Supporting Information for:

Enterococcus faecalis pCF10-encoded Surface Proteins PrgA, PrgB (Aggregation Substance), and PrgC Contribute to Plasmid Transfer, Biofilm Formation, and Virulence

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Supplementary Table. Bacterial strains, plasmids and oligonucleotides used in this study.

Strains or plasmids	Relevant features	Source or Reference
Strains		
E. coli		
DH5α	F-φ80d*lacZ* ΔM15 Δ(*lacZYA-argF*)U169 *deoR recA1 endA1hsdR17(rK-mK+)phoA supE44λ- thi-1gyrA96 relA1*	Gibco-BRL
BL21 (DE3)	F- *ompT hsdSB* (*rB-mB-) gal dcm* (DE3)	Novagen
EC1000	*E. coli* cloning host, provides RepA in *trans*	(Leenhouts *et al.*, 1996)
E. faecalis		
OG1RF	Rif^r Fus^r	(Dunny *et al.*, 1978)
OG1ES	Ery^r Str^r	(Staddon *et al.,* 2006)
OG1SSp	Str^r Spc^r	(Dunny *et al.,* 1978)
TX5536	Rif^r Fus^r, OG1RF deleted of *srtA*	(Kemp *et al.*, 2007)
TX5470	Rif^r Fus^r, OG1RF deleted of *bps*	(Kemp *et al.,* 2007)
TX5537	Rif^r Fus^r, OG1RF deleted of *srtA* and *bps*	(Kemp *et al.*, 2007)
TX5608	Rif^r Fus^r, OG1RF deleted of *ebpABC*	(Nallapareddy *et al.*, 2006)
TX5266	Rif^r Fus^r, OG1RF deleted of *fsrB*	(Qin *et al.,* 2001)
TX5467	Rif^r Fus^r, Cm^r, OG1RF Δ*ace*::*cat*	(Singh *et al.,* 2010)
TX5264	Rif^r Fus^r, OG1RF deleted of *gel*; Gel^- Spr^+	(Sifri *et al.,* 2002)
TX5243	Rif^r Fus^r, Kan^r, OG1RF *sprE* insertional mutant; Gel^+ Spr^-	(Qin *et al.*, 2000)
TX5128	Rif^r Fus^r, Kan^r, OG1RF *gelE* transposon mini-γδ insertion mutant; Gel^- Spr^-	(Singh *et al.,* 1998)
Plasmids		
pCJK47	Spc^r, carries $oriT_{pCF10,}$ *lacZ,* and P-pheS* cassette	(Kristich *et al.,* 2007)
pDLP278p23	Spc^r, pDL278 with *L. lactis* constitutive promoter P_{23}	(Chen *et al.,* 2007)
pCIE	Cm^r, cCF10 inducible P_Q expression vector	Dunny Lab
pET28b(+)	Kan^r, expression vector for His tagging	Novagen
pCF10	Tet^r, pheromone inducible conjugative plasmid	(Dunny *et al.*, 1981)
pCF10Δ*oriT*	pCF10 with a 54-bp deletion within *oriT*	(Staddon *et al.,* 2006)
pCF10Δ*prgA*	pCF10 deleted of *prgA*	This study
pCF10Δ*oriT*Δ*prgA*	pCF10 deleted of *oriT* and *prgA*	This study

pCF10-8, designated here as pCF10Δ*prgB*	Tet^r, pCF10 deleted of *prgB*	(Chuang-Smith *et al.*, 2010)
pCF10Δ*prgC*	Tet^r, pCF10 deleted of *prgC*	This study
pCF10Δ*oriT*Δ*prgC*	Tet^r, pCF10 deleted of *oriT* and *prgC*	This study
pCF10Δ*prgA-C*	Tet^r, pCF10 deleted of *prgA, prgB, prgU and prgC*	This study
pCF10Δ*prgK*	Tet^r, pCF10 deleted of *prgK*	(Laverde Gomez *et al.*, 2014)
pCF10Δ*prgJ*	Tet^r, pCF10 deleted of *prgJ*	(Li *et al.*, 2012)
pCF10Δ*pcfC*	Tet^r, pCF10 deleted of *pcfC*	(Chen *et al.,* 2008)
pMCM1	Spc^r, pCJK47 with *prg*A flanking regions for construction of pCF10 Δ*prgA*	This study
pMCM2	Spc^r, pCJK47 with *prg*C flanking regions for construction of pCF10Δ*prgC*	This study
pMB1	Spc^r, pCJK47 with *prgA* and *prg*C flanking regions for construction of pCF10 Δ*prg*ABUC	This study
pMB2	Spc^r, pDL278p23 expressing P_{23}-*prgA*	This study
pMB3	Spc^r, pDL278p23 expressing P_{23}-*prgB*	This study
pMB4	Spc^r, pDL278p23 expressing P_{23}-*prgC*	This study
pMB5	Cm^r, cCF10 inducible pCIE expressing *prgA*	This study
pMB6	Cm^r, cCF10 inducible pCIE expressing *prgB*	This study
pMB7	Cm^r, cCF10 inducible pCIE expressing *prgC*	This study
pINY1801	Cm^r, fragment of pCF10 cloned in shuttle vector pWM401 confers constitute expression of PrgA, PrgB, and PrgC	(Christie *et al.,* 1988)
Primer name	**Sequence (5' to 3')**	**Used for**
F- prgA up-XbaI	gctctagaatttcgtgcgatgatcttttg	pMCM1
R- prgA up-XmaI	aattattaaccccgggtttattttcctcacttccg	pMCM1
F- prgA down-XmaI	gaaaataaacccggggttaataattgtttgatatagc	pMCM1
R- prgA down-NcoI	catgccatggcctccgctaagttgcttgtt	pMCM1
F- prgC up-XbaI	gctctagagcaccagtagagccatta	pMCM2
R- prgC up-XmaI	ctgattaagctcccggggtaatttcctccaaata	pMCM2
F- prgC down-XmaI	gaggaaattacccccgggagcttaatcagaaagagt	pMCM2
R- prgC down-NcoI	catgccatggaaccaaatgacgcacctg	pMCM2
F-RBS prgA-BamHI	cgcggatccttaaaggaggtattatatgaaaaagattgcaagt	pMB2, pMB5
R-prgA- SphI	acatgcatgcttaactatttttttttacg	pMB2, pMB5

F-RBS prgB- BamHI	cgcggatccttaaaggaggtattatatgaatcaacagactgaag	pMB3, pMB6
R-prgB-SphI	acatgcatgcttattttgtttcttttctacg	pMB3, pMB6
F-RBS prgC- SalI	acgcgtcgacttaaaggaggtattatatgaaaaaaattattttatcaa	pMB4, pMB7
R-prgC-SphI	acatgcatgcttaagctttttttcttattc	pMB4, pMB7
gyrB forward	caagccaaaacaggtcgcc	(Bourgogne *et al.*, 2007)
gyrB reverse	accaacaccgtgcaagcc	(Bourgogne *et al*., 2007)
QL forward	catgtatatgttccccgctttt	(Chatterjee *et al.*, 2013)
QL reverse	cggctcttacgagtagttcca	(Chatterjee *et al*., 2013)
prgD-F RT	cgaagagaataggccgttca	This work
prgD-R RT	gacgcacctgtaaaaacacc	This work
prgJ-F RT	accccaatgactggcttagag	This work
prjJ-R RT	tagacgttagccctgatacgg	This work
pcfC-F RT	gcgcttattggaggagacgag	(Chatterjee *et al.,* 2013)
pcfC-R RT	cggcgccacgtataccac	(Chatterjee *et al.,* 2013)
pcfG-F RT	ggcaacgccgttttagcac	(Chatterjee *et al.,* 2013)
pcfG-R RT	ccatcaccgtgttgtaccc	(Chatterjee *et al.,* 2013)

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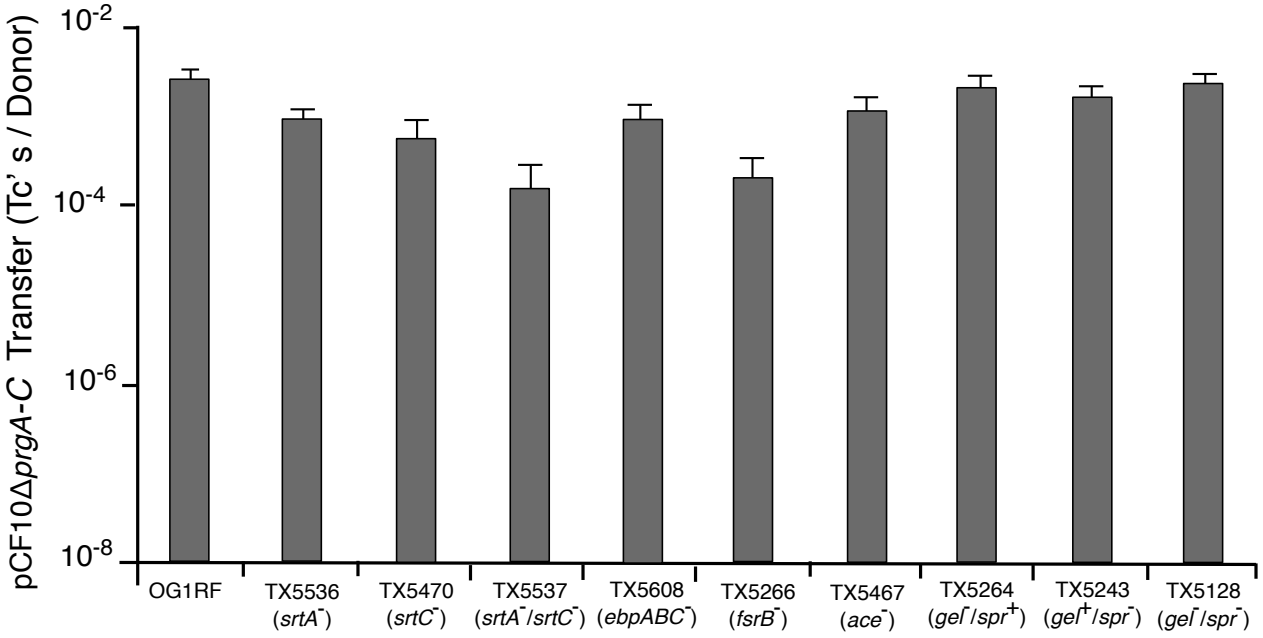

Fig. S1. Contributions of chromosomally-encoded surface proteins to transfer of pCF10Δ*prgA-C*. Transfer frequencies of pCF10Δ*prgA-C* in OG1RF mutant backgrounds in a 2 h liquid mating. Transfer frequencies are presented as the number of transconjugants per donor cell (Tc's/Donor). Experiments were repeated at least 3 times and the histogram depicts average values with standard deviations.

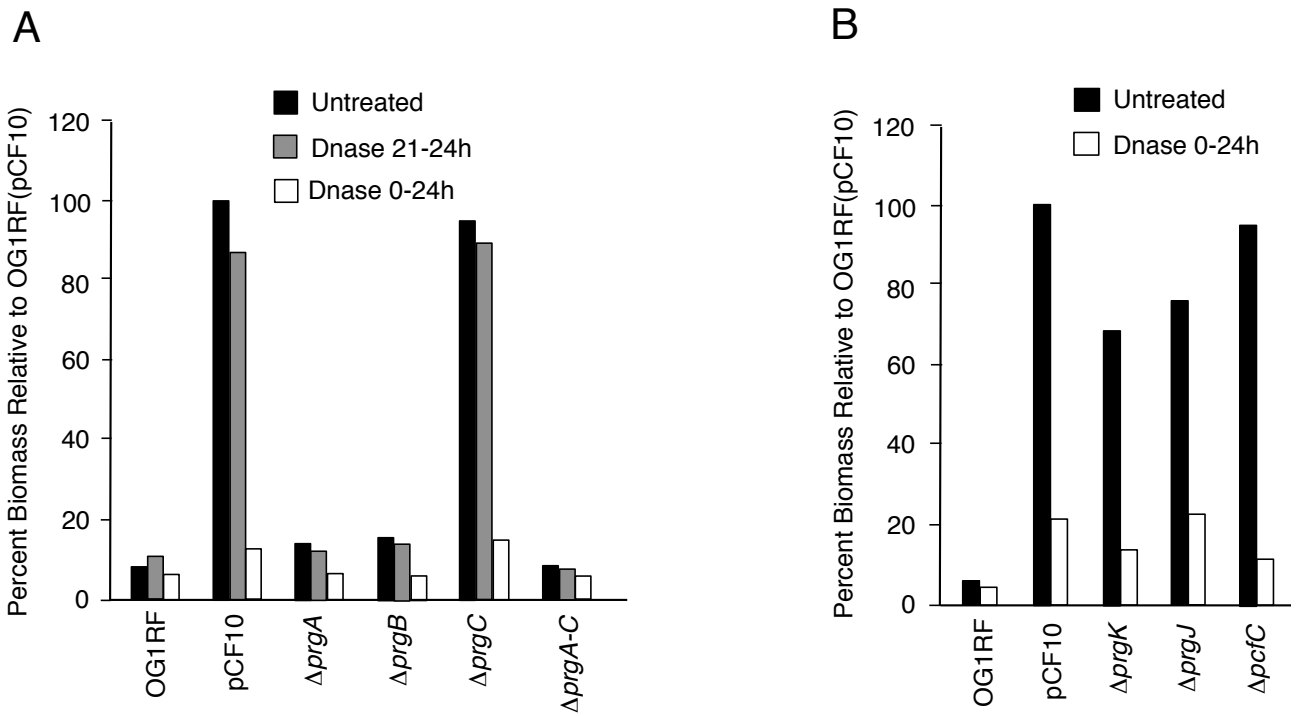

Fig. S2. Effects of DNase treatment on biofilm development by *prgA*, *prgB*, *prgC* and T4SS gene deletion strains. Biofilm formation by **(A)** *prgA*, *prgB*, *prgC* mutants or **(B)** T4SS mutants on polystyrene microtiter plates in the absence or presence of DNase. DNase was added either at the onset of biofilm growth and the cells were allowed to develop for 24 h (DNase 0 - 24 h) or at 21 h after which biofilms development was continued for an additional 3 h (DNase 21 - 24 h). The biofilm biomass was assayed as a function of the crystal violet stain retained. Results are expressed as the percentage biomass relative to strain OG1RF(pCF10). OG1RF, plasmid free parental strain.

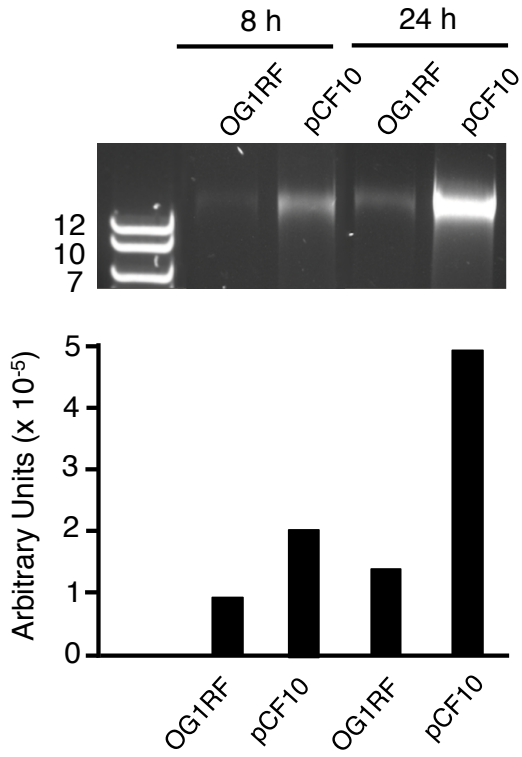

FIG. S3. Contribution of pCF10 to eDNA accumulation during biofilm development. High molecular weight bacterial chromosomal DNA was detected by ethidium bromide staining, after 20-fold concentration of supernatant fractions recovered from resuspended biofilms. Upper: stained gel showing relative amounts of eDNA recovered from OG1RF and OG1RF(pCF10) biofilms grown for 8- and 24-h following pheromone induction. The first lane contains the DNA ladder; sizes (kb) are shown on the left. Lower: Quantification of the eDNA in the upper gel after densitometric analysis and normalization per CFU.

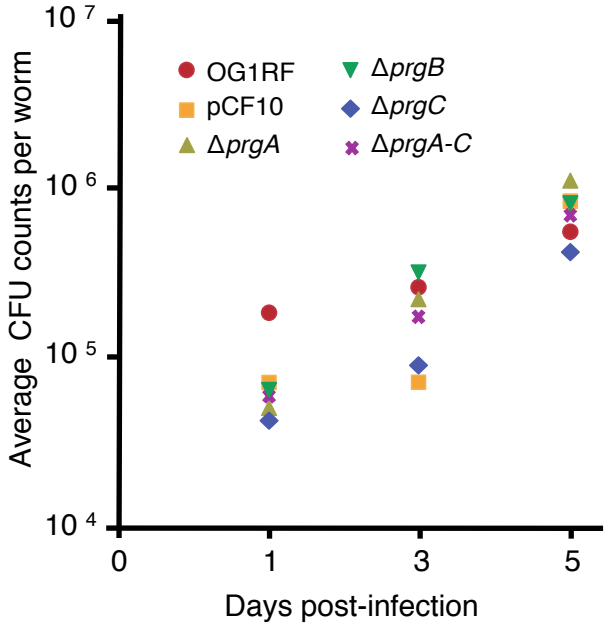

FIG. S4 Proliferation of *prg* mutant strains in the worm. Average colony forming units (CFU) per worm for *E. faecalis* OG1RF without or with pCF10 or Δ*prg* mutant plasmids quantitated at 1, 3 and 5 days post-infection.

Table S1. Quantitation of Biofilm Images

Strain/ Relevant construct	Biofilm Thickness (μM)		Total Fluorescence Intensity As a Percent of OG1RF(pCF10)					
			8 hr biofilms			24 hr biofilms		
	8 h	24 h	Cells	EPS	eDNA	Cells	EPS	eDNA
OG1RF	7	8	21	22	19	30	7	32
(P_Q::*prgA-C*)	11	20	53	14	45	72	20	55
pCF10	14	21	100	100	100	100	100	100

	48 h	48 hr biofilms	
		Total DNA	EPS
OG1RF	10	31	21
(P_Q::*prgA-C*)	26	67	81
pCF10	31	100	100
Δ*prgA*	8	25	18
Δ*prgB*	7	25	24
Δ*prgC*	33	71	84
Δ*prgA-C*	6	12	17

Table S1. For each channel of the biofilm images (Fig. 5, 6) acquired with the Olympus IX 81 fluorescence microscope, total fluorescence intensity (FI) was calculated using Slidebook (version 6) software (see Materials and Methods) and presented as a percentage of the value for OR1RF(pCF10) biofilms in the same experiment. The values listed for eDNA also reflect any DNA associated with lysed cells. The thickness of each biofilm was directly determined using the Slidebook software as the number of 1 μM slices collected.