strains were grown in BHI at 46°C. (B) OG1RF (O) and the Δ *grvR* mutant (Δ) were grown in BHIS at 37°C, in BHI at 46°C, and in BHI with bile salts.

increased in BHIS and at 46°C compared to that of BHI only. Expression of *ace* in bile salts was concentration dependent, as only the higher concentration (0.04%) increased *ace* mRNA. Increased temperature (46°C) resulted in the greatest increase in *ace* mRNA in OG1RF (Fig. 2B). Under all conditions tested, *ace* mRNA in the Δ *grvR* strain was barely detectable, confirming that GrvR is necessary for *ace* induction under these conditions. Currently, we do not know whether *ace* expression is higher at tested stress conditions or the RNA is more stable under these conditions.

Ace surface levels are decreased in the Δ *grvR* mutant. We next investigated whether the level of *ace* transcripts in the Δ *grvR* mutant is correlated with the levels of Ace on the surface and with function. For Western blots of cell wall extracts, OG1RF and its Δ *grvR* mutant were grown in the presence of serum. Ace was present in surface extracts of OG1RF but was not detected in surface extracts of the Δ *grvR* strain (see Fig. S3 in the supplemental material).

Previous studies have shown that Ace mediates adherence of *E. faecalis* to ECM proteins such as collagen IV (4, 6). We then performed an adherence assay to investigate the effect of the deletion of *grvR* on collagen binding using strains grown at 46°C and methods described previously (20). There was no collagen IV binding by the Δ *ace* or Δ *grvR* strain, whereas wild-type OG1RF and its complemented *grvR** strain had comparable levels of binding activity (Fig. 3A). The same binding patterns were observed with collagen I and laminin (data not shown). Previous studies with TX10293 suggested a role for *grvR* in biofilm formation (21). We found that deletion of *grvR* modestly but statistically significantly

reduced biofilm formation; biofilm-forming ability was restored in the *grvR** strain (Fig. 3B).

Deletion of *grvR* causes attenuation in the mouse urinary tract infection. To further investigate the involvement of GrvRS in virulence of *E. faecalis*, we used a mixed infection of OG1RF and the Δ *grvR* strain in the murine UTI model. As shown in Fig. 4A and B, significantly greater numbers of OG1RF were recovered from both kidneys and bladders. Attenuation of the Δ *grvR* strain in the UTI model was restored in the reconstituted (*grvR**) strain (Fig. 4C and D). These results indicate that *grvR* is involved in the pathogenesis of *E. faecalis* in the murine UTI model.

DISCUSSION

Ace of *E. faecalis* is expressed during infection and assumed to contribute to infection by adhering to exposed ECM molecules such as collagen and laminin (4, 6, 22–24). Previous work found that expression of *ace* is dependent on environmental conditions, including the presence of serum and bile salts (8, 9). Bacteria often control gene expression in response to environmental changes via TCSs, which are generally composed of a sensor histidine kinase that recognizes specific environmental signals and a response regulator that mediates a response to gene expression (25, 26). In order to determine whether a TCS is responsible increases in *ace* expression, we investigated TCSs we had previously studied in *E. faecalis* (14). With disruption mutation and an *ace* promoter fusion, we found that GrvRS appeared to control *ace* expression in response to serum, leading to the upregulation of *ace* expression. GrvRS has homology with other TCSs of Gram-positive pathogens, such as CovRS (also called CsrRS) of group A/B streptococci

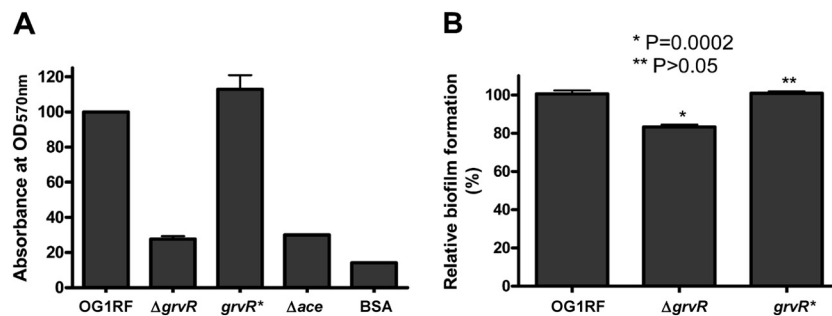


FIG 3 Effect of *grvR* deletion on adherence to collagen IV (A) and biofilm (B). Values for OG1RF were considered 100%, and the relative activities for the other strains are shown. Mean OD₅₇₀ of OG1RF were 1.048 (A) and 1.733 (B). Bars represent the means and standard deviations from two independent experiments representing six wells for each strain. BSA was used as a control for adherence. *P* values are for comparison versus OG1RF.

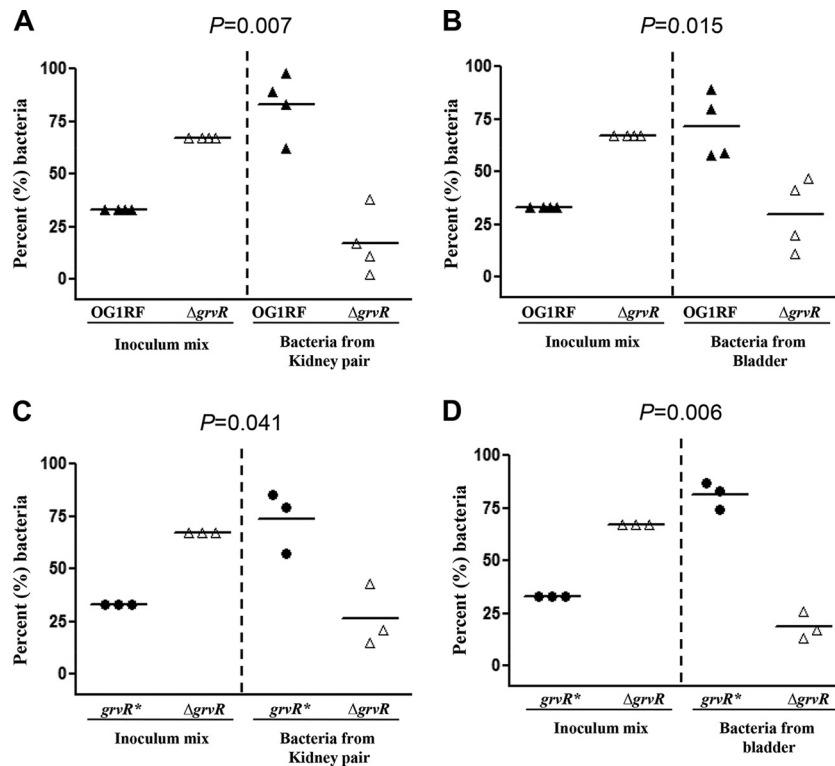


FIG 4 Effect of *grvR* deletion from OG1RF and its restoration in a mouse urinary tract infection using a mixed inoculum. (A and C) Percentage of bacteria recovered from kidneys. (B and D) Percentage of bacteria recovered from bladders. Horizontal bars indicate the means of percentages of each strain in the inoculum and from kidneys and bladders. Percentages were derived from CFU counts of the inoculum mixtures and from the kidney and bladder homogenates and compared using the paired *t* test. Percentage values are obtained from four (A and B) and three (C and D) mice, respectively.

(27–30) and LisRK of *Listeria monocytogenes* (31, 32), which have been shown to be important for their virulence. The CovRS system shows high similarity with GrvRS (70% for GrvR versus CovR and 54% for GrvS versus CovS) (14, 33). The CovRS system has also been shown to respond to the environmental stimuli produced by blood components (34–36).

A $\Delta grvR$ deletion mutant confirmed the involvement of GrvR in the regulation of *ace* expression (Fig. 2). In addition, disruption of *grvR* led to the loss of ECM binding activity and reduced biofilm formation and attenuation in the UTI model (Fig. 3 and 4). Because the *ace* deletion mutant was also attenuated in the UTI model (6), attenuation of the $\Delta grvR$ mutant in this model could be the consequence of reduced *ace* expression in the $\Delta grvR$ mutant. Previously, we showed that a *grvRS* mutant (TX10293) of OG1RF was attenuated in a mouse peritonitis model (14). However, an *ace* mutant was not attenuated in the same peritonitis model (6), indicating that the GrvRS system regulates other virulence determinants in addition to *ace*. Indeed, a *kat* gene encoding catalase was reported to be regulated by the GrvRS system in OG1RF (37). Catalase plays an importance role in protection against cellular oxidative stress by degrading hydrogen peroxide (38). In *E. faecalis* JH2-2, GrvRS was also reported as a negative regulator of the heat shock proteins DnaK and GroEL (39). Therefore, the GrvRS regulon appears to contain many other genes in addition to the genes mentioned above.

The *grvRS* region is part of the *E. faecalis* core genome; of the approximately 250 *E. faecalis* genome sequences available in the NCBI genomic database, the amino acid sequences of GrvRS are

virtually identical in all (data not shown). Therefore, based on the sequence similarity of GrvRS, we expected a similar, if not identical, signaling mechanism of GrvRS in *E. faecalis* strains. However, studies carried out in different *E. faecalis* backgrounds, such as JH2-2 and V583, showed differences between strains in the GrvRS system (24, 39, 40). In strains OG1RF and JH2-2 (14, 24, 39), GrvRS was shown to be involved in stress responses, such as high temperature, low pH, and survival in bile salts; however, in *E. faecalis* V583, the *grvR* mutant did not show an acid-sensitive or heat resistance phenotype, suggesting the existence of a backup system of GrvRS in V583 (40).

In summary, we show here that GrvRS is a major regulator of *ace* and important for *E. faecalis* OG1RF virulence. A better understanding of the regulatory mechanisms controlling virulence gene expression may help us find new strategies to treat and prevent *E. faecalis* infections.

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