Supplementary material

Table S1. Bacterial strains and plasmids used in this study

Strains	**Genotype and/or comments**	**Source or reference**
S. marcescens		
RM66262	*S. marcescens* RM66262; clinical isolate, wild-type	(1)
RM66262 p*PuilS*-gfp	RM66262 pPROBE(NT)::prom*uilS* Km^R	(2)
Δ*cpxR*	RM66262 *cpxR*::pKNOCK-Cm^R	(3)
Δ*cpxR* pP*uilS*-gfp	RM66262 *cpxR*::pKNOCK-Cm^R pPROBE(NT)::prom*uilS* Km^R	(2)
Δ*ureR*	RM66262 *ΔureR*	This work
Δ*ureR* pSU36::*ureR*	RM66262 *ΔureR* pSU36::*ureR* Km^R	This work
Δ*ureR* pP*uilS*-gfp	RM66262 *ΔureR* pPROBE(NT)::prom*uilS* Km^R	This work
Δ*ureR* Δ*cpxR* pP*uilS*-gfp	RM66262 *ΔureR cpxR*::pKNOCK-Cm^R pPROBE(NT)::prom*uilS* Km^R	This work
RM66262 p*PureR*-gfp	RM66262 pPROBE(NT)::prom*ureR* Km^R	This work
Δ*ureR* pP*ureR*-gfp	RM66262 *ΔureR* pPROBE(NT)::prom*ureR* Km^R	This work
Δ*ompR* pP*ureR*-gfp	ompR::pKNOCK-CmR pPROBE(NT)::prom*ureR* Km^R	This work
Δ*shlBA*	RM66262 *shlBA*::Km^R	(4)
RM66262 p*PshlBA*-gfp	RM662662 pPROBE(OT)::proms*hlBA*; Sp^R	(4)
Δ*ureR* p*PshlBA*-gfp	RM662662 *ΔureR* pPROBE(OT)::proms*hlBA*; Sp^R	This work
Δ*ureR* pSU36::*ureR* p*PshlBA*-gfp	RM662662 *ΔureR* pSU36::*ureR* Km^R pPROBE(OT)::proms*hlBA*; Sp^R	This work
S. typhimurium		
14028s	Wild-type	ATCC
Δ*cpxR*	Δ*cpxR*::Cm^R	(5)
14028s pP*uilS*-gfp	14028s pPROBE(NT)::prom*uilS* Km^R	This work
Δ*cpxR* pP*uilS*-gfp	Δ*cpxR*::Cm^R pPROBE(NT)::prom*uilS* Km^R	This work
E. coli		

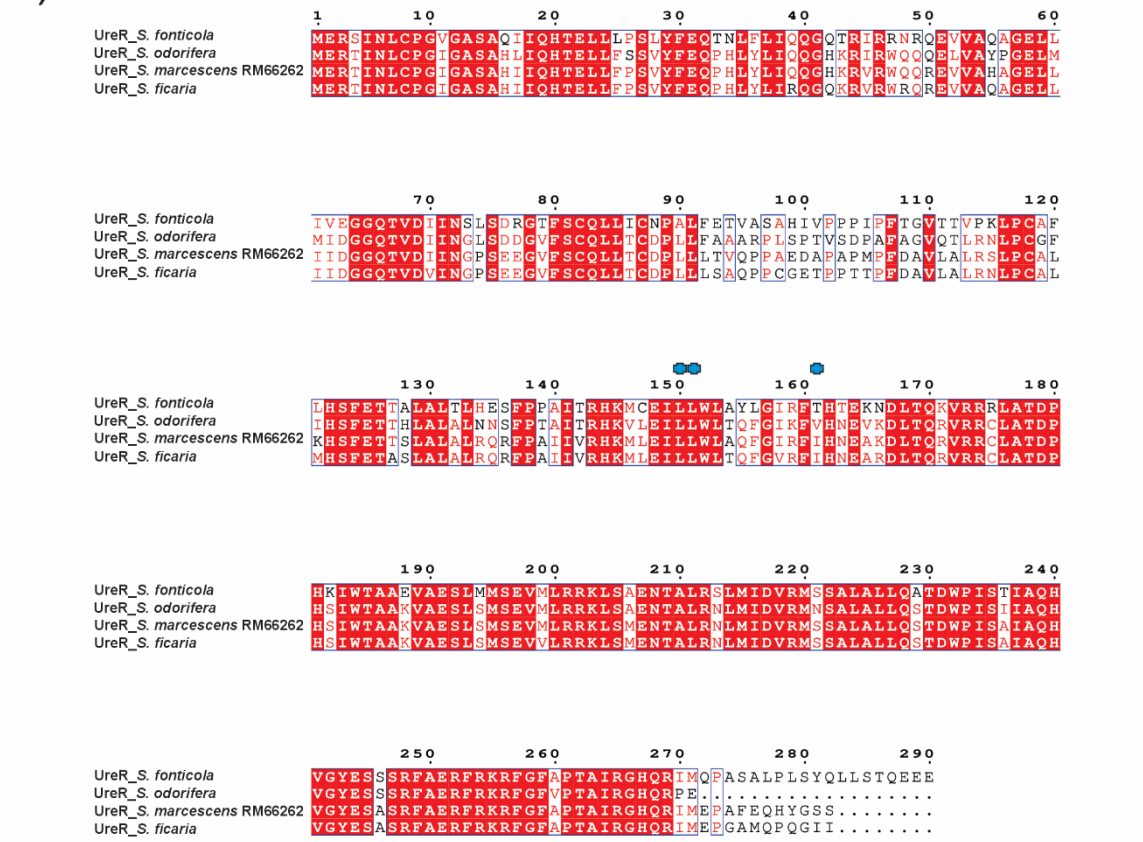
One Shot Top10	F- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*ΔM15 *ΔlacX*74 *nupG recA1 araD*139 *Δ(ara-leu)*7697 *galE*15 *galK*16 *rpsL endA1* Sm^R	Invitrogen
SM10 λpir	*thi J thr leu tonA lacY61 lic recA*::RP4-2-Tc::Mu λpir Km^R	(6)
Plasmids		
pSU36	derived from pACYC184, Km^R	(7)
pSU36::*ureR*	Kn^R	This work
pKNG101	Suicide vector (*oriR6K*), mobilizable (*mobRK2)*, with counterselection marker (*sacB*), Sm^R	(8)
pKNG101::*ureR*	pKNG101 containing the regions flanking the *ureR* gene	This work
pPROBE(NT)	Kn^R	(9)
p*PuilS*-gfp	pPROBE(NT)::prom*uilS* Km^R	(2)
p*PureR*-gfp	pPROBE(NT)::prom*ureR* Km^R	This work
p*PshlBA*-gfp	pPROBE(OT)::proms*hlBA*; Sp^R	(4)
pKNOCK-Cm::*cpxR*	*cpxR*::pKNOCK-Cm^R	(3)

Table S2. Primers used in this study

Primers	**Sequence (5'–3')**
ureR-A	CGCGGATCCCGCTTACGTAACGGCGGT
ureR-B	CCGCCATTGATTAGCTGGAACATCGATATTCATTTCGCAG
ureR-C	CTGCGAAATGAATATCGATGTTCCAGCTAATCAATGGCGG
ureR-D	CGGACTAGTAAGTCGCGCGGATTTTGC
ureR-F	CAGATCGGCCCCAACTAC
ureR-R	AGTGGCGAAAGCAAGAGC
C.*ureR*-Fw	CGGGATCCATGGAACGTACAATTAATCTCTG
C.*ureR*-Rv	CCCAAGCTTTGATTAGCTGGAACCGTAATGC
P.*ureR*-Fw	GCTCTAGACGCTTACGTAACGGCGGTGG
P.*ureR*-Rv	CGGAATTCCATCGATATTCATTTCGCAG
lac-F2	CGGGATCCATGTCCTCAATCTGCAGCGC
lac-R2	CCGCTCGAGAAACGCTGCGCTATCAGC
UreR-fw	CGCGGATCCACTGCTGCTCACCGTTCAGC

UreR-rv	CCGCTCGAGACGTCGATCATCAGGTTGCG

A)



B)

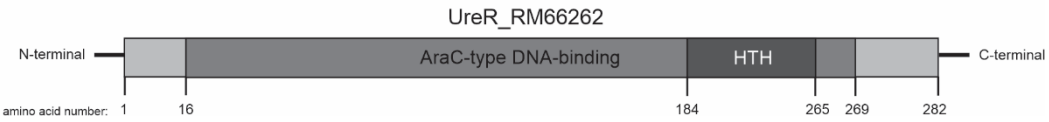
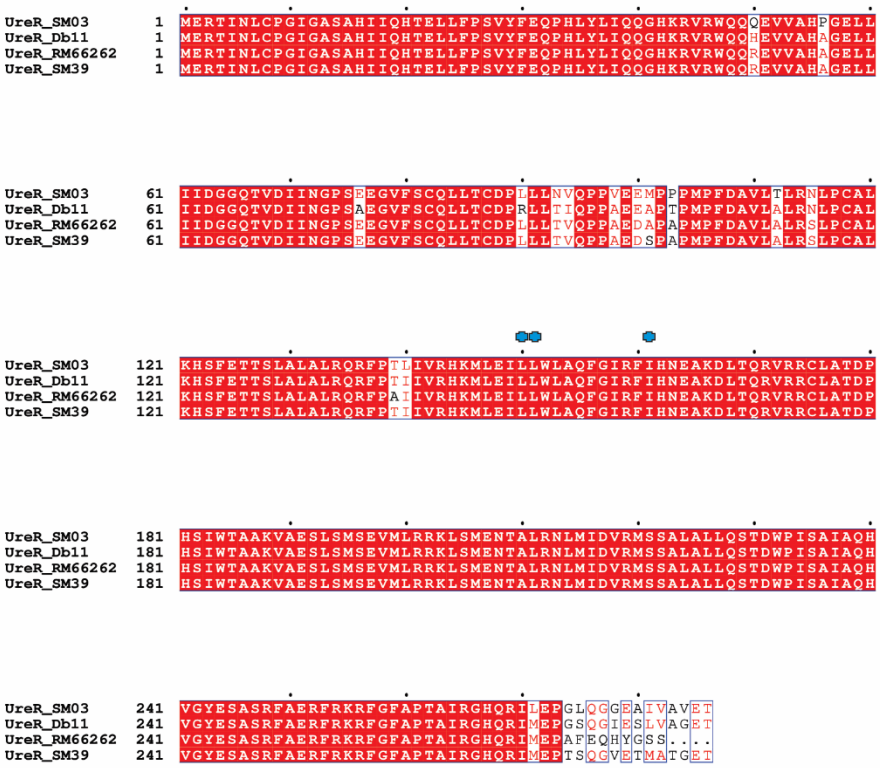


Figure S1. Comparative sequence analysis and domain mapping of UreR. (A) BLASTP-based comparison of UreR (RM66262) to selected *Serratia* homologs. BLASTP searches were performed against UniProtKB/Swiss-Prot and NCBI nr using default parameters (BLOSUM62). The light blue crosses markers indicate residues implicated in dimerization (Leu150, Leu151) and the present Ile161 substitution in RM66262. **(B)** Linear domain map of UreR (RM66262). The full protein (282 aa) is shown as a horizontal bar. The AraC-type DNA-binding domain predicted by NCBI CDD (positions 16–269; E-value 4.52e-14) and the helix turn helix motif (HTH) (positions 184-265; E-value 2.90e-17) are boxed. Domains were annotated with NCBI CDD and Pfam/InterPro.

A)



B)



Figure S2. (A) BLASTP-based comparison of UreR from *S. marcescens* RM66262 with UreR homologs from *S. marcescens* SM39, SM03, and Db11. BLASTP searches were performed against the NCBI non-redundant (nr) protein databases using default parameters (BLOSUM62). Pairwise multiple sequence alignment was generated with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and visualized in ESPript 3.0 (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi), with conserved residues highlighted and critical positions (Leu150, Leu151, Ile161 in RM66262) indicated as light blue crosses. **(B)** Genomic context of the *ureR* gene in *Serratia marcescens* RM66262. The *ureR* locus (green arrow) is flanked by genes encoding proteins associated with flagellar structure and function (yellow arrows). Genes shown in grey correspond to hypothetical proteins with no experimentally confirmed function. RT90_RS03050 encodes a transcriptional regulator of the LysR family, and RT90_RS03045 encodes an FMN-dependent NADH-azoreductase. Arrows indicate the direction of transcription. Gene annotations were obtained from the RAST server and the NCBI database.

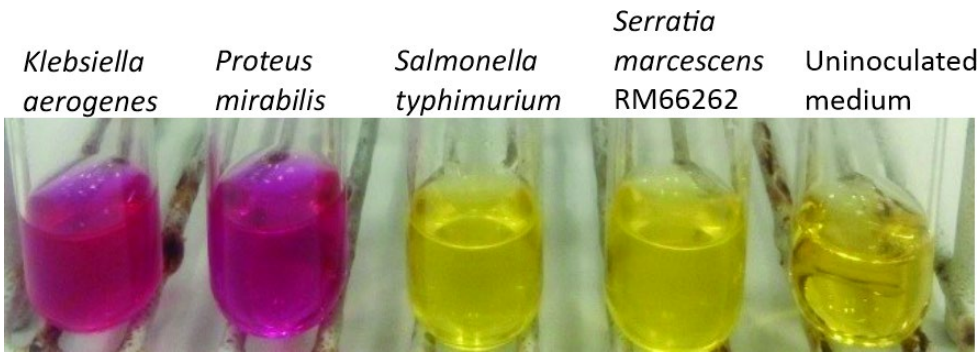


Figure S3. Experimental confirmation of the urease-negative phenotype of *Serratia marcescens* RM66262. Representative Christensen urea medium assay showing the absence of urease activity in RM66262. *Proteus mirabilis* and *Klebsiella aerogenes* were used as urease-positive controls and developed the characteristic pink coloration associated with urea hydrolysis, whereas *Salmonella Typhimurium* and RM66262 remained negative.

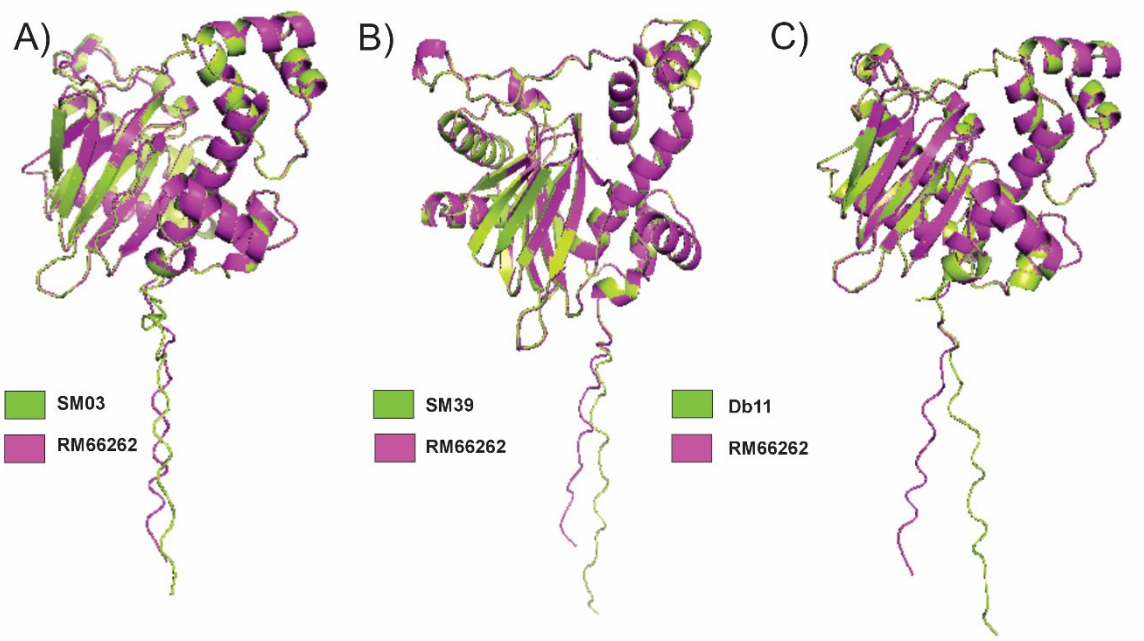


Figure S4. Structural superposition of UreR from *S. marcescens* RM66262 with homologs from other *S. marcescens* strains. **(A)** RM66262 (magenta) and SM03 (green); **(B)** RM66262 (magenta) and SM39 (green); **(C)** RM66262 (magenta) and Db11 (green). Predicted 3D models were retrieved from the AlphaFold Protein Structure Database, exhibiting high-confidence scores (pLDDT > 85% for the core structured domains), and aligned with PyMOL v3.1. RMSD and TM-score values for each comparison were obtained with TM-align (see text).

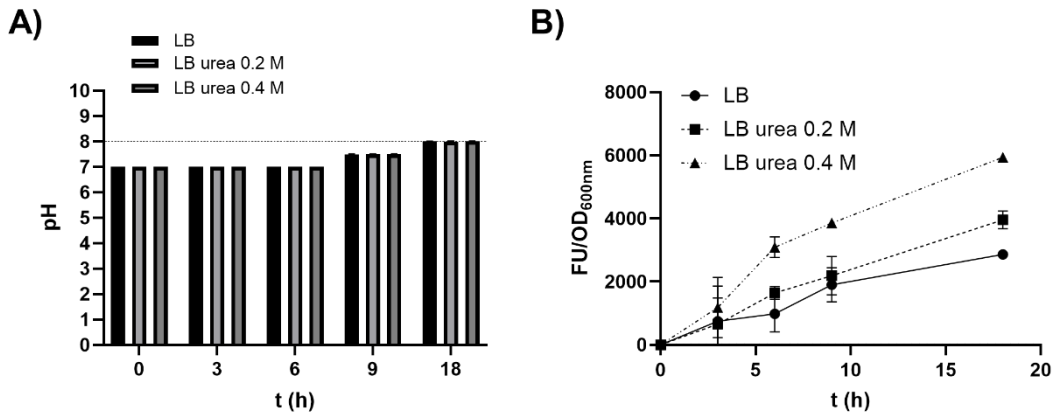


Figure S5. Culture pH and reporter activity are uncoupled from urea supplementation. Wild-type *S. marcescens* carrying the pP*uilS-gfp* reporter was grown in LB or LB supplemented with 0.2 or 0.4 M urea, at 37°C with agitation. (A) Medium pH measured at 0, 3, 6, 9 and 18 h. (B) Transcriptional activity of the reporter (FU/OD600) measured in the same cultures over the same time course. Individual replicate values from three independent cultures per condition are shown.

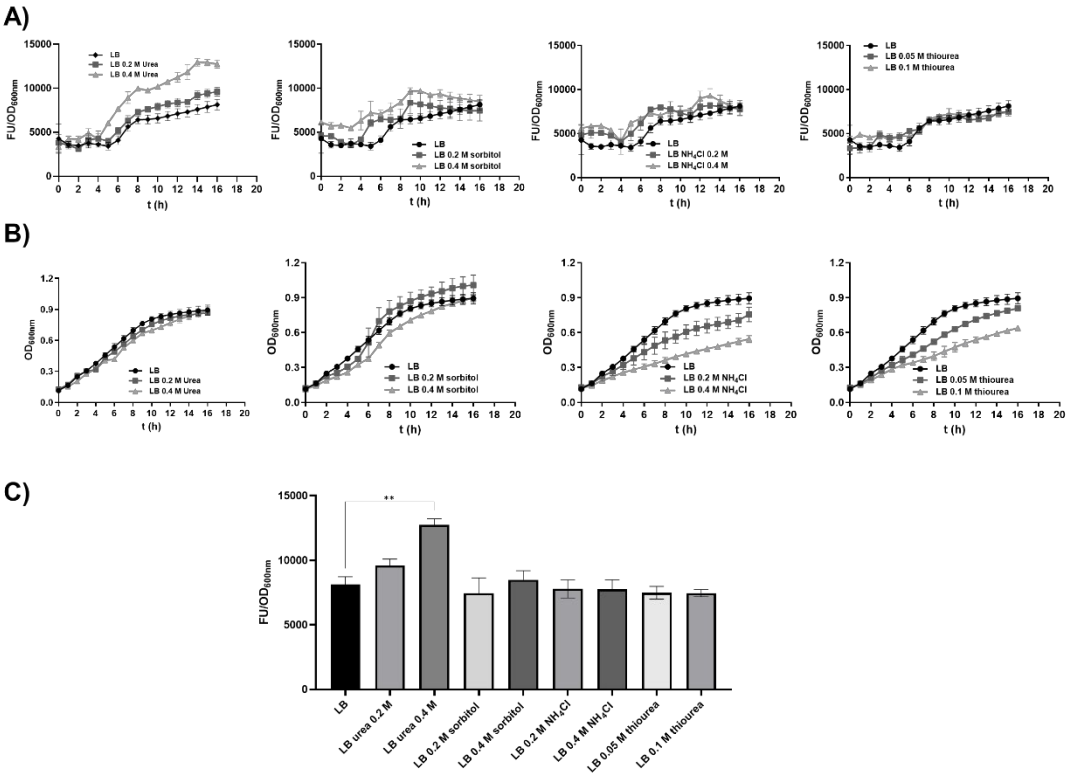


Figure S6. Specificity of the urea-dependent transcriptional response. Wild-type *S. marcescens* carrying the pP*uilS-gfp* reporter was grown in LB supplemented with urea, sorbitol, NH_4Cl or thiourea at the indicated concentrations, at 37°C with agitation, over 18 h Transcriptional activity of the reporter (FU/OD600), measured in the same cultures. (A). Bacterial growth (OD600) (B). Last measurement (18 h) of graph A (C). 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 (Last measured time) with the control LB condition. 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).

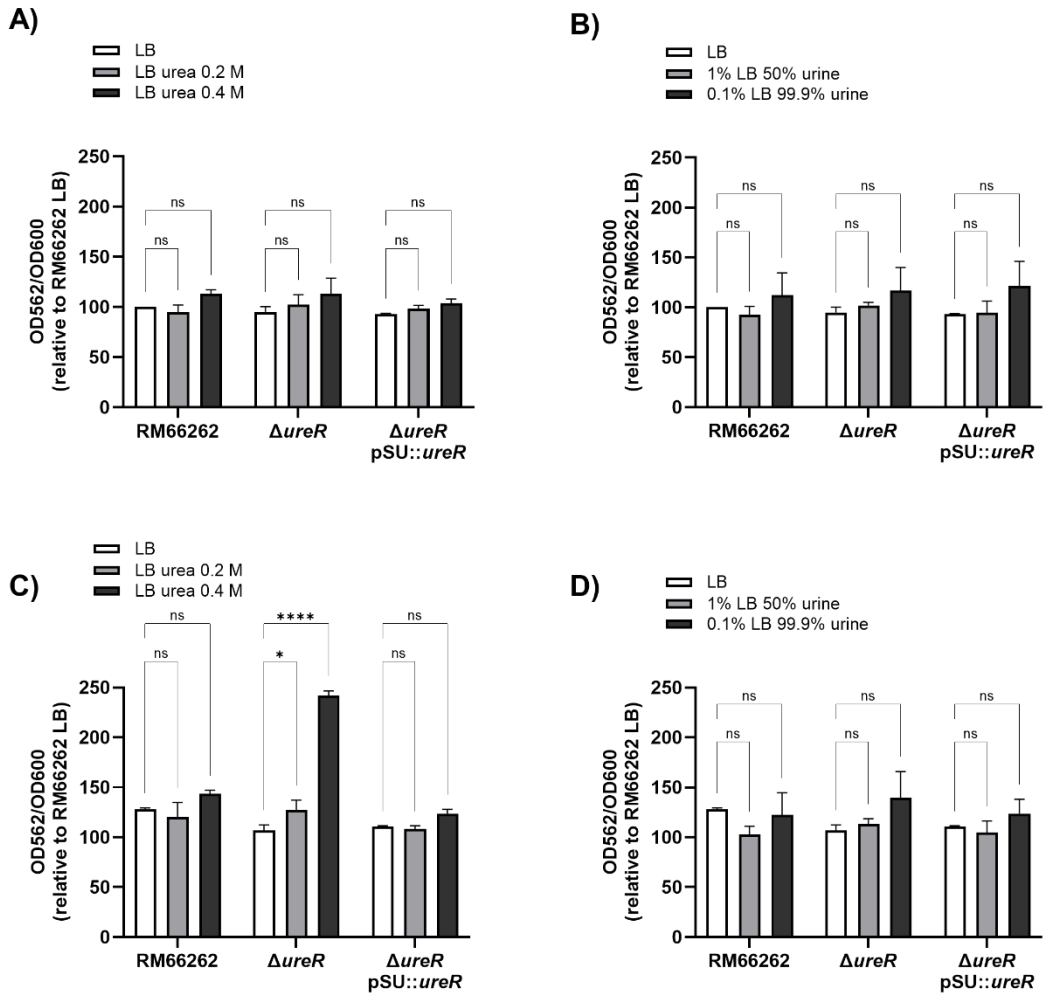


Figure S7. Kinetic monitoring of early biofilm formation (24 h) and maturation phases (48 h) under urea and urine stress. Time-course quantification of biofilm production was evaluated at early incubation stages to capture initial regulatory effects. Cultures of the wild-type strain (RM66262), the Δ*ureR* mutant, and the complemented strain (Δ*ureR* pSU36::*ureR*) were grown statically at 37 °C for 24 h **(A, B)** or 48 h **(C, D)**. Media conditions consisted of baseline LB or LB supplemented with 0.2 M and 0.4 M urea **(A, C)**, and 1% LB / 50% human urine or 0.1% LB / 99.9% human urine **(B, D)**. Biofilm mass was determined via crystal violet staining (OD562/OD600). For direct kinetic comparisons across the entire time-course spectrum, all data points across both time intervals and media compositions were normalized relative to the values obtained for the wild-type strain cultivated in non-supplemented LB broth at 24 h (arbitrarily set to 100%). Means ± SDs from three independent experiments are shown. Statistical significance was evaluated using a two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Asterisks indicate significance thresholds: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$; ns, not significant. All statistical operations were processed using GraphPad Prism (GraphPad Software, San Diego, CA, USA).

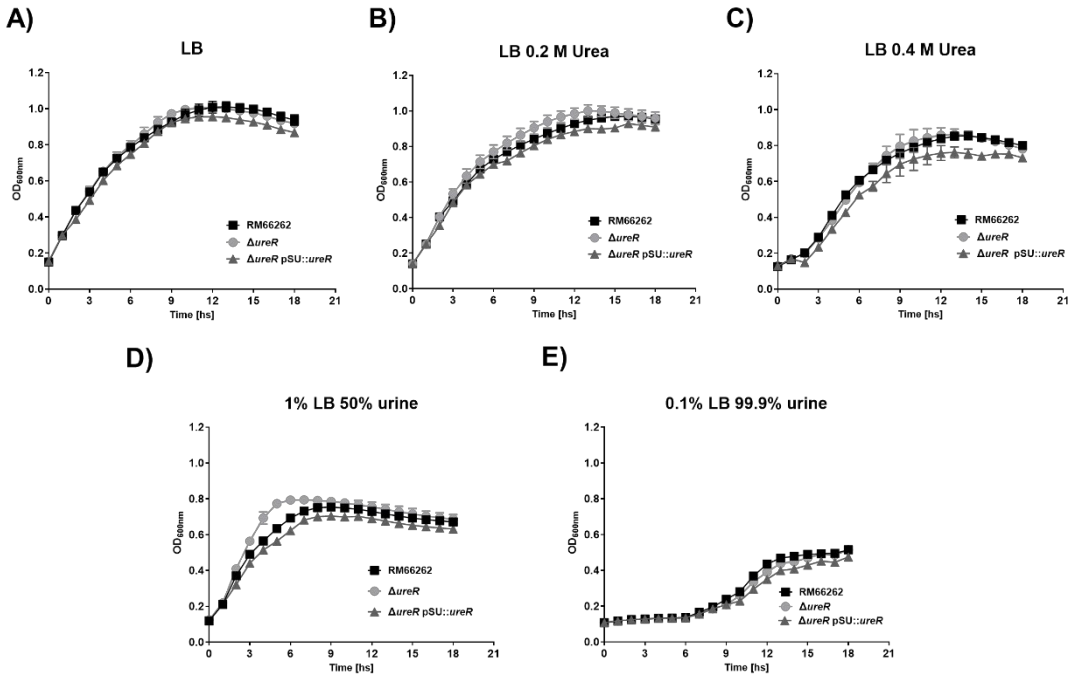


Figure S8. Growth curves. Bacteria were grown for 18 h in LB (A) or LB supplemented with 0.2 (B) or 0.4 M urea (C) or in LB, 1 % LB 50 % urine (D), or 0.1 % LB 99.9 % urine (E), in 96-well microplates, at 37°C with agitation. Growth is shown as OD 600 nm values from the *S. marcescens* RM66262 wild-type, Δ*ureR,* and Δ*ureR* pSU36::*ureR* strains. Means ± SDs from three independent experiments performed in duplicate in each case are shown.

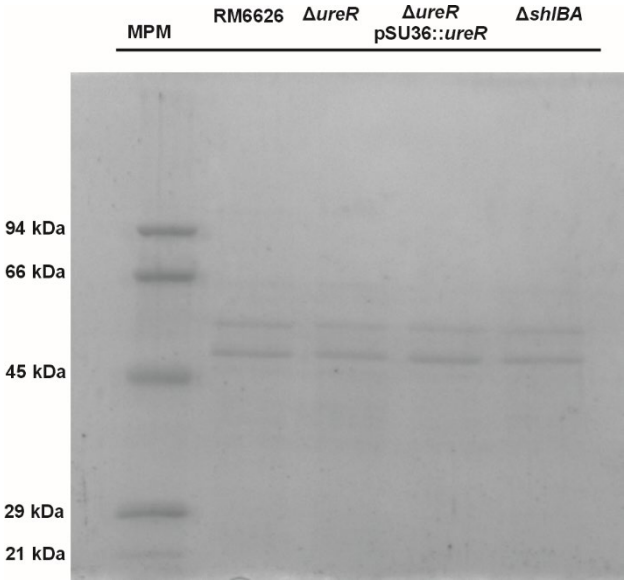


Figure S9. Loading control for ShlA Western blot. SDS-PAGE 10 % (Coomassie staining) of the same extracellular protein samples from wild-type RM66262, Δ*ureR* and complemented (Δ*ureR* pSU36::*ureR*) *S. marcescens* strains used for the anti-ShlA Western blot shown in Fig. 5G, confirming comparable total protein loading across lanes.

References

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