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A novel conjugative plasmid from *Enterococcus faecalis* E99 enhances resistance to ultraviolet radiation

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Abstract

Enterococcus faecalis has emerged as a prominent healthcare-associated pathogen frequently encountered in bacteremia, endocarditis, urinary tract infection, and as a leading cause of antibiotic-resistant infections. We recently demonstrated a capacity for high-level biofilm formation by a clinical *E. faecalis* isolate, E99. This high biofilm forming phenotype was attributable to a novel locus, designated *bee*, specifying a pilus at the bacterial cell surface and localized to a large ~80 kb conjugative plasmid. To better understand the origin of the *bee* locus, as well as to potentially identify additional factors important to the biology and pathogenesis of strain E99, we sequenced the entire plasmid. The nucleotide sequence of the plasmid, designated pBEE99, revealed large regions of identity to the previously characterized conjugative plasmid pCF10. In addition to the *bee* locus, pBEE99 possesses an open reading frame potentially encoding aggregation substance, as well as open reading frames putatively encoding polypeptides with 60 to 99 % identity at the amino acid level to proteins involved in regulation of the pheromone response and conjugal transfer of pCF10. However, strain E99 did not respond to the cCF10 pheromone in clumping assays. While pBEE99 was found to be devoid of any readily recognizable antibiotic resistance determinants, it carries two non-identical *impB/mucB/samB*-type genes, as well as genes potentially encoding a two-component bacteriocin similar to that encoded on pYI14. Although no bacteriocin activity was detected from an OG1RF transconjugant carrying pBEE99 against strain FA2-2, it was approximately an order of magnitude more resistant to ultraviolet radiation. Moreover, curing strain E99 of this plasmid significantly reduced its ability to survive UV exposure. Therefore, pBEE99 represents a novel conjugative plasmid that confers biofilm-forming and enhanced UV resistance traits that might potentially impact the virulence and/or fitness of *E. faecalis*.

Keywords

Enterococcus faecalis; *bee* locus; pBEE99; bacteriocin; ultraviolet radiation resistance; conjugation

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1. Introduction

Enterococcus faecalis is a Gram-positive bacterium that commonly occupies a commensal niche in the gastrointestinal tract. However, *E. faecalis* is notorious for acquiring new traits through horizontal gene transfer that allow the organism to become an opportunistic pathogen capable of causing infections of the bloodstream, urinary tract, and endocardium (Coburn et al., 2007; Gilmore et al., 2002; Tendolkar et al., 2006). In fact, *E. faecalis* is the third leading cause of healthcare-associated infections (Hidron et al., 2008) which are commonly recalcitrant to standard treatments and have a high morbidity rate (Gilmore et al., 2002). *E. faecalis* is particularly adept at acquiring and disseminating genes via bacterial conjugation, a complex process whereby large portions of genetic material are transferred from a donor bacterium to a recipient. The sex-pheromone conjugative plasmid system employed by *E. faecalis* permits the rapid transfer of genes, including genes that encode resistance to antibiotics and genes that enhance the ability to cause disease (Clewell, 1974; Clewell and Dunny, 2002).

Recently, we identified a novel operon, designated *bee* (biofilm enhancer in enterococcus), by Tn917 insertional mutagenesis of the urinary tract *E. faecalis* isolate E99. Tn917 insertion into this locus severely impaired the ability of this strain to form a biofilm. In filter mating experiments, the *bee* locus was shown to transfer by conjugation and enhance biofilm formation by transconjugants. Southern hybridization analysis revealed that the *bee* locus was carried on an approximately 80 kb conjugative plasmid (Tendolkar et al., 2006). The discovery of a conjugative plasmid that enhances the ability of *E. faecalis* to form a biofilm represents another mechanism for enterococcal strains to disseminate the genes necessary to persist in the hospital setting or in susceptible patients. In the current work, we sequenced the *bee*-encoding conjugative plasmid, designated pBEE99, to potentially identify other factors that might enhance the pathogenesis or fitness of *E. faecalis*.

2. Methods

2.1 Bacterial strains, plasmids, and growth conditions

All *E. faecalis* strains were cultivated in Brain Heart Infusion (BHI) medium supplemented with the appropriate antibiotics. *E. faecalis* strain E99 is a clinical isolate obtained from the urine of a patient at the Veterans Administration Hospital in Little Rock, AR. Selection for strain E99 consisted of 25 µg/ml kanamycin. E99 was grown in BHI medium containing kanamycin 25 µg/ml at 45°C for 10 consecutive days (subculturing was done every consecutive day) in order to cure the strain of pBEE99. After 10 days, the culture was plated on BHI containing kanamycin 25 µg/ml and random colonies were picked and screened for the absence of the *bee* locus and the *prgK* (Table 1) gene by PCR and Southern hybridization. The absence of pBEE99 from a single clone that was negative by both PCR and Southern hybridization for the *bee* locus and *prgK* was further verified in biofilm assays. This clone, designated KDS01, was significantly attenuated in biofilm formation, indicating the absence of the *bee* locus.

The plasmid free strain OG1RF (Dunny et al., 1978) and the previously generated transconjugant IG9, that was derived from mating E99 with OG1RF (Tendolkar et al., 2006), were cultivated in the presence of 25 µg/ml rifampicin and 10 µg/ml fusidic acid. OG1RF (pCF10) (Dunny et al., 1981) possesses the 67.6 kb pheromone responsive conjugative plasmid pCF10 that encodes a response to the cCF10 pheromone (Hirt et al., 2005).

Long range PCR products were subcloned into pBluescript for automated sequencing. *Escherichia coli* XL1-Blue (Stratagene, LaJolla, CA) was used as a host for subcloned pBluescript plasmids. *E. coli* was cultivated in Luria Broth (LB) or 2xYT media and supplemented with 100 µg/ml ampicillin.

2.2 DNA purification

The *bee* locus encoding plasmid DNA was purified from strain E99 using the QIAGEN large plasmid purification kit (QIAGEN, Valencia, CA). The pBluescript plasmid clones were purified from XL1-Blue using Promega's Wizard plasmid miniprep kit (Promega, Madison, WI).

2.3 Cloning, Long range PCR, primer walking, and DNA sequencing

We previously identified and sequenced a novel 8,320 bp locus, designated *bee* (Tendolkar et al., 2006). Using inverse PCR and primer walking methodologies, an additional 16,038 bp of sequence data was obtained 5' and 3' to the *bee* locus for a total of 24,358 bp. This was followed by a shotgun sequencing approach in which purified pBEE99 DNA was restricted with HindIII, fragments ligated to HindIII-restricted pBluescript and introduced into *E. coli* by electroporation. Clones were then selected, sequenced, and fragments were assembled into a 36,932 bp contig that interestingly did not overlap with the 24,358 bp contig, and thus represented a distinct region of the pBEE99 plasmid. The two gaps between these two regions were then closed using long range PCR and primer walking. Long range PCR was then used to confirm correct assembly of the fragments into the complete pBEE99 plasmid. Takara LA *Taq* was used for all PCR amplifications, and PCR products generated by long range PCR were purified using Promega's Wizard PCR purification kit (Promega, Madison, WI). Sequencing of purified PCR products and purified pBluescript clones possessing pBEE99 fragments was performed via automated sequencing using an ABI3730 capillary sequencer at the Oklahoma Medical Research Foundation (Oklahoma City, OK). Analysis of pBEE99 sequence was performed using FGENESB from Softberry® (<http://linux1.softberry.com/berry.phtml>). This program predicts genes, operon organization, promoters, tRNA, rRNA, and deduce functions using BLASTP against COG and NR databases at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>). A circular genome viewer java application called CGVIEW (Stothard and Wishart, 2005) was used to Visualize analyzed feature of pBEE99. Additionally, pair-wise nucleotide analysis against related *E. faecalis* plasmids was performed using BLASTN algorithm. Homology searches of the sequenced DNA were performed by BLASTN analyses using National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) and the FGENESB software from Softberry® (<http://linux1.softberry.com/berry.phtml>). The FGENESB program performs several analyses: BLASTP, tRNA and rRNA scans, promoter, terminator and operon prediction. The results were then visualized using the Circular Genome Viewer (CGVIEW) java application (Stothard and Wishart, 2005).

2.4 Clumping assay

Overnight cultures of *E. faecalis* strains OG1RF (pCF10) and E99 were diluted 1:100 in 5 mL Todd Hewitt Broth with or without 10 ng/mL of cCF10 peptide and incubated at 37°C. Cell aggregates appear after 2 hours of incubation.

2.5 Bacteriocin assay

To test whether the pBEE99 plasmid specifies bacteriocin production, we compared strain OG1RF to a previously generated OG1RF transconjugant, designated IG9, possessing the pBEE99 plasmid (Tendolkar et al., 2006) for the ability to inhibit the growth of *E. faecalis* strain FA2-2. Briefly, strain FA2-2 was grown overnight in brain heart infusion medium (BHI) and 50 µl of the overnight culture added to 3 ml of molten BHI soft agar (0.75% [wt/vol]). The soft agar was then poured onto the surface of a BHI agar plate and allowed to solidify at room temperature. Three microliters of either strain OG1RF or IG9 was spotted onto the surface of the plate in triplicate, and the plates incubated at 37°C for 24 hours. Any clearing around the spots was interpreted as bacteriocin production.

2.6 Ultraviolet sensitivity assay

In order to determine whether the pBEE99 plasmid confers a UV resistant phenotype, we compared the UV sensitivity of strain OG1RF to IG9 [OG1RF (pBEE99)]. We performed a qualitative UV irradiation killing assay essentially as described by Scott et al (Scott et al., 2008). Strains OG1RF and IG9 were grown overnight in Todd-Hewitt broth (Difco) supplemented with 2% yeast extract (THY medium), diluted 1:20 in fresh, pre-warmed THY, and allowed to grow to an OD₆₀₀ of 0.6. Cultures were then centrifuged at 5,000 × g for 15 minutes at 4°C, resuspended in ice-cold 0.1 M MgSO₄, and incubated on ice for 10 minutes. For each strain, 5 ml of the resuspended culture was placed in a sterile glass 100 × 15 mm petri dish and exposed to a calibrated 254 nm UV lamp (300 μW/cm²). At 30, 60, 90, and 120 seconds, 1 ml aliquots were removed and serially diluted 10-fold in 0.1 M MgSO₄. Two microliters of each dilution from 10⁻¹ to 10⁻⁴ was spotted onto the surface of a THY plate and incubated in the dark at 37°C for 24 hours.

In order to precisely quantify the difference observed between strain OG1RF and IG9 and to determine whether curing pBEE99 from strain E99 results in increased UV sensitivity, we performed a quantitative UV radiation resistance assay as follows. Strains E99, KDS01, OG1RF, and IG9 were grown overnight in BHI broth supplemented with the appropriate antibiotics, diluted 1:20 in fresh, pre-warmed BHI, and allowed to grow to an OD₆₀₀ of 0.6. Cultures were then centrifuged at 5,000 × g for 15 minutes at 4°C, resuspended in ice-cold 0.1 M MgSO₄, and incubated on ice for 10 minutes. For each strain, 1 ml of the resuspended culture was placed in a sterile plastic 35 × 10 mm petri dish and exposed to a calibrated 254 nm UV lamp (300 μW/cm²). At 30, 60, 90, and 120 seconds, 20 μl aliquots were removed, serially diluted 10-fold in 0.1 M MgSO₄, and colony forming units (CFU) per milliliter determined by the track dilution method (Jett et al., 1997). The statistical significance of the results was determined by performing pairwise comparisons at each time point using the Student's t-test.

3. Results and Discussion

3.1 DNA sequence and organization of plasmid pBEE99 from *E. faecalis* strain E99

The molecular size of pBEE99 was determined to be 80,600 base pairs, and the first nucleotide was defined as a C located 245 bp 5' to the start codon of the *repA* (*prgW*) gene. The pBEE99 plasmid possesses 95 putative ORFs identified using the FGENESB software from Softberry© (<http://linux1.softberry.com/berry.phtml>). Comparative BLASTN analysis of pBEE99 against other *E. faecalis* plasmids pCF10, pTEF1, pTEF2, pTEF3, pRE25 and pAM373, demonstrated that pBEE99 is more closely related to pTEF2 and pCF10 (Hirt et al., 2005; Dunny, 2007; Paulsen et al., 2003; Schwarz et al., 2001; De Boever et al., 2000). The putative replication initiation protein A (RepA) demonstrates 59% identity and 74% similarity to PrgW from pCF10 (Hirt et al., 2005). Within the putative RepA coding sequence is the direct repeat sequence 5' – CGAAAGTGGGAAAACCAAC – 3' occurring thrice in tandem and suggestive of the direct repeats within the origin of replication (*oriV*) of pAD1 (Francia et al., 2001; Francia et al., 2004). While the precise location of the *oriV* remains to be determined experimentally, we predict that this region of pBEE99 corresponds to the *oriV*.

pBEE99 possesses a conjugal transfer region including an open reading frame potentially encoding aggregation substance (98% identity to PrgB from pCF10 and pTEF2) (Table 1), and open reading frames putatively encoding polypeptides with 60 to 99 % identity at the amino acid level to proteins involved in regulation of the pheromone response and conjugal transfer of pCF10 (Table 1) (Hirt et al., 2005). Interestingly, pBEE99 carries two non-identical open reading frames that show a high degree of identity and similarity to a putative ImpB/MucB/SamB family protein on pTEF2 (Table 1). The first of the 1315 bp genes begins at position 54,379 and ends at 55,695, and the second at position 76,497 to 77,813 (Table 1 and Fig. 1).

E. coli UmuC protein is the prototypical member of this family of genes that encode a class of DNA polymerases that are capable of replicating through UV-induced DNA lesions (Smith and Walker, 1998; Wang, 2001), and thus allow the organism to survive lethal doses of ultraviolet radiation. The *E. coli* MucB, the *Salmonella typhimurium* ImpB, and the *S. typhimurium* SamB proteins are all homologs of the UmuC protein; however, they are all plasmid associated (Sarov-Blat and Livneh, 1998; Koch et al., 1995; Smith and Walker, 1998). A cognate ImpA/MucA/SamA protein was not found in association with either copy of the ImpB/MucB/SamB family protein. Another pBEE99 open reading frame possibly related to UV resistance shows 95% identity and 96% similarity to a putative UvrC protein from pCF10. The pCF10 open reading frame is 95% identical and 98% similar to UvrC from pAD1, which has been demonstrated to function as a negative regulator of the *uvrA* gene (Ozawa et al., 1997). The pAD1 *uvrA* gene product increases the resistance of *E. faecalis* to DNA-damaging UV light (Ozawa et al., 1997). Further, pBEE99 possesses another open reading frame with 68% identity and 84% similarity to a putative UvrC family transcriptional regulator from pTEF2 (Table 1).

A set of open reading frames that potentially encode a bacteriocin similar to the Bacteriocin 41 from pYI14 (Tomita et al., 2008) were identified at position 43,207 to 49,672 on pBEE99. These open reading frames include two copies of *bacL1* (the second a possible pseudogene), *bacL2*, two hypothetical proteins, *bacA*, and *bacI* with identities ranging from 77% to 100% to the pYI14 genes. Finally, pBEE99 possesses 24 hypothetical proteins, and although no antibiotic resistance genes were readily apparent on pBEE99, we cannot rule out the possibility that these hypothetical genes could potentially encode novel resistance determinants.

3.2 pBEE99 does not encode a mating response to the peptide sex pheromone cCF10

Given the degree of identity and similarity to pCF10, we sought to determine whether pBEE99 specifies a mating response to the sex pheromone cCF10 by performing a cell aggregation assay in the presence and absence of 10 ng/ml cCF10. Overnight cultures of *E. faecalis* strains OG1RF (pCF10) and E99 were diluted 1:100 in 5 mL THB broth containing 10 ng/mL of cCF10 peptide, or no peptide, and incubated at 37°C. As expected, cell aggregation was observed after 2 hours of incubation for strain OG1RF (pCF10) in the presence, but not in the absence of 10 ng/ml cCF10 (data not shown). On the contrary, E99 was not observed to clump in the presence of cCF10, indicating that pBEE99 does not encode a mating response to this pheromone. Moreover, in our previous work, transfer of pBEE99 from E99 to OG1RF was not detected in liquid broth, suggesting that pBEE99 might have lost the ability to specify a response to sex pheromones. Alternatively, transfer of pBEE99 from the E99 background might be inefficient. Whether broth transfer from the OG1RF background to another recipient of a different strain background can occur remains to be determined.

3.3 *E. faecalis* strain OG1RF (pBEE99) does not elaborate detectable bacteriocin activity against *E. faecalis* strain FA2-2

A feature that distinguishes pBEE99 from pTEF2 and pCF10, in addition to the *bee* locus, is a set of genes encoding a putative bacteriocin similar to the Bacteriocin 41 from pYI14. Bacteriocin 41 consists of two lytic subunits, BacL1 and BacL2, an activator component, BacA, and was demonstrated to only be active against *E. faecalis* (Tomita et al., 2008). Two additional open reading frames, ORF9 and ORF10 are found in between the *bacL2* and *bacA* genes on pYI14, and likewise two hypothetical proteins are similarly situated between the putative *bacL2* and *bacA* genes of pBEE99. Functions for ORF9 and ORF10 on pYI14 have not yet been elucidated. Producer self-protection is afforded by BacI, a homolog of which is also found on pBEE99. While the pBEE99 *bacL1* gene possesses a frameshift mutation located towards the 3' end at position 44,724 that could potentially result in a nonfunctional polypeptide, we nonetheless sought to test whether the pBEE99 plasmid specifies bacteriocin production. We

compared strain OG1RF to a previously generated OG1RF transconjugant, designated IG9, possessing the pBEE99 plasmid (Tendolkar et al., 2006) for the ability to inhibit the growth of *E. faecalis* strain FA2-2, which was previously shown to be sensitive to Bac41 (Tomita et al., 2008). However, neither the parental strain OG1RF nor the transconjugant IG9 [OG1RF (pBEE99)] produced any zones of inhibition on a lawn of FA2-2 (data not shown), suggesting that neither strain produces bacteriocin activity against strain FA2-2 under the conditions tested. BacL1 from pYI14 shows a high degree of identity and similarity to lytic enzymes from gram-positive bacteria, such as muramidase and lysozyme (Lopez and Garcia, 2004), and possesses three characteristic domains: a signal sequence, catalytic, and ligand binding domain consisting of three proline-rich SH3 repeat regions (Tomita et al., 2008). Alignment of the pBEE99 BacL1 with the pYI14 BacL1 reveals that the pBEE99 version possesses all three domains; however, a frameshift mutation following amino acid 505 results in a truncated polypeptide missing the C-terminal 90 amino acids. This mutation deletes the third SH3 repeat region from the ligand binding domain and thus might alter or abrogate receptor binding. This might explain the lack of observed bacteriocin activity against strain FA2-2. Work is currently in progress to address this possibility, as well as other possible explanations for the observed lack of activity, such as whether the pBEE99 bacteriocin genes are expressed, that FA2-2 possesses a chromosomally-encoded immunity determinant, or whether the pBEE99 Bac41 possesses altered target specificity.

3.4 pBEE99 enhances ultraviolet radiation resistance of OG1RF and E99

The presence of open reading frames with a high degree of identity and similarity to genes involved in UV resistance suggests that pBEE99 might enhance the ability of *E. faecalis* to survive UV radiation and therefore enhance the fitness of *E. faecalis* in the environment. In order to determine whether the pBEE99 plasmid confers an increased resistance to UV, we initially compared the UV sensitivity of strain OG1RF to strain IG9. We performed a UV irradiation killing assay essentially as described by Scott *et al* (Scott et al., 2008). As shown in Fig. 2, strain IG9 was approximately an order of magnitude more resistant to ultraviolet radiation than OG1RF. In order to quantify the difference between these two strains, an aliquot was taken at each time point and CFU/ml determined by track dilution. As shown in Fig. 3, OG1RF demonstrated a more precipitous decline in viability over time relative to IG9, and by 60 seconds was statistically significantly lower ($P = 0.048$). These results support a role for the pBEE99 plasmid in enhancing the resistance of strain OG1RF to ultraviolet radiation. To further show that pBEE99 imparts an enhanced UV resistance phenotype, we cured strain E99 of pBEE99 by repeated passaging of E99 at 45°C. The resultant strain, designated KDS01, demonstrated significantly greater sensitivity to UV light than the parental strain E99 after 30 seconds of UV exposure (Fig. 3; $P = 0.038$). At 120 seconds, the CFU per milliliter for all 4 strains was below the threshold of detection ($<10^3$ CFU/ml), and therefore this time point is not shown on the graph. While these results ascribe a role for pBEE99 as a whole in enhancing UV resistance of the host, the role(s) of specific gene(s) in this process will be the subject of future studies.

Recently, we reported that the pBEE99 plasmid harbors the novel *bee* gene cluster that encodes the ability to enhance biofilm formation by *E. faecalis* (Tendolkar et al., 2006) and thus pBEE99 represents a conjugative plasmid capable of disseminating a trait associated with the pathogenesis of this species (Mohamed et al., 2004). This locus, consisting of a cluster of 5 genes encoding three putative surface proteins and two sortases, respectively, bears a striking similarity to the gene clusters encoding pili in a number of pathogenic Gram-positive organisms (Tendolkar et al., 2006; Lauer et al., 2005; Ton-That and Schneewind, 2003). BLAST analysis of this gene cluster suggested that the three structural genes might have been acquired from *Leuconostoc mesenteroides* (Tendolkar et al., 2006). Recent genetic, mutational, and complementation analyses by our laboratory have confirmed that the *bee1*, *bee2*, and *bee3*

genes encode surface-localized proteins that are polymerized into a pilus structure (manuscript in revision).

4. Conclusions

In summary, we sequenced and characterized in *E. faecalis*, a novel 80 kb conjugative plasmid (pBEE99) that specifies an enhanced ability to form biofilms and resistance to ultraviolet radiation. This plasmid bears global similarity to pCF10; however, pBEE99 differs from pCF10 in that it is missing the Tn925 element and possesses a gene cluster that might have been acquired from *L. mesenteroides* (Tendolkar et al., 2006), as well as an operon potentially specifying a Bac41-like bacteriocin. The discovery of a conjugative plasmid that enhances the ability of *E. faecalis* to form a biofilm and to resist ultraviolet radiation represents another mechanism for enterococcal strains to disseminate the genes necessary to persist in the hospital setting or in susceptible patients.

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References

- Clewell, DB.; Dunny, GM. Conjugation and Genetic Exchange in Enterococci. In: Gilmore, MS.; Clewell, DB.; Courvalin, P.; Dunny, GM.; Murray, BE.; Rice, LB., editors. *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance*. Washington, D.C.: ASM Press; 2002. p. 265-300.
- Clewell DB, Yagi Y, Dunny GM, Schultz SK. Characterization of three plasmid deoxyribonucleic acid molecules in a strain of *Streptococcus faecalis*: identification of a plasmid determining erythromycin resistance. *J Bacteriol* 1974;117:283–289. [PubMed: 4202995]
- Coburn PS, Baghdayan AS, Dolan GT, Shankar N. Horizontal transfer of virulence genes encoded on the *Enterococcus faecalis* pathogenicity island. *Mol Microbiol* 2007;63:530–544. [PubMed: 17163979]
- De Boever EH, Clewell DB, Fraser CM. *Enterococcus faecalis* conjugative plasmid pAM373: complete nucleotide sequence and genetic analyses of sex pheromone response. *Mol Microbiol* 2000;37:1327–1341. [PubMed: 10998166]
- Dunny G, Funk C, Adsit J. Direct stimulation of the transfer of antibiotic resistance by sex pheromones in *Streptococcus faecalis*. *Plasmid* 1981;6:270–278. [PubMed: 6796985]
- Dunny GM. The peptide pheromone-inducible conjugation system of *Enterococcus faecalis* plasmid pCF10: cell-cell signalling, gene transfer, complexity and evolution. *Philos Trans R Soc Lond B Biol Sci* 2007;362:1185–1193. [PubMed: 17360276]
- Dunny GM, Brown BL, Clewell DB. Induced cell aggregation and mating in *Streptococcus faecalis*: evidence for a bacterial sex pheromone. *Proc Natl Acad Sci U S A* 1978;75:3479–3483. [PubMed: 98769]
- Francia MV, Fujimoto S, Tille P, Weaver KE, Clewell DB. Replication of *Enterococcus faecalis* pheromone-responding plasmid pAD1: location of the minimal replicon and *oriV* site and RepA involvement in initiation of replication. *J Bacteriol* 2004;186:5003–5016. [PubMed: 15262938]
- Francia MV, Haas W, Wirth R, Samberger E, Muscholl-Silberhorn A, Gilmore MS, Ike Y, Weaver KE, An FY, Clewell DB. Completion of the nucleotide sequence of the *Enterococcus faecalis* conjugative virulence plasmid pAD1 and identification of a second transfer origin. *Plasmid* 2001;46:117–127. [PubMed: 11591137]
- Gilmore, MS.; Coburn, PS.; Nallapareddy, SR.; Murray, BE. Enterococcal virulence. In: Gilmore, MS.; Clewell, DB.; Courvalin, P.; Dunny, GM.; Murray, BE.; Rice, LB., editors. *The Enterococci*:

Pathogenesis, Molecular Biology, and Antibiotic Resistance. Washington, D.C.: ASM Press; 2002. p. 301-354.

- Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemiol* 2008;29:996–1011. [PubMed: 18947320]
- Hirt H, Manias DA, Bryan EM, Klein JR, Marklund JK, Staddon JH, Paustian ML, Kapur V, Dunny GM. Characterization of the pheromone response of the *Enterococcus faecalis* conjugative plasmid pCF10: complete sequence and comparative analysis of the transcriptional and phenotypic responses of pCF10-containing cells to pheromone induction. *J Bacteriol* 2005;187:1044–1054. [PubMed: 15659682]
- Jett BD, Hatter KL, Huycke MM, Gilmore MS. Simplified agar plate method for quantifying viable bacteria. *Biotechniques* 1997;23:648–650. [PubMed: 9343684]
- Koch WH, Henrikson E, Eisenstadt E, Cebula TA. *Salmonella typhimurium* LT7 and LT2 strains carrying the *imp* operon on *colla*. *J Bacteriol* 1995;177:1903–1905. [PubMed: 7896721]
- Lauer P, Rinaudo CD, Soriani M, Margarit I, Maione D, Rosini R, Taddei AR, Mora M, Rappuoli R, Grandi G, Telford JL. Genome analysis reveals pili in Group B *Streptococcus*. *Science* 2005;309:105. [PubMed: 15994549]
- Lopez R, Garcia E. Recent trends on the molecular biology of pneumococcal capsules, lytic enzymes, and bacteriophage. *FEMS Microbiol Rev* 2004;28:553–580. [PubMed: 15539074]
- Mohamed JA, Huang W, Nallapareddy SR, Teng F, Murray BE. Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by *Enterococcus faecalis*. *Infect Immun* 2004;72:3658–3663. [PubMed: 15155680]
- Ozawa Y, Tanimoto K, Fujimoto S, Tomita H, Ike Y. Cloning and genetic analysis of the UV resistance determinant (*uvr*) encoded on the *Enterococcus faecalis* pheromone-responsive conjugative plasmid pAD1. *J Bacteriol* 1997;179:7468–7475. [PubMed: 9393713]
- Paulsen IT, Banerjee L, Myers GS, Nelson KE, Seshadri R, Read TD, Fouts DE, Eisen JA, Gill SR, Heidelberg JF, Tettelin H, Dodson RJ, Umayam L, Brinkac L, Beanan M, Daugherty S, DeBoy RT, Durkin S, Kolonay J, Madupu R, Nelson W, Vamathevan J, Tron B, Upton J, Hansen T, Shetty J, Khouri H, Utterback T, Radune D, Ketchum KA, Dougherty BA, Frase CM. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. *Science* 2003;299:2071–2074. [PubMed: 12663927]
- Sarov-Blat L, Livneh Z. The mutagenesis protein MucB interacts with single strand DNA binding protein and induces a major conformational change in its complex with single-stranded DNA. *J Biol Chem* 1998;273:5520–5527. [PubMed: 9488676]
- Schwarz FV, Perreten V, Teuber M. Sequence of the 50-kb conjugative multiresistance plasmid pRE25 from *Enterococcus faecalis* RE25. *Plasmid* 2001;46:170–187. [PubMed: 11735367]
- Scott J, Thompson-Mayberry P, Lahmamsi S, King CJ, McShan WM. Phage-associated mutator phenotype in group A *Streptococcus*. *J Bacteriol* 2008;190:6290–6301. [PubMed: 18676670]
- Smith BT, Walker GC. Mutagenesis and more: *umuDC* and the *Escherichia coli* SOS response. *Genetics* 1998;148:1599–1610. [PubMed: 9560379]
- Stothard P, Wishart DS. Circular genome visualization and exploration using CGView. *Bioinformatics* 2005;21:537–539. [PubMed: 15479716]
- Tendolkar PM, Baghdayan AS, Shankar N. Putative surface proteins encoded within a novel transferable locus confer a high-biofilm phenotype to *Enterococcus faecalis*. *J Bacteriol* 2006;188:2063–2072. [PubMed: 16513736]
- Tomita H, Kamei E, Ike Y. Cloning and genetic analyses of the bacteriocin 41 determinant encoded on the *Enterococcus faecalis* pheromone-responsive conjugative plasmid pYI14: a novel bacteriocin complemented by two extracellular components (lysin and activator). *J Bacteriol* 2008;190:2075–2085. [PubMed: 18203826]
- Ton-That H, Schneewind O. Assembly of pili on the surface of *Corynebacterium diphtheriae*. *Mol Microbiol* 2003;50:1429–1438. [PubMed: 14622427]
- Wang Z. Translesion synthesis by the UmuC family of DNA polymerases. *Mutat Res* 2001;486:59–70. [PubMed: 11425512]

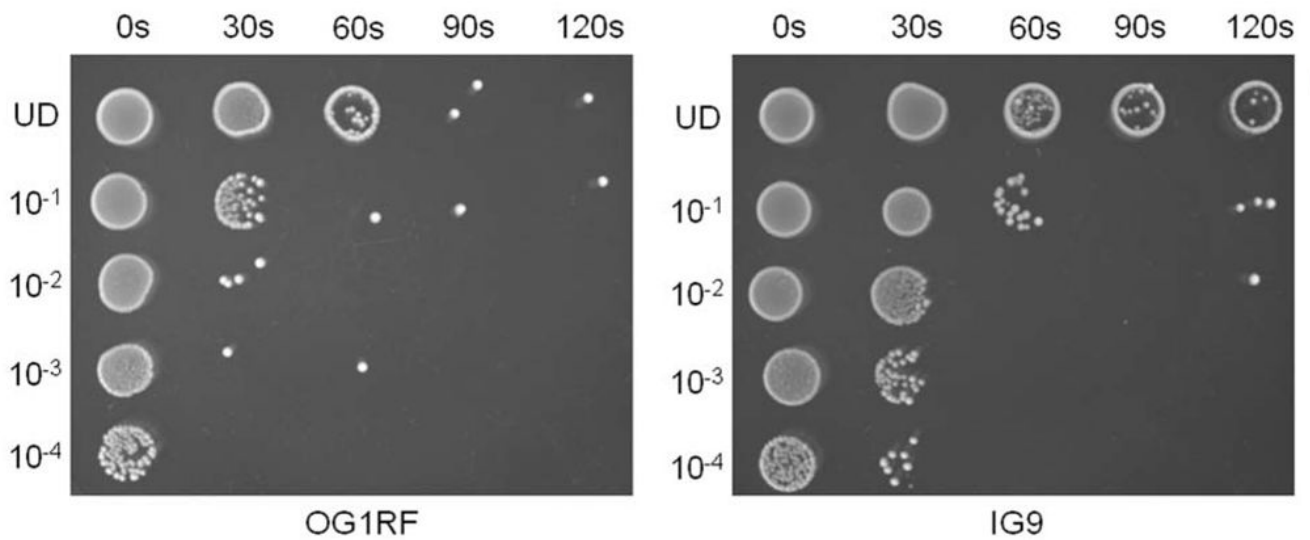


Fig. 2. Comparison of the UV resistance of strain OG1RF to that of strain IG9 (an OG1RF transconjugant possessing pBEE99). IG9 demonstrated approximately an order of magnitude greater UV resistance than the parental strain OG1RF. Shown is a representative of two independent experiments.

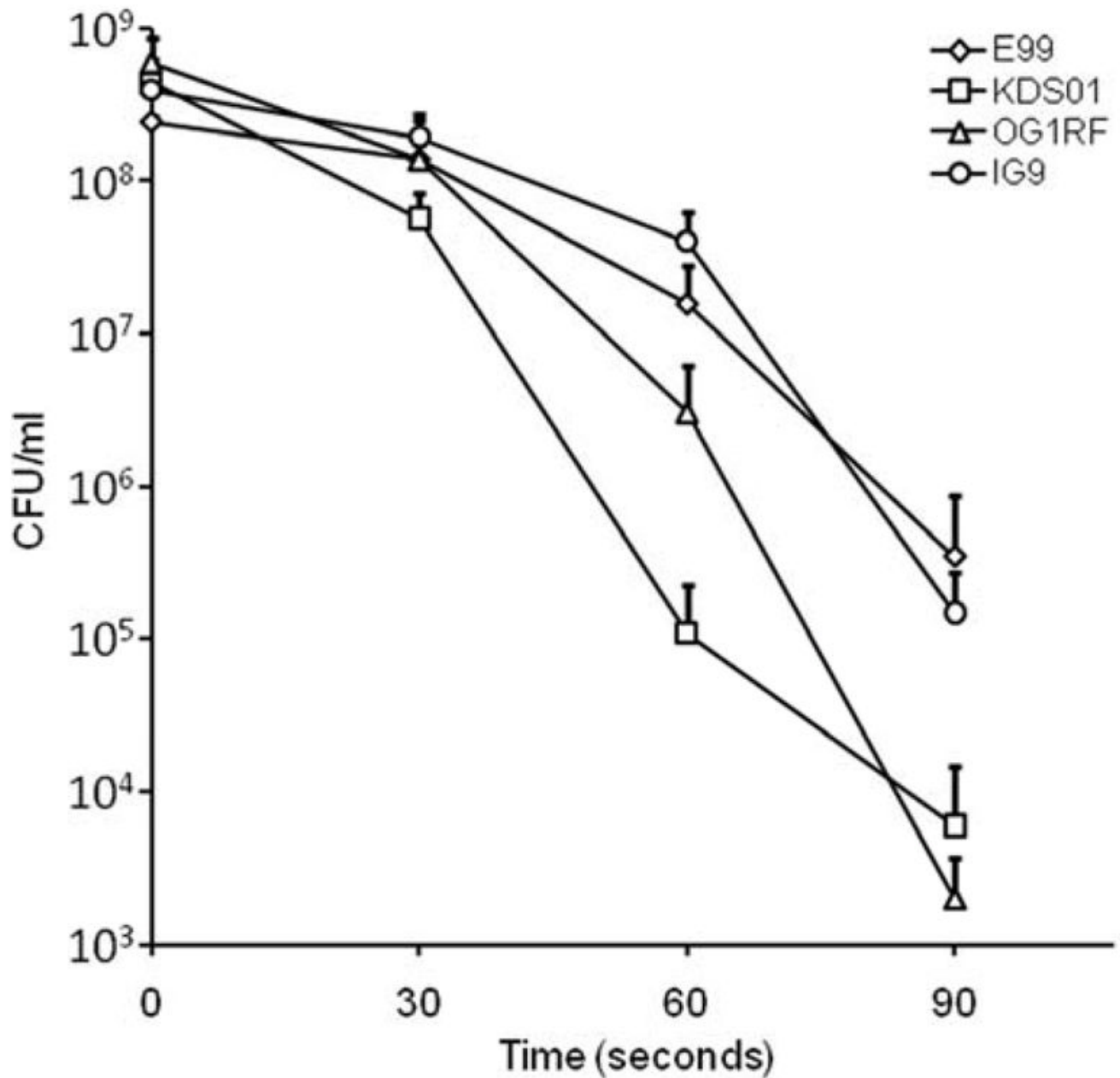


Fig. 3. Plasmid pBEE99 specifies enhanced resistance to ultraviolet radiation. Strain E99 demonstrated significantly greater resistance than the pBEE99-cured strain KDS01 ($P = 0.038$), and strain IG9 was significantly more resistant to UV than was the parental strain OG1RF ($P = 0.048$). Experiments were conducted in triplicate and the error bars indicate the mean \pm standard deviation. Levels of significance were determined using a Student's *t*-test.

Table 1

pBEE99 open reading frames. Asterisks indicate possible pseudogenes.

#	Start	End	Length	Strand	Match	Affiliation	E_value	%Identity
1	246	1259	1012	+	gb AAA25553.1 PrgW	<i>E. faecalis</i>	3.00E-164	59%
2	1390	2058	667	+	gi 22536356 ref NP_687207.1 Protease, putative	<i>S. agalactiae</i>	1.00E-20	38%
3	2100	3728	1627	+	gi 58616130 ref YP_195766.1 PrgZ	<i>E. faecalis</i>	2.00E-185	59%
4	3731	4714	982	-	ref YP_003329053.1 PrgX	<i>E. faecalis</i>	5.00E-63	99%
5	5534	5914	379	+	gi 29377901 ref NP_817027.1 PrgR	<i>E. faecalis</i> V 583	3.00E-66	96%
6	5965	6186	220	+	pii C56272 PrgS	<i>E. faecalis</i> plasmid pCF10	5.00E-35	97%
7	6539	6829	289	+	gi 69246067 ref ZP_00603794.1 Transposase IS3/IS911	<i>E. faecium</i> DO	3.00E-47	100%
8	6865	7701	835	+	gi 69246262 ref ZP_00603875.1 Innegrase, catalytic region	<i>E. faecium</i> DO	5.00E-140	89%
9	8127	12044	3916	+	gi 29377905 ref NP_817031.1 PrgB	<i>E. faecalis</i> V 583	0	89%
10*	12142	12470	311	+	gb AAW51309.1 PrgU	<i>E. faecalis</i>	9.00E-15	100%
11	12498	13355	856	+	gi 29377906 ref NP_817032.1 PrgC	<i>E. faecalis</i> V 583	3.00E-86	68%
12	13397	14296	898	+	gi 58616088 ref YP_195777.1 PrgD	<i>E. faecalis</i>	5.00E-120	97%
13	14316	14750	433	+	gi 29377908 ref NP_817034.1 hypothetical protein EF_B0014	<i>E. faecalis</i> V 583	6.00E-67	88%
14	14797	15024	226	+	ref YP_195779.1 PrgF	<i>E. faecalis</i>	4.00E-32	100%
15	15038	15334	295	+	gi 29377910 ref NP_817036.1 hypothetical protein EF_B0016	<i>E. faecalis</i> V 583	2.00E-48	96%
16	15349	16149	799	+	gi 58616084 ref YP_195781.1 PrgH	<i>E. faecalis</i>	4.00E-127	87%
17	16151	16504	352	+	gi 58616092 ref YP_195782.1 PrgI	<i>E. faecalis</i>	1.00E-47	82%
18	16458	18848	2389	+	gi 58616093 ref YP_195783.1 PrgJ	<i>E. faecalis</i>	0	97%
19	18860	21373	2512	+	gi 58616094 ref YP_195784.1 PrgK	<i>E. faecalis</i>	0	89%
20	21509	21838	328	-	No Hits Found			
21	22097	24031	1933	+	gi 21693271 gb AAM75218.1 Group II intron reverse transcriptase	<i>E. faecalis</i>	2.00E-250	66%
22	24304	24930	625	+	gi 58616095 ref YP_195785.1 PrgL	<i>E. faecalis</i>	3.00E-84	78%
23	24908	25159	250	+	ref YP_195786.1 PrgM	<i>E. faecalis</i>	6.00E-35	97%
24	25146	25754	607	+	gi 58616097 ref YP_195787.1 PrgA	<i>E. faecalis</i>	3.00E-93	87%
25	25867	26622	754	+	gi 90962837 ref YP_536752.1 hypothetical protein LSL_2159	<i>L. salivarius</i>	6.00E-11	39%
26	26639	27097	457	+	gi 58616114 ref YP_195805.1 PrgS	<i>E. faecalis</i>	9.00E-50	67%
27	27143	27298	154	+	No hits found			

#	Start	End	Length	Strand	Match	Affiliation	E_value	%Identity
28	27364	27852	487	+	113706806 ref YP_195788.2 PcfB	<i>E. faecalis</i>	4.00E-85	99%
29	27852	29681	1828	+	gi 58616099 ref YP_195789.1 PcfC	<i>E. faecalis</i>	0	91%
30	29732	31891	2158	+	gi 58616100 ref YP_195790.1 PcfD	<i>E. faecalis</i>	0	96%
31	31924	32196	271	+	ref YP_195791.1 PcfE	<i>E. faecalis</i>	2.00E-43	98%
32	32442	32798	355	+	gi 58616102 ref YP_195792.1 PcfF	<i>E. faecalis</i>	4.00E-56	100%
33	32799	34484	1684	+	gi 29377923 ref NP_817049.1 PcfG	<i>E. faecalis</i> V583	2.00E-248	76%
34	34517	34867	349	+	gi 58616104 ref YP_195794.1 PcfH	<i>E. faecalis</i>	4.00E-49	77%
35	35387	36727	1339	-	gi 58616106 ref YP_195796.1 PcfI	<i>E. faecalis</i>	7.00E-239	90%
36	36727	37500	772	-	gi 29377927 ref NP_817053.1 hypothetical protein EF_B0034	<i>E. faecalis</i> V583	5.00E-103	71%
37	37605	38195	589	-	gi 29377928 ref NP_817054.1 hypothetical protein EF_B0035	<i>E. faecalis</i> V583	3.00E-108	100%
38	38219	38410	190	-	gb AAW51348.1 PcfM	<i>E. faecalis</i>	8.00E-28	98%
39	38395	38580	184	-	ref YP_195800.1 PcfN	<i>E. faecalis</i>	3.00E-27	100%
40	38876	39076	199	+	ref YP_195802.1 PcfP	<i>E. faecalis</i>	5.00E-31	100%
41	39178	39408	229	-	ref YP_195803.1 PcfQ	<i>E. faecalis</i>	2.00E-37	100%
42	39541	39957	415	+	gi 58616113 ref YP_195804.1 PcfR	<i>E. faecalis</i>	6.00E-58	84%
43*	40023	40475	449	+	gi 29377935 ref NP_817061.1 PcfS	<i>E. faecalis</i> V583	2.00E-64	100%
44	40489	40641	151	+	dbj BAH02365.1 hypothetical protein pMG2200_57	<i>E. faecalis</i>	2.00E-20	100%
45	40653	41243	589	+	ref YP_195806.1 PcfT	<i>E. faecalis</i>	9.00E-84	82%
46	41249	41569	319	+	gi 58616116 ref YP_195807.1 PcfU	<i>E. faecalis</i>	8.00E-53	94%
47	41596	41664	67	+	ref NP_815781.1 hypothetical protein EF2118	<i>E. faecalis</i> V583	2.00E-09	86%
48	41677	41922	244	+	dbj BAH02368.1 hypothetical protein pMG2200_60	<i>E. faecalis</i>	5.00E-40	100%
49	41976	42158	181	+	dbj BAG12397.1 hypothetical protein pMG2200_61	<i>E. faecalis</i>	1.00E-24	100%
50	42353	43069	715	+	gi 169635863 dbj BAG12398.1 hypothetical protein pMG2200_62	<i>E. faecalis</i>	3.00E-104	82%
51*	43207	44994	1784	+	gi 169635864 dbj BAG12399.1 BacL1	<i>E. faecalis</i>	7.00E-289	95%
52	45180	45815	634	+	gi 169635865 dbj BAG12400.1 BacL2	<i>E. faecalis</i>	2.00E-105	100%
53	45838	46269	430	+	gi 169635866 dbj BAG12401.1 putative hollin	<i>E. faecalis</i>	7.00E-49	79%
54	46272	46799	526	+	gi 169635867 dbj BAG12402.1 hypothetical protein pMG2200_66	<i>E. faecalis</i>	7.00E-84	90%
55	46842	49022	2179	+	gi 169635868 dbj BAG12403.1 BacA	<i>E. faecalis</i>	0	100%
56	49130	49672	541	+	gi 169635869 dbj BAG12404.1 BacI	<i>E. faecalis</i>	8.00E-70	77%
57	49739	50314	574	+	gi 169635870 dbj BAG12405.1 hypothetical protein	<i>E. faecalis</i>	2.00E-88	87%

#	Start	End	Length	Strand	Match	Affiliation	E_value	%Identity
58	50457	50789	331	+	gi 29377949 ref NP_817075.1 hypothetical protein EF_B0057	<i>E. faecalis</i> V583	5.00E-57	100%
59	50953	51555	601	+	gi 29377950 ref NP_817076.1 site-specific recombinase	<i>E. faecalis</i> V583	3.00E-84	83%
60	51622	52416	793	+	No Hits Found			
61	52956	53126	169	-	No Hits Found			
62	53406	54218	811	+	No Hits Found			
63	54379	55695	1315	+	gi 29377952 ref NP_817078.1 UvrA	<i>E. faecalis</i> V583	3.00E-237	97%
64	55700	56206	505	+	gi 29377891 ref NP_817018.1 hypothetical protein EF_C0016	<i>E. faecalis</i> V583	2.00E-85	90%
65	56154	56384	229	+	ref NP_817080.1 UvrC	<i>E. faecalis</i>	1.00E-17	68%
66	56462	56875	412	-	gi 29377892 ref NP_817019.1 hypothetical protein EF_C0017	<i>E. faecalis</i> V583	3.00E-59	85%
67	56882	57121	238	-	ref NP_817020.1 RepB	<i>E. faecalis</i> V583	9.00E-36	100%
68	57159	57845	685	-	gi 29377809 ref NP_816937.1 Transposase IS1216	<i>E. faecalis</i> V583	3.00E-126	97%
69	58530	61781	3250	+	gi 72388797 gb AAZ68037.1 Bee1	<i>E. faecalis</i>	0	100%
70	61783	62514	730	+	gi 72388798 gb AAZ68038.1 Bee2	<i>E. faecalis</i>	5.00E-113	100%
71	62579	64066	1486	+	gi 72388799 gb AAZ68039.1 Bee3	<i>E. faecalis</i>	2.00E-283	100%
72	64152	65348	1195	+	gi 72388800 gb AAZ68040.1 Str1	<i>E. faecalis</i>	6.00E-229	100%
73	65345	66466	1120	+	gi 72388801 gb AAZ68041.1 Str2	<i>E. faecalis</i>	2.00E-188	100%
74	67237	68196	958	+	gb AAA68982.1 Transposase IS6770	Insertion sequence IS6770	2.00E-185	99%
75	68193	68423	229	+	ref ZP_00874586.1 PadR	<i>S. sitis</i>	1.00E-13	60%
76	68765	69697	931	-	gi 167746666 ref ZP_02418793.1 hypothetical protein ANACAC_01377	<i>A. cacciae</i> DSM 14662	8.00E-102	55%
77	69793	70422	628	+	gi 124485630 ref YP_001030246.1 hypothetical protein Mlab_0808	<i>M. labreanum</i> Z	7.00E-74	64%
78	70604	71197	592	+	gi 57854758 ref YP_187535.1 Site-specific recombinase	<i>S. epidermidis</i> RP62A	1.00E-52	65%
79	71169	72206	1036	+	gi 33416281 ref NP_878027.1 Transposase Tn552	<i>S. aureus</i>	2.00E-114	59%
80	72184	72612	427	+	gi 67077955 ref YP_245575.1 putative transposase	<i>B. cereus</i> E33L	2.00E-31	54%
81	72605	73423	817	+	gi 49483988 ref YP_041212.1 ATP-binding protein	<i>S. aureus</i>	9.00E-94	62%
82	73566	74162	595	+	gb AAW51342.1 PcfY	<i>E. faecalis</i>	5.00E-93	87%
83	74152	74466	313	+	gi 58616121 ref YP_195812.1 PcfZ	<i>E. faecalis</i>	8.00E-45	94%
84	74460	74681	220	+	ref YP_195813.1 UvrC	<i>E. faecalis</i>	3.00E-35	95%
85	74745	74954	208	+	gb AAO83067.1 hypothetical protein EF_A0075	<i>E. faecalis</i> V583	1.00E-29	94%
86	75424	75546	121	+	No Hits Found			
87	75601	76038	436	-	No Hits Found			

#	Start	End	Length	Strand	Match	Affiliation	E_value	%Identity
88	76130	76378	247	+	ref NP_817016.1 hypothetical protein EF_C0014	<i>E. faecalis</i> V583	9.00E-36	95%
89	76497	77813	1315	+	gi 29377952 ref NP_817078.1 UvrA	<i>E. faecalis</i> V583	1.00E-206	93%
90	77817	78332	514	+	gi 158021643 gb ABW08102.1 hypothetical protein EF_C0016	<i>E. faecalis</i>	2.00E-88	91%
91	78280	78447	166	+	gb ABW08103.1 hypothetical protein ABW08103	<i>E. faecalis</i>	2.00E-06	65%
92	78559	78645	85	+	gb ABW08104.1 hypothetical protein ABW08104	<i>E. faecalis</i>	3.00E-18	100%
93	78903	79217	313	+	gi 29377955 ref NP_817081.1 PrgN	<i>E. faecalis</i> V583	1.00E-38	80%
94	79455	80237	781	+	gi 29377956 ref NP_817082.1 ParA	<i>E. faecalis</i> V583	4.00E-142	100%
95	80230	80586	355	+	gi 29377957 ref NP_817083.1 hypothetical protein EF_B0065	<i>E. faecalis</i> V583	8.00E-60	97%