SUPPLEMENTAL MATERIAL

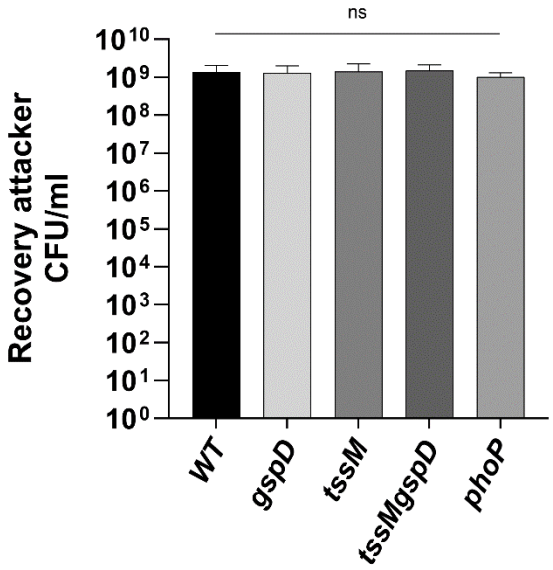


FIG. S1. Control of attacker bacterial growth. Wild-type (WT), T2SS mutant (*gspD*), T6SS mutant (*tssM*), double mutant (*gspD tssM*), and *phoP* mutant strains of *S. marcescens* RM66262 were used as attackers, while *Escherichia coli* MC4100 served as the prey strain. Attacker and prey cells were co-incubated at a 5:1 ratio (attacker:prey) at 37°C for 4 h, and attacker survival was determined by plating on the corresponding selective medium. Means ± SD of three independent experiments performed in duplicate in each case are shown. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test (*, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$; ns, not significant).

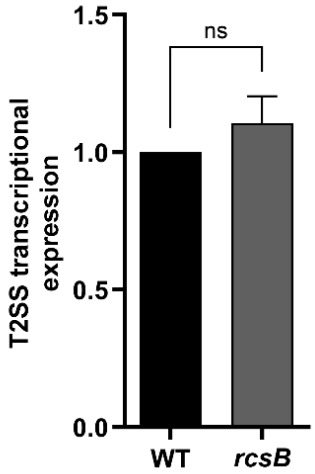


FIG. S2. Transcriptional expression of the T2SS promoter. Bacteria were grown for 16 h in LB medium, in 96-well microplates, at 37°C with shaking. Transcriptional activity was calculated as the ratio of GFP fluorescence to OD_{600}, measured in strains carrying the *pPgsp-gfp* reporter plasmids, and expressed relative to the wild-type strain. Means ± SD of two independent experiments performed in triplicate in each case are shown. Means ± SD from two independent experiments, each performed in triplicate, are shown. No significant differences relative to the reference condition were detected, as determined by paired *t* test.

Table S1. Strains and plasmid used in this study

Strains	Genotype and/or comments	Source or reference
Serratia marcescens		
wild type (WT)	*S. marcescens* RM66262; clinical isolate	(1)

gspD	RM66262 Δ*gspD*	This work
tssM	RM66262 Δ*tssM*	This work
gspD tssM	RM66262 Δ*gspD* Δ*tssM*	This work
gspD/pGspD	RM66262 Δ*gspD*/pSU36::*gspD*	This work
phoP	RM66262 *phoP::*pKNOCK-Gm	(2)
WT/pP*gsp-gfp*	RM66262 WT/pP*gsp-gpf*	This work
phoP/pP*gsp-gfp*	RM66262 *phoP*::pKNOCK-Gm^R/pP*gsp-gfp*	This work
WT/pP*gsp*.mut-*gfp*	RM66262 WT/pP*gsp*.mut-*gfp*	This work
phoP/pP*gsp*.mut-*gfp*	RM66262 *phoP*::pKNOCK-Gm^R/pP*gsp*.mut-*gfp*	This work
Db11	Wild type, environmental isolate. Spontaneous Sm^R derivative of strain Db10	(3)
Escherichia coli		
MC4100	Model laboratory *E. coli* K-12 strain; Sm^R (*rpsL150*)	(4)
DH5α	General cloning strain	Laboratory Stock
One Shot TOP10	*F-, mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu) 7697 galE15 galK16 rpsL endA1; Sm^R*	Invitrogen
SM10 λpir	*thi J thr leu tonA lacY 61lic recA::RP4-2-Tc::Mu; λpir; Km^R*	(5)
Acinetobacter nosocomialis		
M2	Wild type	(6)
tssD	M2 Δ*tssD*	(7)

Pseudomonas aeruginosa		
PAO1	Wild type	(8)
Plasmids		
pGspD	pSU36::*gspD*; Km^R	This work
p*Pgsp-gfp*	pPROBE-OT::prom*gsp,* Sp^R	This work
p*Pgsp*.mut.*gfp*	pPROBE-OT::prom*gsp*.mut, Sp^R	This work

TABLE S2. Primers used in this study

Primer	Sequence (5'–3')[a]
gspD-A (BamHI)	CGCGGATCCCATGCTCCAGCTGGGATG
gspD-B	CGCGGAACGGCGCTTCACGGCGCCAATGCGCGCCATAAGC
gspD-C	GCTTATGGCGCGCATTGGCGCCGTGAAGCGCCGTTCCGCG
gspD-D (SpeI)	CGGACTAGTCGGCGATATCCAGCTTCG
gspD-F[b]	CACGTGAGCAAGCGGTT
gspD-R[b]	GACCTTCAGCGCCATGC
tssM A[b]	CGCGGATCCGCAGGTGGCGATCGGTAACCCGG
tssM B	CGCCAATGCGCGCCATAAGCTTTCCGGTTCCTTGCGGCAAGC
tssM C	GCTTATGGCGCGCATTGGCGCGAAAGGACATGACCATGAGCAC
tssM D[b]	CGGACTAGTGACAATATCTCGGAATCTGC

Tipo VI 1295[b]	TCCATCAGCAGCGTATCAGG
prom.gsp-fw	CCCAAGCTTAGCGATGCCTACTCGCCGATG
prom.gsp-rv	TGCTCTAGAGTCCATCTCTCGCCTATCAGT
Prom phoP serr fwd EcoRI	ACCGAATTCGCGCTTAACCCGCTCG
Prom phoP serr rev BamHI	AGGGATCCCATGGCGAACTCCTGTG
nuclease Fw (XbaI)	GCTCTAGAGGCAAGACGCGCAACTGG
nuclease Rv (XhoI)	CCGCTCGAGGAAATCGGCGCCCTTCGG
gspD-Fw.BamHI	CGGGATCCTTGGCGGCGGCGGCCGTCATG
gspD-Rv.HindIII	CCCAAGCTTTCACGGGCGGGCTCCCCGCGGAT
prom.gsp.mut - F	TCATGTTTTTAAATGATATTATGAATCTAAGGCGATGC
prom.gsp.mut - R	CCTTAGATTCATAATATCATTTAAAAACATGATGATAGCC

[a] Restriction sites are underlined.

[b] Primers used to confirm the mutants by PCR.

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