"Unraveling the Role of UilS, a urea-induced acyl-homoserine lactonase that enhances *Serratia marcescens* fitness, interbacterial competition and urinary tract infection"

SUPPLEMENTAL MATERIAL

Table S1: *S. marcescens* strain RM66262 genes regulated by 0.4 M urea obtained by transcriptomic analysis (Table S1 is provided as an Excel file).

Table S2. Bacterial strains and plasmids used in this study.

Strains	**Genotype and/or comments**	**Source or reference**
S. marcescens		
wild-type (wt)	*S. marcescens* RM66262; clinical isolate	[1]
wt pSU36::*uilS*	RM66262/ pSU36::*uilS* Kn^R	This work
wt P-gfp	RM66262 pPROBE(NT) Km^R	This work
wt p*uilS*-gfp	RM66262 pPROBE(NT)::prom*uilS* Km^R	This work
wt p*PuilS*i-gfp	RM66262 pPROBE(KT)::prom*uilS* Km^R	This work
wt p*luxR*-gfp	RM66262 pPROBE(NT)::prom*luxR* Km^R	This work
wt p*luxI*-gfp	RM66262 pPROBE(NT)::prom*luxI* Km^R	This work
cpxR	*cpxR*::pKNOCK-Cm^R	[2]
cpxR pSU36::*luxR*	*cpxR*::pKNOCK-Cm^R pSU36::*luxR*	This work
cpxR pBB5::*cpxR*	*cpxR*::pKNOCK-Cm^R /pBB5::*cpxR* Kn^R	[3]
cpxR luxR	*cpxR*::pKNOCK-Cm^R *ΔluxR*	This work
cpxR luxR pSU36::*luxR*	*cpxR*::pKNOCK-Cm^R *ΔluxR* /pSU36::*luxR*	This work
cpxR luxR pBB5::*cpxR*	*cpxR*::pKNOCK-Cm^R *ΔluxR* /pBB5::*cpxR*	This work

cpxR pP*uilS*-gfp	*cpxR*::pKNOCK-Cm^R pPROBE(NT)::prom*uilS* Km^R	This work
luxI	*ΔluxI*	This work
luxI pP*uilS*-gfp	*ΔluxI* pPROBE(NT)::prom*uilS* Km^R	This work
luxR	*ΔluxR*	This work
luxR pP*uilS*-gfp	*ΔluxR* pPROBE(NT)::prom*uilS* Km^R	This work
luxR pBB1-lacI^q::*luxR*	*ΔluxR* pBB1-lacI^q::*luxR* Cm^R	This work
luxR pBB1-lacI^q::*luxR* pPuilSi-gfp	*ΔluxR* pBB1-lacI^q::luxR Cm^R pPROBE(KT)::promuilS Km^R	This work
luxR pBB5::*cpxR*	*ΔluxR/*pBB5::*cpxR*	This work
luxR pSU36::*nlpE*	*ΔluxR/*pSU36::*nlpE*	This work
ompR pP*uilS*-gfp	*ompR*::pKNOCK-Cm^R pPROBE(NT)::prom*uilS* Km^R	This work
phoP pP*uilS*-gfp	*phoP*::pKNOCK-Gm^R [4] pPROBE(NT)::prom*uilS* Km^R	This work
rcsB	*rcsB*::pKNOCK-Gm^R	[4]
rcsB pP*uilS*-gfp	*rcsB*::pKNOCK-Gm^R pPROBE(NT)::prom*uilS* Km^R	This work
rssB pP*uilS*-gfp	*rssB*::pKNOCK-Gm^R pPROBE(NT)::prom*uilS* Km^R	This work
tssM	*tssM*::pKNOCK-Gm^R	[2]
uilS	*uilS*::pKNOCK-Cm^R	This work

uilS luxR	*uilS*::pKNOCK-Cm *ΔluxR*	This work
uilS pSU36::*uilS*	*uilS*::pKNOCK-Cm^R /pSU36::*uilS* Kn^R	This work
Pseudomonas aeruginosa		
PAO1	*P. aeruginosa* PAO1 strain	[5]
Agrobacterium tumefaciens		
NT1/pZLR4	AHLs long chain biosensor	[6]
Chromobacterium violaceum		
VIR07	AHLs long chain biosensor	[7]
E. coli		
One Shot Top10	F- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 *ΔlacX74 nupG recA1 araD139* Δ*(ara-leu)7697 galE15 galK16 rpsL endA1* Sm^R	Invitrogen
SM10 λ_{pir}	*thi J thr leu tonA lacY61 lic recA::RP4-2-*Tc::Mu *λ*pir Km^R	[8]
HB101/pRK2013	F- *mcrB mrr hsdS20(rB- mB-) recA13 leuB6 ara-14 proA2 lacY1 galK2 xyl-5 mtl-1 rpsL20* (Sm^R) *glnV44* λ-; genes mob; Km^R	[9]
M15/pRep4	F- ϕ80*lacZ*ΔM15 *thi lac mtl recA*- p*lacI* Km^R	Qiagen
Plasmids		
pSU36	derived from pACYC184, Km^R	[10]

pSU36::*uilS*	Km^R	This work
pSU36::*luxR*	Km^R	This work
pKNOCK-Cm	Suicide vector (ori R6K), mobilizable	[11]
pKNOCK-Cm::*uilS*	Internal fragment of *uilS*, cloned in pKNOCK-Cm^R	This work
pKNG101	Suicide vector (oriR6K), mobilizable (mobRK2), with counterselection marker (*sacB*), Sm^R	[12]
pP-gfp	pPROBE(NT) Km^R	[13]
pP*uilS*-gfp	pPROBE(NT)::prom*uilS* Km^R	This work
pP*uilS*i-gfp	pPROBE(KT)::prom*uilS* Km^R	This work
pP*luxI*-gfp	pPROBE(NT)::prom*luxI* Km^R	This work
pP*luxR*-gfp	pPROBE(NT)::prom*luxR* Km^R	This work
pBB1-$lacI^q$	pBBR1-MCS1::$lacI^q$ Cm^R	[14]
pBB1-$lacI^q$::*luxR*	pBBR1-MCS1::$lacI^q$::*luxR* Cm^R	This work
pBB5::*cpxR*	pBBR1-MCS5::$lacI^q$::*cpxR* Gn^R	[3]

7 **Table S3. Primers used in this study**

Primers	**Sequence (5'–3')**
uilS-Fw.BamHI	CGGGATCCATGTCCTCAATCTGCAGCGC
uilS-Rv.XhoI	CCGCTCGAGAAACGCTGCGCTATCAGC

uilS-Fw	CAGCGGGCATTCCGCTGAGC
uilS-Rv	TGCGCATCGTCGCCAATACC
C.uilS-Fw	CGGGATCCATGGCATTAACCACGCATAA
C.uilS-Fv	TCCAAGCTTTTACAGGTGCTCGTTGAACCA
luxI-A Fw	ACGGGATCCGACAGGTCCGAGGACATACTG
luxI-B Rv	GAACGCAGGTTCGTCGCTCATTGATGCGTAGTTGGTGCTG
luxI-C Fw	CAGCACCAACTACGCATCAATGAGCGACGAACCTGCGTTC
luxI-D Rv	GGACTAGTCCGATGGCTATACCTTCGTG
luxR-A Fw	CGCGGATCCTGAAATCGTTGGTGACCA
luxR-B Rv	CGAACCTGCGTTCTAATCACTATTCCATTAACTGCCTGCC
luxR-C Fw	GGCAGGCAGTTAATGGAATAGTGATTAGAACGCAGGTTCG
luxR-D Rv	CGGACTAGTCTACGCATCAATGAGCAC
luxR-Fw	AGATTCGACGTCTTCCAG
luxR-Rv	GGCATGCTCGTAGTGAAA
C.luxR-Fw	CCGCTCGAGATGGAATATGAAGAAAATATCAGTC
C.luxR-Rv	CGCGGATCCTCACCCCACCGGTTTAATCAGCT
C2.luxR-Fw	CGGGATCCATGGAATATGAAGAAAATATCAG

C2.luxR-Rv	TCCAAGCTTTAATCACCCCACCGGTTTAA
P.uilS-Fw	CCCAAGCTTCACCAAGGCGACGCTGAATG
P.uilS-Rv	TGCTCTAGACATGTTAACTTCCTGTTTTTTA
P.uilS-Rv2	CGAGCTCCATGTTAACTTCCTGTTTTTTA
P.luxI-Fw	CCCAAGCTTGCACCGCTCATTTTACTCAGC
P.luxI-Rv	TGCTCTAGATCAGAGAAGTTTCACTACGAGCA
P.luxR-Fw	CCCAAGCTTCGGTGTCCAATACGATGATGTC
P.luxR-Rv	TGCTCTAGACATTAACTGCCTGCCCCGCTAC
uilS.RTqPCR-Fw	GGCACATCGTTTGGCGGTTG
uilS.RTqPCR-Rv	CTCTTTGCCGGTGGCCTGTT
prtA.RTqPCR-Fw	TTACCCGTGAGAACCAAACC
prtA.RTqPCR-Rv	TGTAGTTGCCGAAGGTGATG
fliA.RTqPCR-Fw	GTGAGCGATCTGTATACCG
fliA.RTqPCR-Rv	CGCAGCTCGTCGAGCATCGC
fliC.RTqPCR-Fw	CGGGATCCGGCGCAGAACAACCTGAAC
fliC.RTqPCR-Rv	ACGCCCGGGCGTTCAGTGCGCCTTC
flhD.RTqPCR-Fw	TCGCCCGGGATGGGGAATATGGGTAC
flhD.RTqPCR-Rv	ACGCCCGGGCTTTGGTCAGGCGTTC

slpE.RTqPCR-Fw	AAACCTGGAATGGCGTGCAC
slpE.RTqPCR-Rv	CAAGGCATACGCTTGTCCTG
slpD.RTqPCR-Fw	GGGTAAAATCCGCGATAAC
slpD.RTqPCR-Rv	GCGCGATGATTGATATCG
lipB.RTqPCR-Fw	TCACCAAGTTTGTGCGCATG
lipB.RTqPCR-Rv	TTTCCAGCAGCTTGACCAAC
gyrB.RTqPCR-Fw	ATTCTGGCCAAGCGTCTGCG
gyrB.RTqPCR-Rv	TCGGGTGGATCGGGGTTTTG
rpoD.RTqPCR-Fw	GACATCGCCAAGCGCATCGA
rpoD.RTqPCR-Rv	AAGCCGGTGATCAGGTCGGA

8 SUPPLEMENTAL FIGURES AND LEGENDS

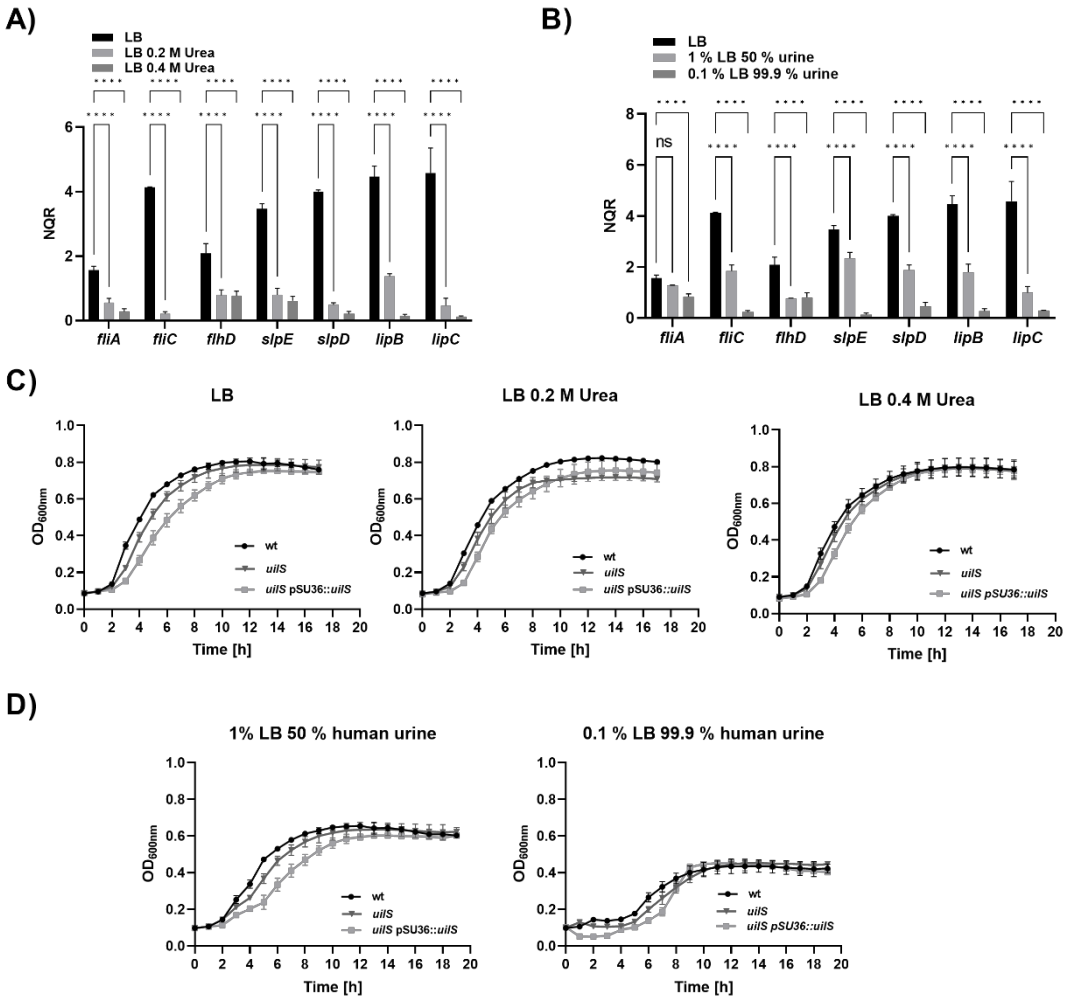


Figure S1. Urea-dependent modulation of *S. marcescens* gene expression. A-B) qRT-PCR analysis of *fliA, fliC, flhD, slpE, slpD, lipB,* and *lipC* of *S. marcescens* RM66262 cultured in LB broth, LB broth supplemented with 0.2 or 0.4 M urea (A) or cultured in LB broth, 1 % LB 50 % urine or 0.1 % LB 99.9 % urine (B). The data presented are the mean ± standard deviation (SD) of normalized relative quantities (NRQ) derived from transcript levels calculated using the qBASE method. Three independent samples were used, and two technical replicates were performed for each sample. Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Asterisks indicate the significance levels for the statistical analysis: ∗, P < 0.05; ∗∗, P < 0.01; ∗∗∗, P < 0.001; and ∗∗∗∗, P < 0.0001, the analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A P < 0.05 was considered significant. Data are presented as mean ± SD. C-D) Growth curves. Bacteria were grown for 18 h in LB or LB supplemented with 0.2 or 0.4 M urea (A)or in LB, 1 % LB 50 % urine, or 0.1 % LB 99.9 % urine (B), in 96-well microplates, at 37°C with agitation. Growth is shown as OD 600 nm values from the *S. marcescens* RM66262 wild-type (wt), *uilS,* and *uilS* pSU36::*uilS*. Means ± SDs from three independent experiments performed in duplicate in each case are shown.

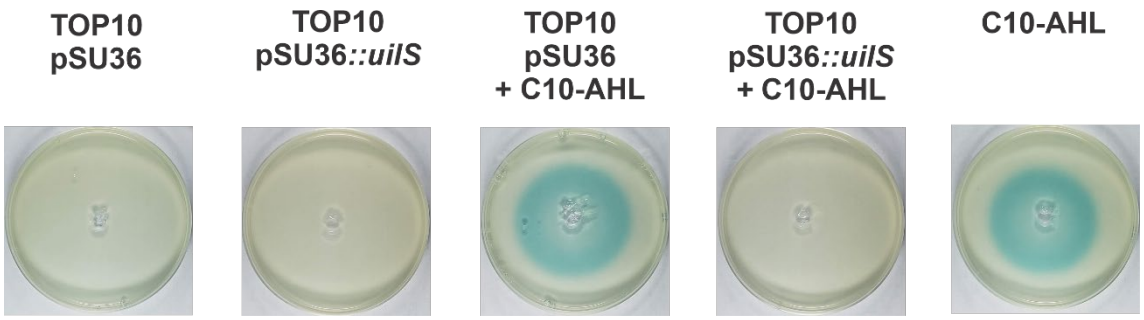


Figure S2. UilS expressed in *E. coli.* Detection by *A. tumefaciens* biosensor assay of quorum quenching activity after incubation with C10-AHL.

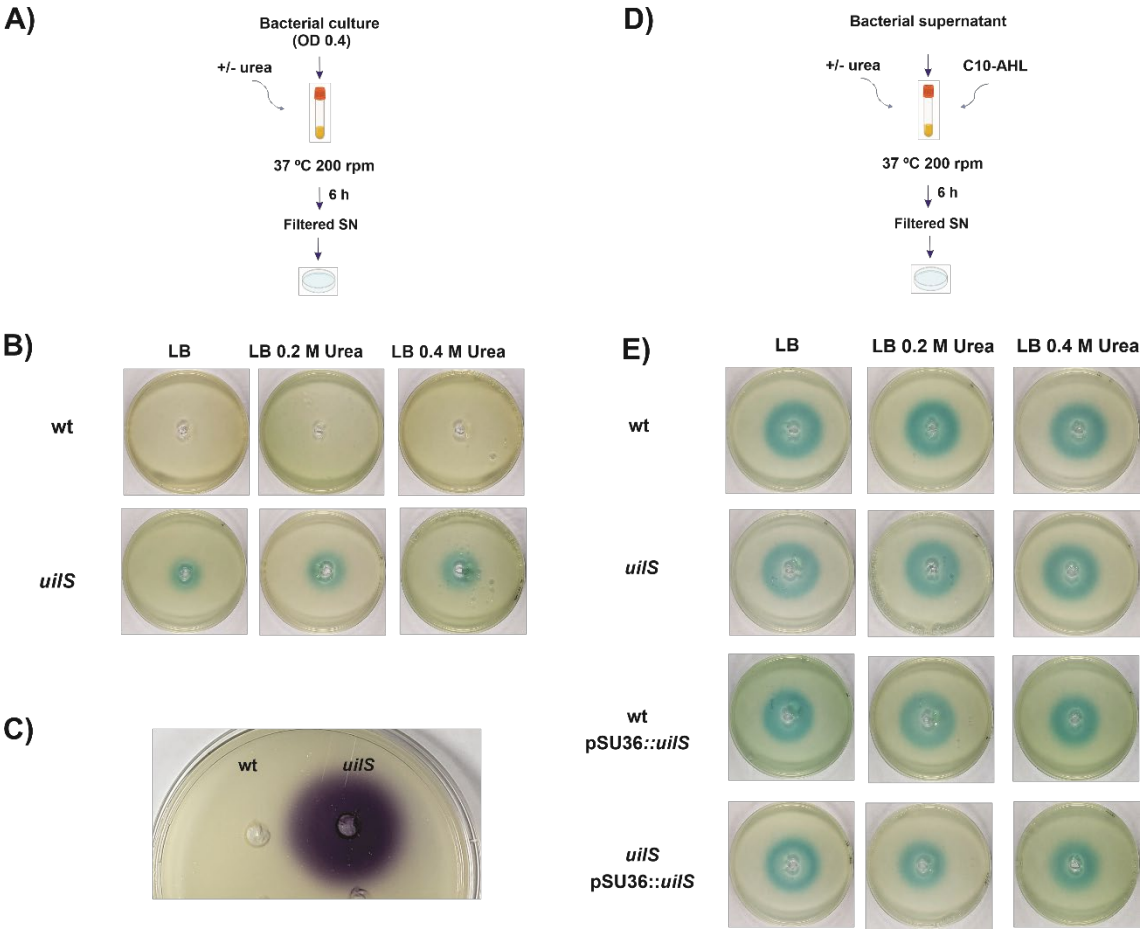


Figure S3. AHLs degradation by urea-induced UilS expression. A) Schematic protocol, determination of AHLs in the filtered supernatant (SN) of *S. marcescens* wt and *uilS* strains grown for 18 hours at 37ºC shaking using the *A. tumefaciens* NT1 (pZLR4) biosensor (B) or *C. violaceum* VIR07 biosensor (C). Plates were inspected and photographed after 24 h at 30 °C. Representative results of three independent experiments are shown. D) Schematic protocol of AHL degradation by *S. marcescens* culture SN. The filtered SN of wt, *uilS,* and *uilS* /pSU36::*uilS* strains was incubated at 37ºC shaking in LB or LB supplemented with urea 0.2 and 0.4 M and C-10 AHL. After 6 h, AHLs were determined using the plate-biosensor assay. Plates were inspected and photographed after 24 h at 30 °C (E). Representative results of three independent experiments are shown.

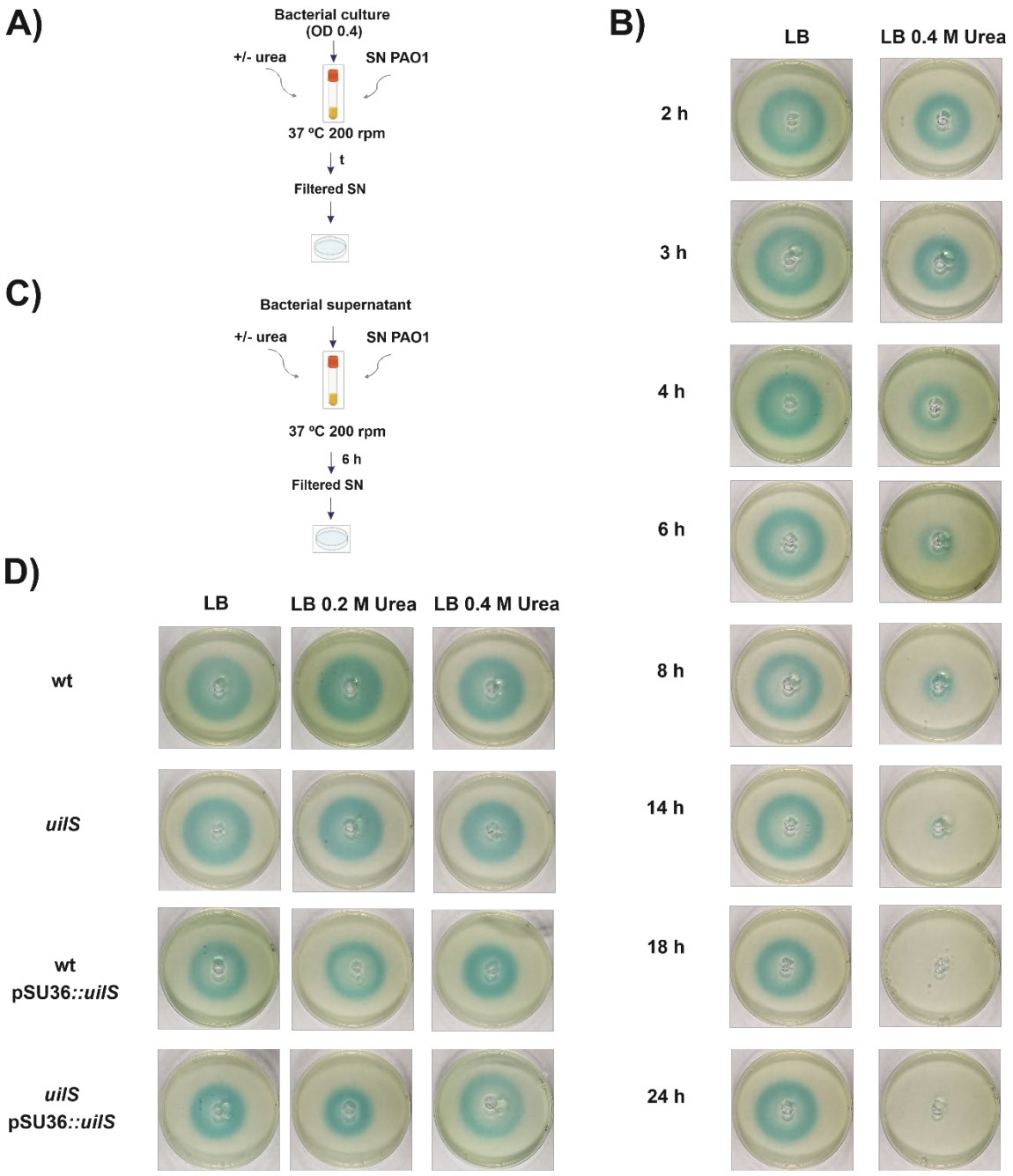
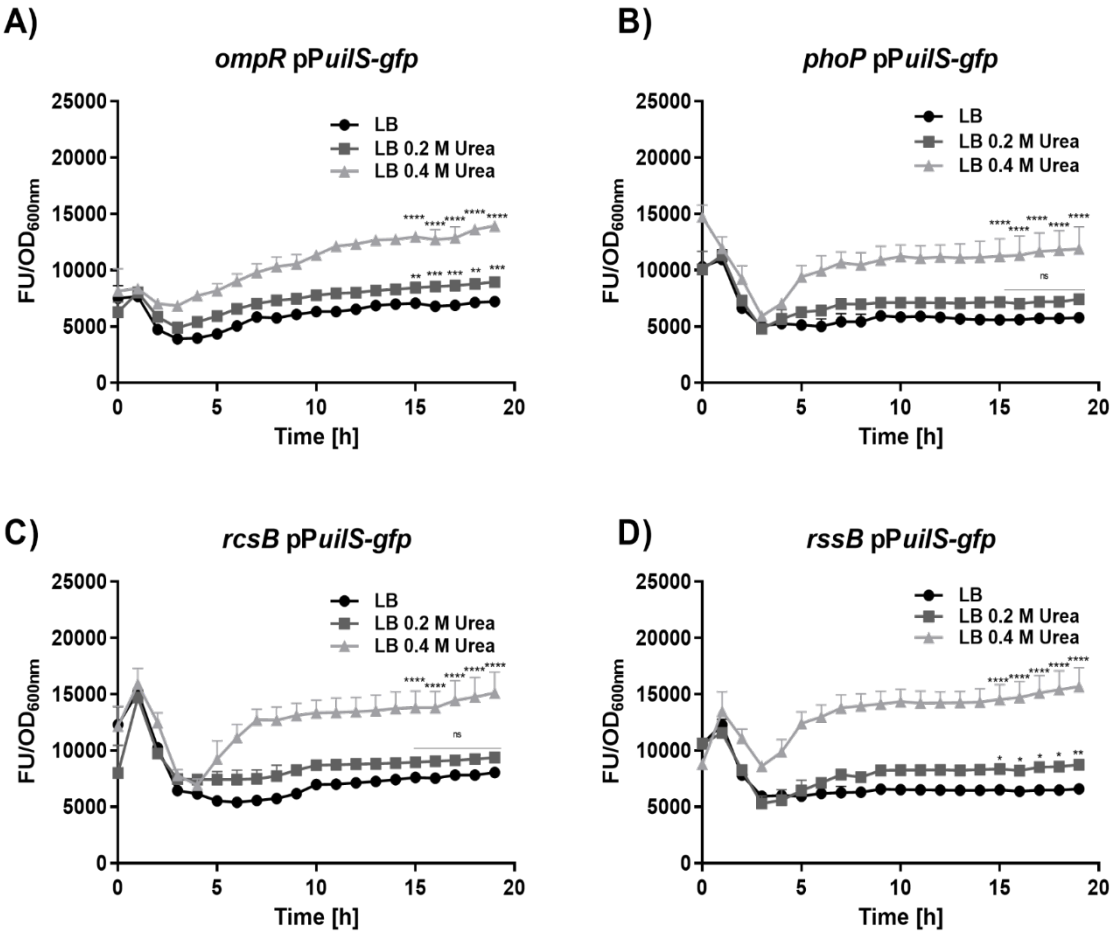


Figure S4. *P. aeruginosa* PAO1 AHLs degradation by *S. marcescens* culture supernatant. A) Scheme of the protocol, *S. marcescens* wt strain culture was inoculated in LB broth supplemented with PAO1 filtered SN in the presence or absence of 0.4 M urea at 37ºC 200 rpm. At the times indicated in figure B, aliquots were taken, and the presence of AHLs was then determined in the filtered SN using the *A. tumefaciens* NT1 (pZLR4) biosensor (B). C) Scheme of the protocol. The filtered SN of wt, *uilS* and *uilS* pSU36::*uilS* strains was incubated at 37ºC shaking in LB or LB supplemented or not with urea 0.2 and 0.4 M and PAO1 filtered SN. After 6 h, AHLs were determined using the plate-biosensor

assay. Plates were inspected and photographed after 24 h at 30 °C. Representative results of three independent experiments are shown (D).

Figure S5. Transcriptional expression of *uilS* in *ompR, phoP, rcsB*, or *rssB* genetic backgrounds. Bacteria were grown for 18 h in LB or LB supplemented with 0.2 or 0.4 M urea, in 96-well microplates, at 37°C with agitation. Transcriptional activity was calculated as the ratio of GFP fluorescence values and OD600 (FU/OD600) measured



from the *S. marcescens ompR* (A), *phoP* (B), *rcsB* (C) and *rssB* (D) strains carrying the P*uilS*-gfp reporter plasmid. Means ± SDs from three independent experiments performed in duplicate in each case are shown. Statistical significance ($P < 0.05$) was determined by two-way ANOVA followed by Tukey's multiple comparison test, comparing each mean (every measured time) with the control LB condition. The last 5 points are shown. Significance was indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ employing GraphPad Prism (GraphPad Software, San Diego, CA, USA).

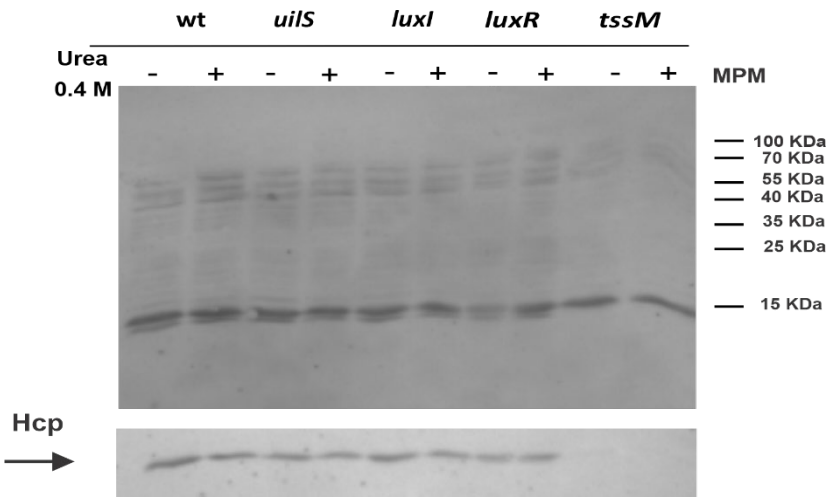


Figure S6. Hcp protein levels were not affected by urea. Filtered supernatants from saturated cultures of the indicated *S. marcescens* RM66262 wild-type (wt), *uilS, luxI, luxR,* and *tssM* strains were precipitated and 20 µg of total protein was loaded into a 18% SDS-PAGE gel. Hcp levels were determined by immunodetection using rabbit Hcp polyclonal antisera. Ponceau red-stained nitrocellulose membrane is shown (top), and immunoprecipitation (bottom). A representative image of the assay is shown.

SUPPLEMENTAL MATERIAL BIBLIOGRAPHY

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