The multi-output incoherent feedforward loop constituted by the transcriptional regulators LasR and RsaL confers robustness to a subset of quorum sensing genes in *Pseudomonas aeruginosa*

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Running Head: Regulatory properties of the *las* QS system

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SUPPLEMENTAL MATERIAL

Table S1. Bacterial strains used in this study

Table S2. Plasmids used in this study

Table S3. Oligonucleotides used in this study

Table S1. Bacterial strains used in this study

Strains	Relevant characteristics	Reference/Source
E. coli		
S17.1 λ*pir*	Conjugative strain for suicide plasmids.	*39*
SM10 (pFLP2)	Strain carrying the pFLP2 plasmid (FLP; Ap^R/Cb^R) for Flp-mediated recombination.	*43*
P. aeruginosa		
PAO1	wild type strain ATCC15692.	ATCC
PA14-R3	Biosensor strain for $3OC_{12}$-HSL quantification.	*49*
Δ*vfr*	PAO1 mutant strain with in frame clear deletion of the *vfr* gene.	*37*
Δ*rsaL*	PAO1 mutant strain with in frame clear deletion of the *rsaL* gene, obtained by using the pDM4Δ*rsaL* plasmid (Table S2).	This study
Δ*rsaL*Δ*vfr*	PAO1 double mutant strain with in frame clear deletion of both the *rsaL* and *vfr* genes, obtained by using the pDM4Δ*rsaL* plasmid (Table S2) in PAO1 Δ*vfr*.	This study
Δ*lasR*	A 344 bp region encompassing the *lasR* gene, corresponding to nucleotides 1558474-1558819 of *P. aeruginosa* PAO1 genome, has been deleted from PAO1 chromosome by using the pEX18Δ*lasR* plasmid (Table S2).	This study
Δ*lasR*Δ*rsaL*	A 685 bp region encompassing the *lasR* and *rsaL* gene, corresponding to nucleotides 1558473-1559112 of *P. aeruginosa* PAO1 genome, has been deleted from PAO1 chromosome by using the pDM4Δ*lasR*Δ*rsaL* plasmid (Table S2).	This study
$lasR^{ind}$	PAO1 Δ*lasR* mutant strain carrying the *araC*-P_{BAD}*lasR* transcriptional fusion for L-arabinose-dependent induction of *lasR*, integrated into the chromosome. Generated by using the mini-CTX1-*araC*-P_{BAD}*lasR* (Table S2).	This study
Δ*rsaL* $lasR^{ind}$	PAO1 Δ*lasR*Δ*rsaL* mutant strain carrying the *araC*-P_{BAD}*lasR* transcriptional fusion for L-arabinose-dependent induction of *lasR*, integrated into the chromosome. Generated by using the mini-CTX1-*araC*-P_{BAD}*lasR* (Table S2).	This study

Table S2. Plasmids used in this study

Plasmid	Relevant characteristics and plasmid construction	Reference/Source
pDM4	Suicide vector for construction of deletion mutants; *sacBR*; *oriR6K*; Cm^R.	*42*
pDM4Δ*rsaL*	pDM4-derivative plasmid for in frame clear deletion of the *rsaL* gene. The upstream (UP) and the downstream (DW) DNA regions of *rsaL* were PCR amplified from *P. aeruginosa* PAO1 genome using primers FW61G/RV62G and FW63G/RV64G, respectively (Table S3). The UP and DW PCR fragments were subsequently cloned in pDM4 by XhoI-BamHI and BamHI-XbaI restriction, respectively.	This study
pEX18Δ*lasR*	pEX18Tc-derivative plasmid for deletion of the *lasR* gene from nucleotides 1558474-1558819 of *P. aeruginosa* PAO1 genome.	*44*
pDM4Δ*lasR*Δ*rsaL*	pDM4-derivative plasmid for deletion of the *lasR* and *rsaL* genes from nucleotides 1558473-1559112 of *P. aeruginosa* PAO1 genome. The upstream (UP) and downstream (DW) regions of the genetic *locus* containing the *lasR* and *rsaL* genes was PCR amplified from *P. aeruginosa* PAO1 genome using primers FW675/RV765 and RV62G/FW61G, respectively (Table S3). The UP and DW PCR fragments were subsequently cloned in pDM4 by SpeI-BamHI and BamHI-XhoI restriction, respectively.	This study
mini-CTX1-*araC*-P_{BAD}*tolB*	mini-CTX1-derivative plasmid carrying the *araC*-P_{BAD}*tolB* cassette from pBEM9, cloned by XhoI/EcoRI restriction.	*70*
mini-CTX1-*araC*-P_{BAD}*lasR*	mini-CTX1-*araC*-P_{BAD}-derivative plasmid containing a 720 bp DNA fragment encompassing the *lasR* gene. *lasR* was PCR amplified from *P. aeruginosa* PAO1 genome using primers FW763 and RV764 (Table S3) and cloned in the mini-CTX1-*araC*-P_{BAD}*tolB* plasmid by HindIII-EcoRI restriction.	This study
pMP190	Promoter probe vector; IncQ; 15 kb; Sm^R Cm^R.	*71*

Plasmid	Relevant characteristics and plasmid construction	Reference/Source
pP*lasI*190	A DNA fragment encompassing the *lasI* promoter region (P*lasI*) was PCR amplified and cloned in the pMP190 plasmid upstream of the promoterless *lacZ* gene.	*19*
pP*lasR*190	A DNA fragment encompassing the *lasR* promoter region (P*lasR*) was PCR amplified from *P. aeruginosa* PAO1 genome with primers FW324 and RV325 (Table S3) and cloned by SalI-BglII restriction in the pMP190 plasmid upstream of the promoterless *lacZ* gene.	This study

Table S3. Oligonucleotides used in this study

Name	Sequence (5'-3') [a]	Position [b]	Restriction site
FW324	NNNACGCGTCGACGGTCGCACGCGTGGCG	1557812	SalI
RV325	NNNNGGAAGATCTCAACCAAGGCCATAGCGC	1558166	BglII
FW675	GGACTAGTACCTATGCGCCGCCGTTG	1557572	SpeI
RV676	CGCGGATCCAGCGCTACGTTCTTCTTAAACT	1558149	BamHI
RV765	GCGGGATCCACTCGTGCTGCTTTCGCGT	1558454	BamHI
FW763	TATAAGCTTATGGCCTTGGTTGACGGTTT	1558171	HindIII
RV764	TATGAATTCTCAGAGAGTAATAAGACCCAAA	1558869	EcoRI
FW61G	CCGCTCGAGCGCATCGCCTCCAGCGT	1559614	XhoI
RV62G	TATGGATCCGTGTGAAGCCATTGCTCTG	1559111	BamHI
FW63G	TATGGATCCTTGCATTTCTATATAGAAGG	1558932	BamHI
RV64G	TGCTCTAGACTGGGAACCGTCCATCTAC	1558441	XbaI

[a] Engineered restriction sites are underlined.

[b] Position with respect to *P. aeruginosa* PAO1 chromosome (www.pseudomonas.com). [72]

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[a] Please note that other references cited in this Supplemental Material file are listed in the Reference Section of the manuscript main body.