SUPPLEMENTARY Information

Detailed description of the materials and methods used:

Bacterial strains, plasmids, media and growth conditions. The bacterial strains and the plasmid vectors and their derivatives used in this study are summarized in Table S1. *E. coli* and *P. aeruginosa* strains were routinely propagated at 37 °C on Luria-Bertani (LB) agar or LB broth (1) and all liquid cultures were grown with shaking (225 rpm). When required, antibiotics were used in selective media at the following concentrations (μg ml^{-1}) for *E. coli*: tetracycline (Tc) 30, ampicillin (Am) 200 and chloramphenicol (Cm) 25; for *P. aeruginosa*: carbenicillin (Cb) 200, gentamicin (Gm) 100 and tetracycline 50.
For experiments assessing the effect of temperature, PPGAS medium (phosphate-limited-peptone-glucose-ammonium) (2) was inoculated to an OD_{600} of 0.1 with *P. aeruginosa* overnight cultures grown in LB supplemented with the appropriate antibiotics and incubated at 30°C or at 37 °C. For assays of β- galactosidase activity, bacterial cultures were monitored spectrophotometrically by measuring their optical density at 600 nm (OD_{600}) at 2 h intervals for 24 h (Fig. S6).

DNA manipulations and genetic techniques. DNA manipulations were carried out as described elsewhere (3). The plasmid DNA was prepared by a Qiaprep miniprep kit (Qiagen). DNA digestion and ligation were performed according to manufacturer's instructions (New England Biolabs). Polymerase chain reactions (PCR) were carried out using high fidelity DNA polymerase (Phusion Hot Start II) as specified by the manufacturer (Thermo Scientific). The PCR products were purified by Qiaquick PCR purification kits or Qiaquick gel extraction kits (Qiagen). DNA sequencing reactions for all constructs were undertaken by the DNA sequencing and oligonucleotide synthesis unit at the Instituto de Biotecnología, UNAM.
The oligonucleotides used are listed in Table S4. Restriction sites were added at the 5' ends of primers to facilitate the cloning of the PCR products. A fragment corresponding to the promoter region of *lecA* (nucleotides −445 to +63) was amplified by PCR using PAO1 genomic DNA as the template and the primers lecAF/lecAR. The resulting fragment was cloned into pJET1.2/blunt in accordance with the manufacturer's instructions (Fermentas) and then excised by digestion with *Kpn*I and *Xho*I. The 508-bp

product was eluted from an agarose gel after electrophoresis, followed by cloning into the *Kpn*I and *Xho*I restriction sites of the promoter-less pLP170 vector (4) to generate a transcriptional fusion to the *lacZ* gene, plasmid pLECA-70. For construction of the pHZA1-20 plasmid, a 1,782 bp fragment from nucleotide +244 of the *phzM* coding region (divergent to *phzA1*), that is equivalent to nucleotide -696 with respect to *phzA1*, to nucleotide +81 relative to the *phzA1* translation start codon was generated by PCR amplification using the primers phzA1EcoRI/phzA1KpnI. This fragment was cloned in pJET1.2/blunt, obtained by digestion and cloned into the *Eco*RI and *Kpn*I restriction sites of pMP220 vector (5). The resulting plasmids were introduced into *E. coli* and *P. aeruginosa* strains as described previously (6, 7).

Plasmid construction. The inserts for plasmids pBO-*rhlA*, pBO-*rhlA*(ΔG78) and pBO-*rhlA*(G92A) were constructed by annealing single-stranded oligonucleotides. Briefly, 10 pmol µl-1 of each of the following pairs of oligonucleotides: rhlA-pBADSc/rhlA-pBADaSc, rhlA-Sc-G78/rhlAaSc-G78 or rhlA-S-G92A/ rhlA-aS-G92A (Table S4) were mixed and heated at 60 °C for 30 min and annealed by slowly cooling down to room temperature. The resulting double stranded oligonucleotide was then ligated into *Nhe*I-*Eco*RI digested pBAD-18lacZ841 (Table S1). To construct plasmid pBOC-*rhlA* and pBOC*rhlA*(G92A), primers rhlA-FL-WT/rhlA-RLc-WT and rhlA-FL-WT/rhlA-RL-G92Ac (Table S4) were used to amplify a fragment of 110 bp corresponding to the complete ROSE-element present in the *rhlA* 5' UTR. PCR products digested with *Eco*RI and *Nhe*I were cloned into the corresponding sites of pBAD-18lacZ841. Plasmid pBO-*rhlA,rhlB* was generated by PCR amplification of a fragment containing the *rhlA* coding region with the small version of the ROSE-element and following an in-frame *lacZ* fusion starting with the AUG codon of *rhlB* using the rhlATSpBAD18/rhlB-TRpBAD18 primers (Table S4). To create pBO-Δ*rhlA,rhlB*, plasmid pBO-*rhlA,rhlB* purified from *E. coli* strain IBEC58 (8) was digested with *Msc*I in order to eliminate an internal fragment within the coding region of the *rhlA* gene (+548 to +874). Site-directed mutagenesis to generate plasmid pBOC-*rhlA*(ΔG78) with a deletion of the unpaired G78 was performed using the QuikChange XL site-directed mutagenesis kit (Agilent Technologies) according to the manufacturer's instructions. Plasmid pBOC-*rhlA* was used as the template and the mutagenesis was done with the primers rhlA-lΔG78F/rhlA-lΔG78R (Table S4). After verification of the DNA sequences, plasmids were transformed into *E. coli* DH5α.

Transformants were selected on LB agar plates containing ampicillin (200 µg ml^{-1}) and X-gal (40 µg ml^{-1}) in the presence of 0.04% (w/v) L-arabinose. All β-galactosidase assays for *E. coli* carrying pBAD-18*lacZ*481 *rhlA* fusions were carried out in cells grown to exponential growth phase at 30 °C (OD_{600} 0.5). L-arabinose was added to 0.04% and one sample of 25 ml was maintained at 30 °C. Another sample of equal volume was transferred to 37 °C, and both samples were incubated for 60 min.

To construct plasmid pBOC-*lasI* and pBOC-*lasI*[C84A], primers lasI-Ter Fw/lasITer-Rv and lasI-C84A-Fw/lasI-C84A-Rv (Table S4) were used, respectively. Cloning into pBAD-18lacZ841 and site-directed mutagenesis was undertaken as described above for the *rhlA* ROSE-element. The β-galactosidase activities for *P. aeruginosa* cells carrying the different promoter-probe plasmids (Table S1) were determined spectrophotometrically using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as the substrate, normalized to the OD_{600} of the bacterial culture and expressed in Miller Units (1).

To determine the effect of growth temperature on the expression of different promoter probe constructs, *E. coli* DH5α was transformed with the plasmids and grown at 30 °C in LB medium until the exponential phase (optical density of 0.5 at 600 nm). Subsequently, L-arabinose was added to the culture to induce transcription at a concentration of 0.04% (unless in experiments shown in figure S6 where 0.01% was used). Then, half of the culture was maintained at 30 °C (white bars) and the other half was transferred to 37 °C (black bars) for 60 minutes before measuring β-galactosidase activity. The data are expressed as the mean of at least three independent experiments, each performed in duplicate.

β-galactosidase assays for *E. coli* carrying pBAD-18*lacZ*481 *lasI* fusions were performed using Fluorescein di-β D-galactopyranoside (FDG) as substrate. Briefly, 15 ml of LB was inoculated to an OD_{600} of 0.01 with overnight cultures in LB supplemented with the appropriate antibiotics and incubated at 30 °C and 37 °C. The cells were grown to the exponential growth phase (OD_{600} 0.5) and were cooled on ice. β-galactosidase activity was assayed by transferring 20 µl of each culture to the wells of a black 96-well microplate, previously charged with stock solutions of FDG (150 mM in 8:1:1 H2O/DMSO/ethanol). All measurements were performed in a 200 µl total volume. The samples were incubated at 30 °C and 37 °C for 12 h. Blanks and controls without cells, substrate or inducer were used to eliminate signal background. Fluorescence was

determined with a FL600 fluorescence microplate reader (BioTek Instruments) controlled by an external PC running the data reduction software package Gen5 (BioTek Instruments). Fluorescein production by the hydrolysis of FDG was monitored using a fluorescein filter set (Ex 485±20/Em 528±20) in the plate reader. The sensitivity setting was varied as required and the data were collected from the bottom. All assays were performed in triplicate. The results, normalized to OD_{600}, were plotted as relative fluorescence units (RFU) and represent the average of triplicate determinations.

To generate plasmid pBOC-*lasR* (Table S1), oligonucleotides lasR-Ter-Fw/ lasR-Ter-Rv (Table S4) were used to amplify a fragment of 110 bp of the *lasR* 5' UTR. The PCR product was digested with *Eco*RI and *Nhe*I and cloned into the corresponding sites into pBAD-18lacZ841. To construct the negative control plasmid pBOC-*gyrA*, consisting of a sequence whose translation is not temperature-dependent (9), the primers gyrAEC-FW/gyrAEC-Rv (Table S4) were used. β-galactosidase assays for *E. coli* carrying the pBAD-18lacZ481 *gyrA* fusions were carried out as described above for *rhlA* fusions.

RNA extraction and quantitative real-time PCR (qPCR). To perform real-time quantitative PCR (qPCR) analysis, the bacteria were cultured and total RNA was purified as described for transcriptome analysis. For cDNA synthesis, 1 μg of RNA was reverse transcribed using a mix of specific reverse primers (Table S5) and the Revert-Aid H minus First Strand cDNA Synthesis kit (Thermo Scientific) according to the manufacturer's instructions. Differential expression of the *rhlA, rhlR, rhlI, lasI, lasB* and *phzA1* genes was examined by quantitative real-time RT-PCR using a Maxima SYBR Green/ROX qPCR Master Mix (2X) (Thermo Scientific) according to the manufacturer's instructions and a Light Cycler 480 II Instrument (Roche). Primer pairs were designed using the Primer3 program (10) and subjected to a BLAST analysis against the *P. aeruginosa* PAO1 genome sequence to eliminate the possibility of nonspecific binding. The cycling conditions used were one cycle of 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 60 s. The data were acquired during the 60 °C annealing step. Each analysis was performed at least three times from three independent cultures. As a reference gene to normalize the results, we used *lasR*, because its expression was very similar at both temperatures tested and its concentration was not affected by

temperature. The quantification technique used to analyze data was the $2^{-\Delta, \Delta CT}$ method (11).

Microarray experiments and data analysis. For microarray analysis of mRNA concentration, cells were grown in 15 ml of LB at 37 °C with shaking for 16 h. Overnight cultures were diluted to an OD_{600} of 0.05 in PPGAS medium and grown at 25 °C and 37 °C. Samples were harvested at an OD_{600} of 1.5 (Fig. S5) and the cells from 2.0 ml of each culture were suspended in 0.5 ml of fresh PPGAS media and added to 1.0 ml of RNA Protect bacteria solution (Qiagen). Total RNA was purified using RNAeasy spin columns (Qiagen) according to the manufacturer's instructions, and residual DNA was eliminated by DNase treatment using RNase-free DNaseI (Thermo Scientific). RNA quantity and quality were evaluated by spectrophotometry using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). RNA quality and integrity were confirmed on a denaturing agarose gel and RNA Nano chips in an Agilent Bioanalyzer 2100, respectively. cDNA synthesis, fragmentation, 3′-end labeling and hybridization to Affymetrix GeneChip *P. aeruginosa* genome arrays were carried out by a commercial Affymetrix Genechip service supplier (Affymetrix, Santa Clara, CA). Washing, staining and scanning of the microarrays were performed with the Affymetrix fluidic station. All experiments were performed in triplicate. Data analyses from three biological replicates for each of the conditions were performed after normalizing and summarizing probe level measurements using a Robust Multiarray Average (RMA). Only genes that fit stringent criteria (expression cutoff: 50–100% stringency; p-value ≤0.01 of one-way ANOVA data corrected by Benjamini Hochberg FDR; fold-change ≥1.4) were selected for further analysis. The microarray data were analyzed using the microarray software package Partek Genomic Suite 6.5 (Partek Inc., St. Louis, MO). The fold change was considered to be significant when the pair PAO1-25 °C versus PAO1-37 °C was at least 1.4-fold or greater up- or down-regulated ($p \leq 0.05$). The list of genes whose expression was significantly altered is shown in Table S2. The microarray data is MIAME compliant and has been deposited in NCBI's Gene Expression Omnibus (GEO) and is accessible through GEO Series accession number GSE45695.

SDS gel electrophoresis and western blot analysis. For the preparation of crude cell extracts, cultures were grown in PPGAS medium at 30° C and 37 °C to an OD_{600} of 1.5.

The cells were harvested by centrifugation and re-suspended in PA buffer (10 mM sodium phosphate buffer, 30 mM NaCl, 0.25% Tween-20, 10 mM EDTA, 10 mM β-mercaptoethanol, pH 7.5) prior to cell disruption through sonication. Cellular debris was removed by centrifugation (13,000 x *g* for 15 min at 4 °C) and the supernatant (containing the soluble protein fraction) was mixed 1:1 v/v with Laemmli loading buffer (12). Total protein concentration was determined using a protein assay kit (Bio-Rad) with bovine serum albumin as the standard. Equal amounts of protein (30 µg) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the gels were electro-transferred onto Hybond-C Extra nitrocellulose membranes (Amersham Biosciences). The membrane was blocked by 5% nonfat milk and incubated with a 1:1000 dilution of the rabbit anti-RhlR, anti-LasR or anti-RhlA polyclonal antibodies.

Goat anti-rabbit immunoglobulin G (Santa Cruz Biotechnology) secondary antibody conjugated to horseradish peroxidase was used at a 1:10,000 dilution. Detection was performed using the chemiluminescence-based system Super-Signal West Femto (Pierce) followed by exposure to X-ray film (Amersham Biosciences) for autoradiography.

Virulence factors production. Pyocyanin was extracted from culture supernatants and measured as previously described based on its absorbance at 520 nm in acidic solution (13). The pyocyanin assay is based on the absorbance of pyocyanin at 520 nm in acidic solution. A 5 ml sample of culture supernatant was extracted with 3 ml of chloroform and then re-extracted into 1 ml of 0.2 N HCl to give a pink to deep red solution. The absorbance of this solution was measured at 520 nm. Concentrations, expressed as µg of pyocyanin produced per ml of culture supernatant, were determined by multiplying the optical density at 520 nm (OD_{520}) by a factor of 17.072.

The concentration of rhamnolipids in the sample was determined by the orcinol method (14). A 333 µl portion of the filtered supernatant was extracted twice with 1 ml of diethyl ether. The diethyl-ether extract was evaporated to dryness and dissolved in 1 ml of deionized water. To 100 µl of each sample, 900 µl of a solution containing 0.19% orcinol (in 53% sulfuric acid) was added. The samples were heated at 80 °C in a water-bath for 30 min and cooled for 15 min at room temperature. The A_{421} was measured.

The concentrations of rhamnolipids were determined by comparing the data with a standard curve obtained with L-rhamnose standards between 0 and 50 µg ml^{-1}.

The elastolytic activity of LasB elastase was determined using Elastin Congo Red (ECR) as substrate; the procedure was modified from that described previously (15). Briefly, the cells were grown to anOD_{600} of 1.5 in LB broth at 30 °C and 37 °C and samples of the filter-sterilized supernatants were diluted 1:10 with LB and 1 ml was added to 10 mg of ECR (Sigma) in glass tubes. The mixture was incubated at 37 °C for 16 h with constant shaking (225 rpm). The insoluble substrate was pelleted by centrifugation (13,000 x *g* for 10 min at 4 °C) and the absorbance of the supernatant was measured at 495 nm with a spectrophotometer using an ECR sample incubated with medium alone as the blank. The experiment was performed three times in triplicate with supernatants from three independent cultures. The values from the triplicate experiments were averaged and used as one value to represent each of the three experiments.

***N*-Acyl-Homoserine Lactone (AHL) extraction and analytical thin-layer chromatography.** To evaluate the profiles of AHLs, cells were grown in PPGAS medium at 30 °C and 37 °C for 24 h (Fig. S5). A 10 ml sample of culture supernatant was extracted twice with equal volumes of acidified ethyl acetate and then dried in a fume hood. The residues from the extraction were then dissolved in 100 µl ethyl acetate and a 5 µl sample was analyzed by thin layer chromatography (TLC). Analytical TLC was performed on reverse phase aluminium-backed RP18 F254S TLC plates (20 cm X 20 cm; Merck) (16). The chromatograms were developed with methanol:water (60:40, v:v) and were then air-dried in a fume hood. The TLC plate was then overlaid with a thin film of agar seeded with the AHL reporter strain *Chromobacterium violaceum* CV026 that produces the purple-colored violacein in response to AHLs with N-acyl side chains between 4 and 8 carbons in length (17). After incubation of the plate at 30 °C for 24 h, AHLs were located as purple spots on a white background. Alternatively, TLC plates were overlaid with a thin layer of agar seeded with a culture of an *E. coli* biosensor strain containing the lux-based bioluminescence AHL reporter plasmid (pSB1075). This reporter contains the *P. aeruginosa lasR* gene and the *lasI* promoter fused to *luxCDABE* from *Photorhabdus luminescens* and detects 3-oxo-substituted AHL derivatives with acyl chain lengths from 4 to 12 carbons (18). Light emission was

detected using a system for chemiluminescence detection Gel Logic 112 (Kodak). Each experiment was performed at least twice.

Computer methods. Sequences for computer analysis were retrieved from Pseudomonas Genome Database v 2 (http://www.pseudomonas.com). RNA secondary structures were predicted using Mfold version 3.2 (19) and the RNA-fold program of the Vienna RNA package (20).

Statistical analysis. Student's t-test was used to analyze statistical differences between samples grown at two different temperatures. A *p*-value of <0.05 was considered statistically significant.

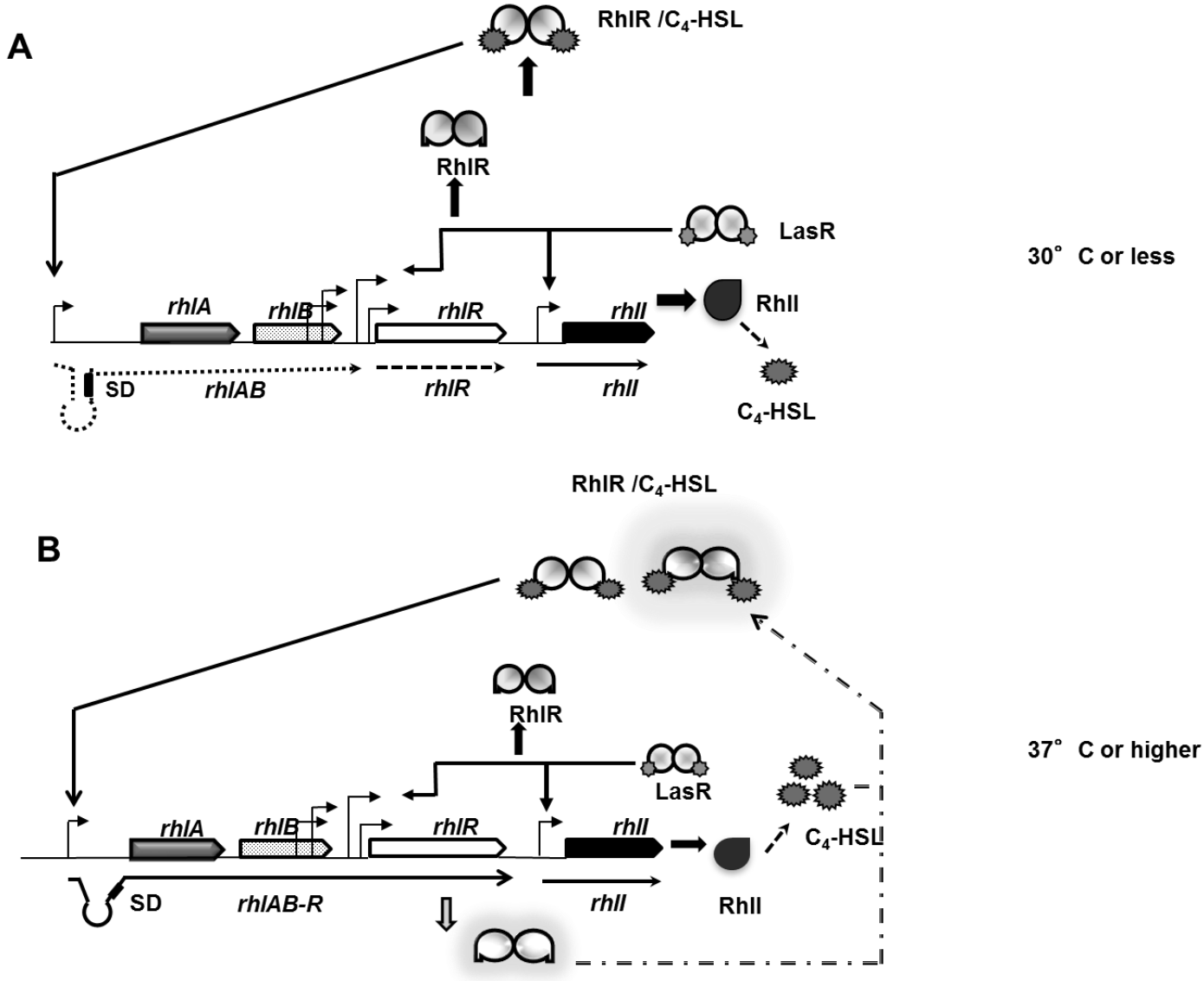

Figure S1. Schematic representation of *rhlR* transcriptional regulation from its five promoters and the role of the ROSE-element at 5' *rhlA* UTR in the autoregulatory loop present at 37 ºC. Panel A shows the pattern of expression at 30 ºC or lower, where the structure formed by the ROSE-element blocks the *rhlA* Shine-Dalgarno (SD) sequence. Panel B represents the expression of the *rhlAB-R* operon at 37 ºC or higher that results in an increased RhlR concentration due to the instability of the ROSE-element at this temperature and the exposure of the *rhlA* SD sequence.

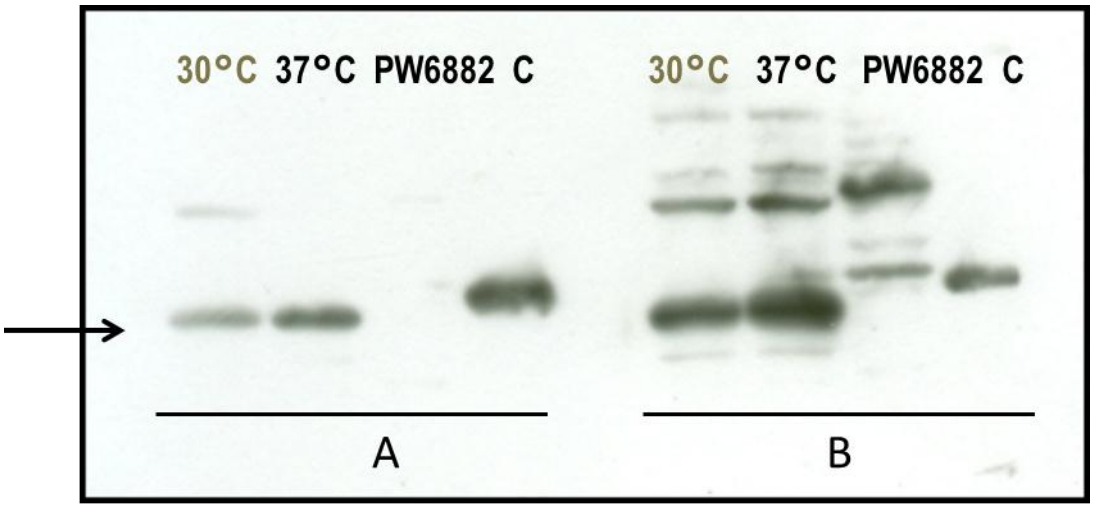

Figure S2. Effect of growth-temperature on RhlA intracellular concentration (30 °C and 37 °C) determined by western blot. RhlA concentration in extracts of PAO1 was determinate from cells grown to an OD_{600} of 1.5 in PPGAS medium at 30 °C and 37 °C. Panels A and B correspond to two separate determinations. The same amount of proteins was used in the case negative control [*P. aeruginosa* strain PW6882 which is an *rhlR* mutant]. Positive control (C) [*E. coli* (pET28RhlA)].

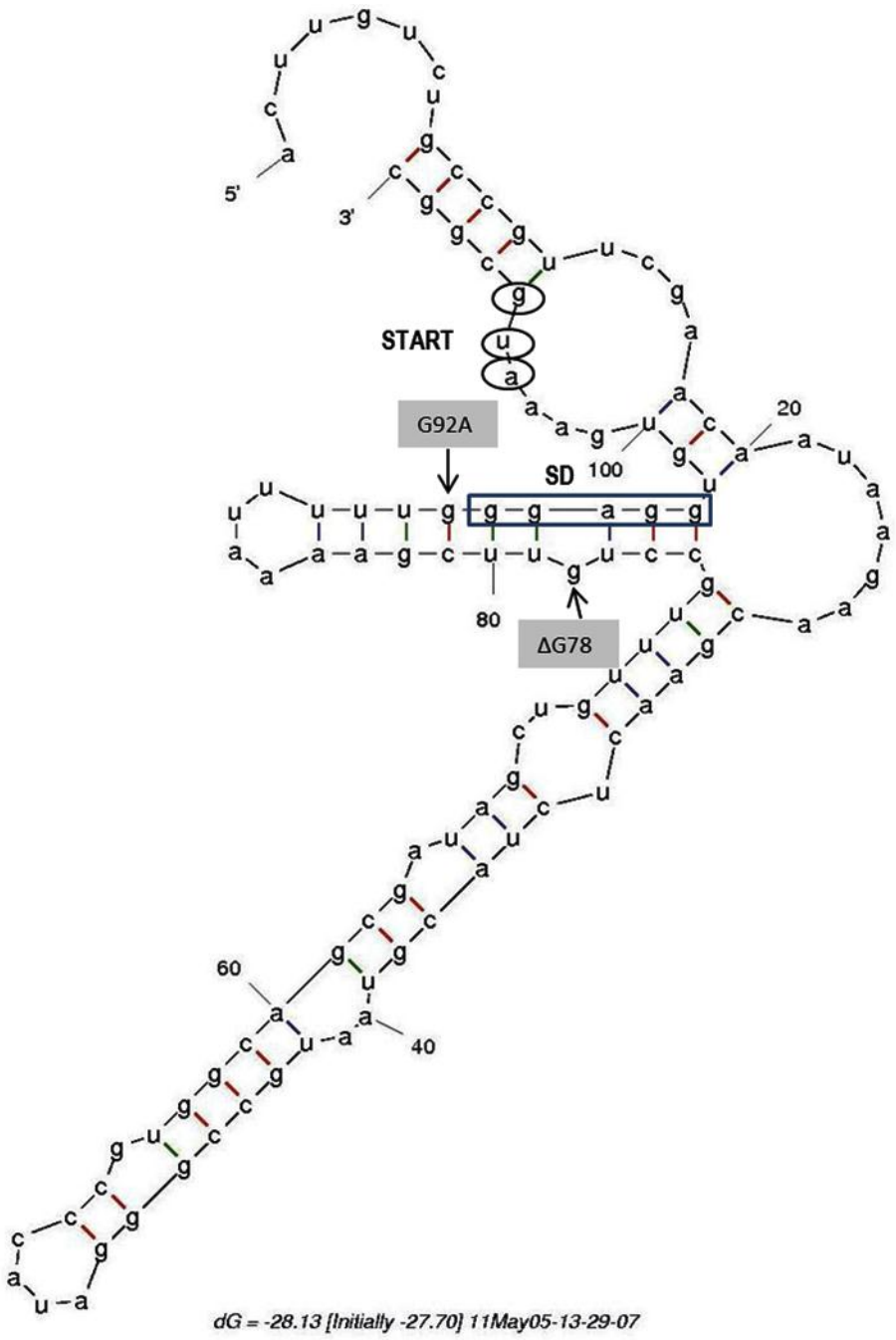

Figure S3A. Secondary structure of the ROSE element present in the 5' UTR of *rhlA*. The Shine-Dalgarno sequence (SD) and the translation start site (START) are shown. Mutations are described in the text.

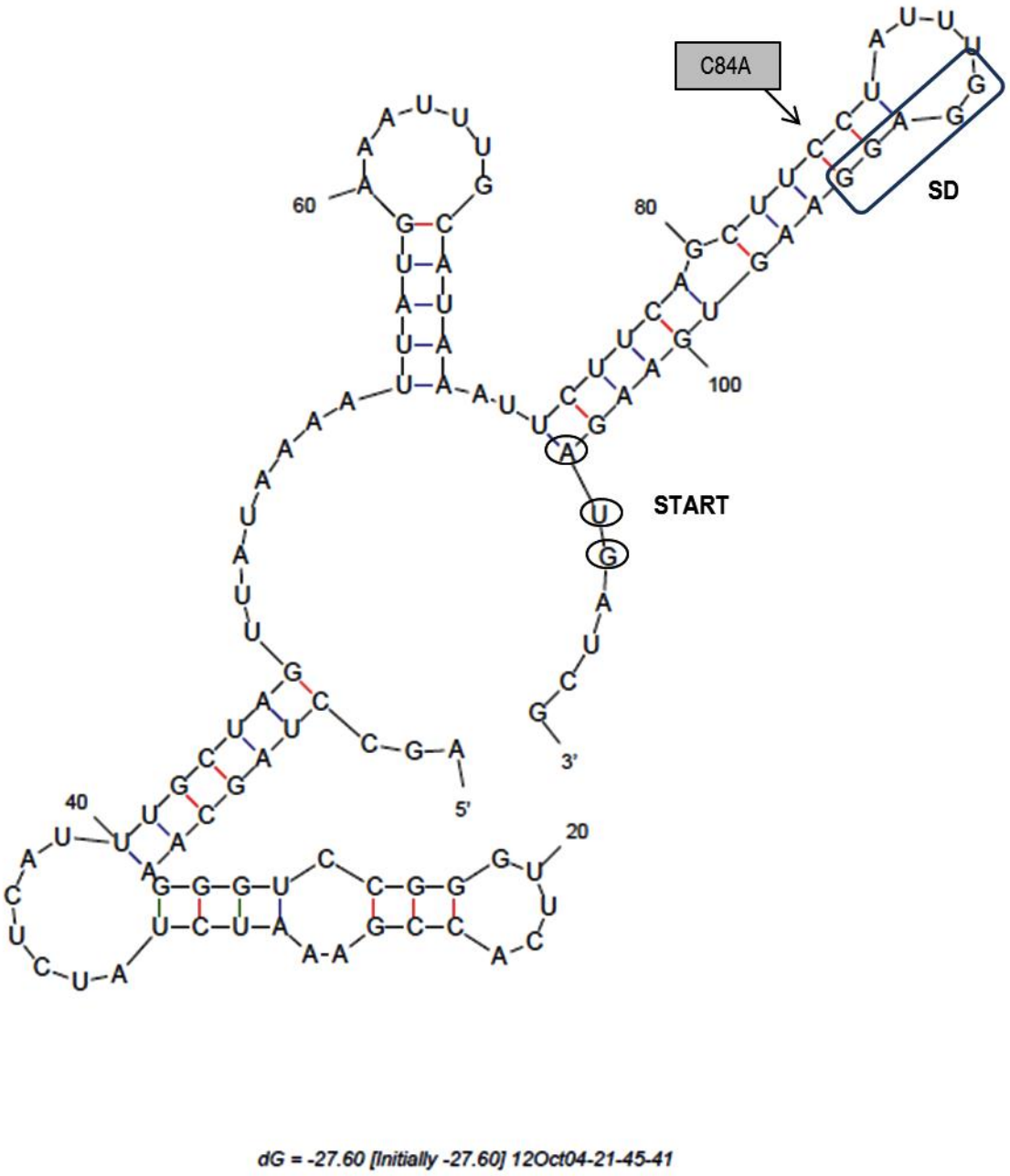

Figure S3B. Secondary structure of ROSE-like element present in the 5' UTR of *lasI*. The Shine-Dalgarno sequence (SD) and the translation start site (START) are shown. Mutations are described in the text.

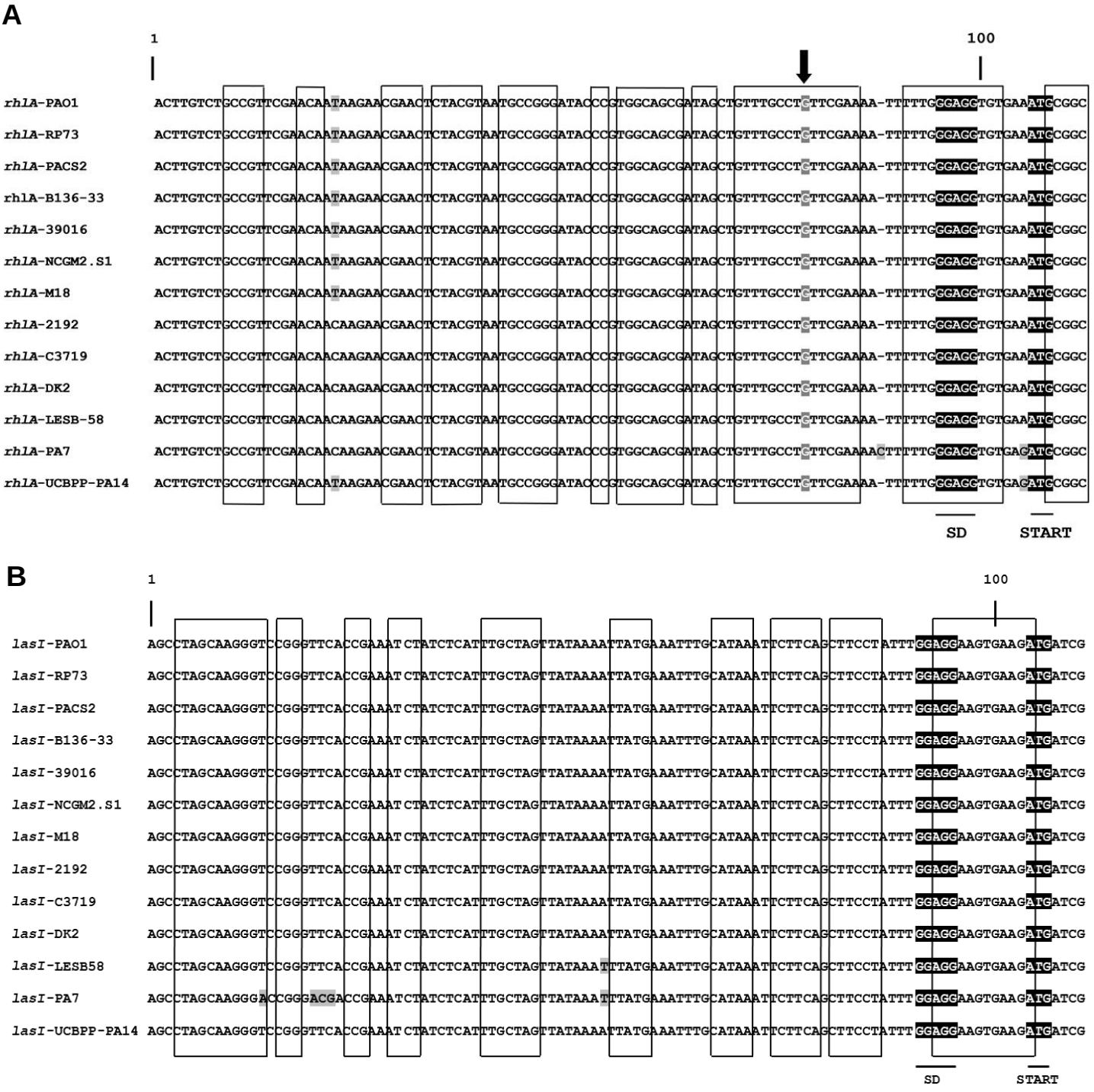

Figure S4. Alignment of the ROSE-like elements presents in the 5' UTR of *rhlA* (A) and *lasI* (B) in *P. aeruginosa* strains. The Shine-Dalgarno sequences (SD) and the translation start sites (START) are indicated. Boxes in the alignment mark regions involved in base pairing in the secondary structure prediction. The conserved G residue opposite to the SD sequence in the 5´UTR of *rhlA* is shaded and indicated by a filled arrow. Non-conserved residues in the alignment are shaded.

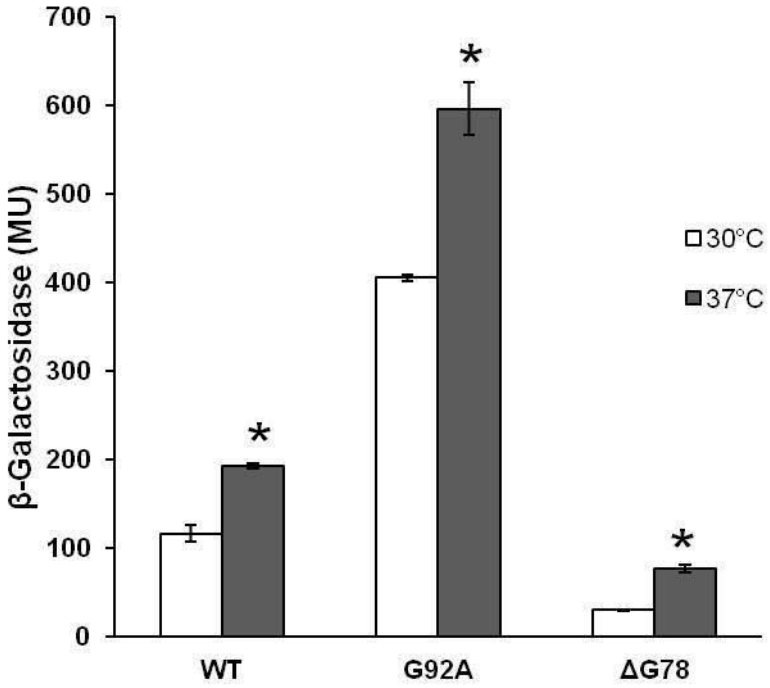

Figure S5. Characterization of the effect of RNA-thermometers present in the 5' UTR of *rhlA* in thermoregulation of gene expression. β-galactosidase activity levels of cells harboring plasmids with different versions of the complete 5´-UTR of *rhlA* fused to *lacZ* (pBOC-*rhlA*). Cells of *E. coli* DH5α transformed with the corresponding plasmids, were grown at 30 °C in LB until exponential phase (optical density of 0.5 at 600 nm). After addition of 0.04% L-arabinose, half of the culture was maintained at 30 °C (white bars) and the other half was transferred to 37 °C (grey bars) for 60 minutes before measuring β-galactosidase activity. An asterisk (*) indicates a value of $P < 0.05$ by Student's t test.

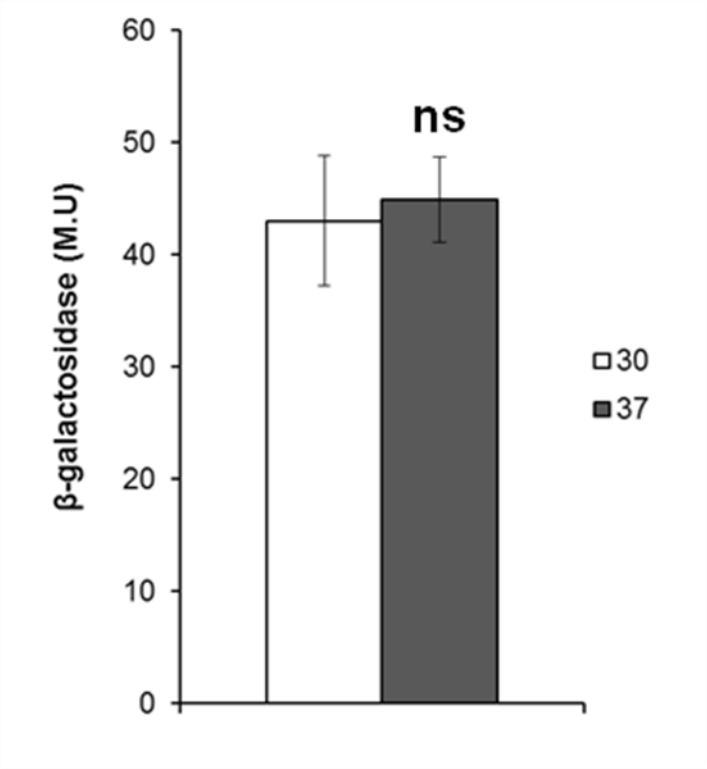

Figure S6. Characterization of the effect of the sequence present in the 5' UTR of *lasR* in thermoregulation of gene expression. β-galactosidase activity levels of cells harboring plasmid pBOC-*lasR*. Cells were grown as described for pBOC-*rhA*, except that L-arabinose addition was 0.01%. An asterisk (*) indicates a value of $P < 0.05$ by Student's t test; ns, means not significant.

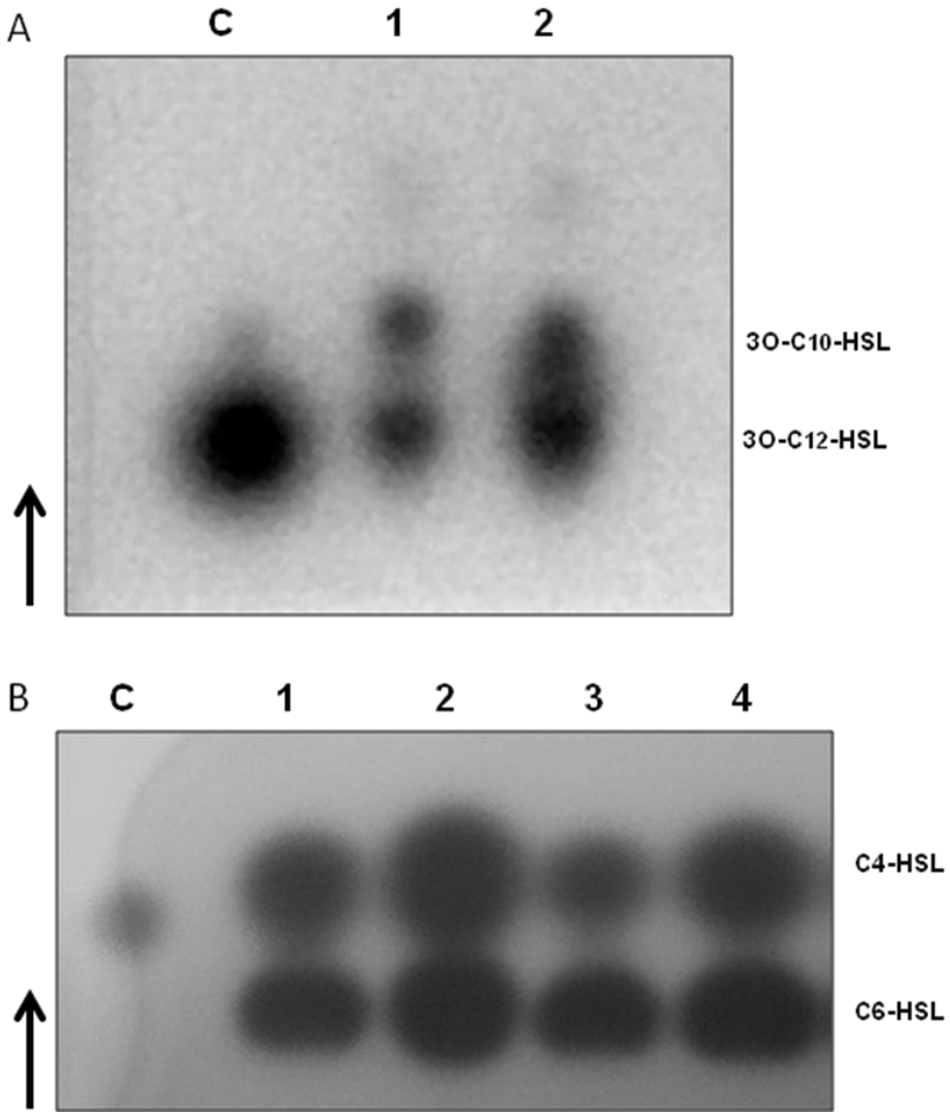

Figure S7. Temperature effect on autoinducers production by *P. aeruginosa* PAO1 and its *rhlR* mutant. In (A), the silica TLC plate was run in 60% methanol for 4 h and overlaid after chromatography with the long-chain AHL biosensor *E. coli* (pSB1075) and in (B) the plate was overlaid after chromatography with the short chain AHL biosensor *C. violaceum* CV026. (A) Lanes: C, positive control 3-oxo-C12-HSL (5µl of 10 mM standard); 1 and 2, extracts from cultures in PPGAS at 30°C and 37°C respectively. (B) Lanes: C, positive control C4-HSL (5 µl of 100 mM standard); 1 and 2, extracts from PAO1 cultures in PPGAS at 30 °C and 37 °C for 24 h, respectively; 3 and 4, extracts from cultures of *rhlR* mutant (PW6882) in PPGAS at 30 °C and 37 °C for 24 h, respectively. Direction of solvent migration is marked with an arrow.

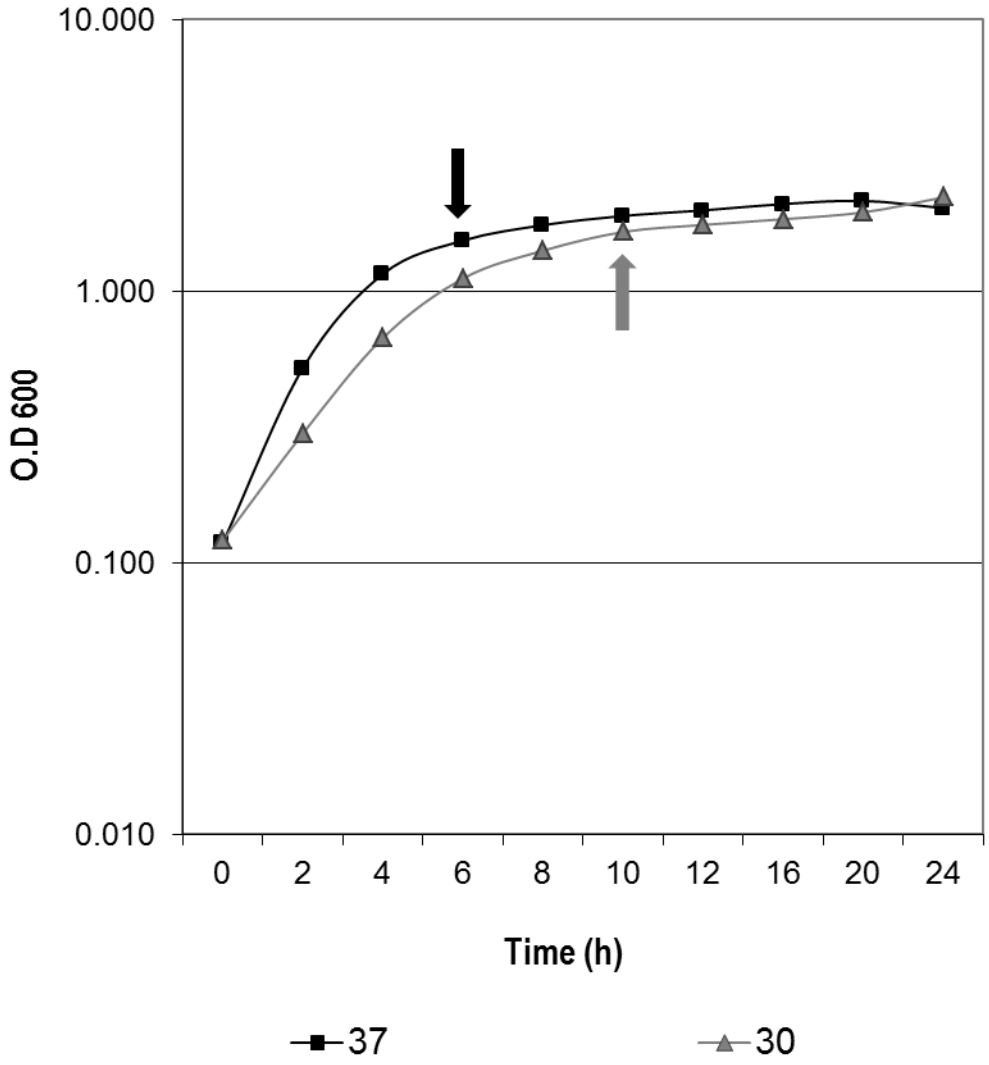

Figure S8. Growth-curve of *P. aeruginosa* PAO1 cultured at 30 ºC and 37 ºC in PPGAS medium. Arrows show the point where cultures reach an OD_{600} of 1.5.

Table S1. Bacterial strains and plasmids used in this work.

Bacterial strain or plasmid	Relevant characteristics	Reference
E. coli		
DH5α	*φ80ΔlacZΔM15Δ[lacZYA-argF]U169 endA recA1 hsdR17 deoR thi-1 supE44*	Invitrogen
IBEC58	*gyrA96 relA1 Δdam Δdcm ΔhsdRMS* derivative of BW25113. No DNA methylation	8
C. violaceum		
CV026	Double mini-Tn5 mutant derived from ATCC 31532, Kan^R, Hg^R, *cvil*::Tn5 *xyl*E, Str^R AHL biosensor, produces violacein pigment only in the presence of exogenous AHL	17
P. aeruginosa		
PAO1	Wild type strain of the MPAO1 subline	21
rhlA::Gm	PAO1 derived *rhlA* : : Ω mutant, Gm^r	22
PW6882	PAO1 *rhlR* : :Tn9lacZ mutant	21
PW8143	PAO1 *phzA1*::*ISphoA/hah* mutant	21
Plasmids		
pLP170	*lacZ* transcriptional fusion vector that contains an origin of replication for both *P. aeruginosa* and *E.coli,* Ap^R, Cb^R	23
pMP220	Broad-host-range, low-copy-number promoter-probe vector; IncP replicon,*lacZ* Tc^RTra-	5
pPCS1002	*rhlR* promoter region (nt -500 to +242) cloned in pLP170 to create a *rhlR´-lacZ* transcriptional fusion	23
pLECA-70	*lecA* promoter region (nt −445 to +63) cloned in pLP170 to create a *lecA´-lacZ* transcriptional fusion	This study
pHZA1-20	*phzM* (divergent to *phzA1*) coding region (nt + 244) and -696 to +81 pb of *phzA1* gene cloned in pMP220 to create *phzA1´-lacZ* transcriptional fusion	This study
pMPCG	0.8-kb *Hin*dIII-*Bam*HI fragment from plasmid pUO58 containing the *rhlAB* promoter cloned in pMP220 to create an *rhlA´-lacZ* transcriptional fusion	24
pUCP20	Expression vector with plac able to replicate in *P. aeruginosa* and *E.coli*; Ap^R, Cb^R	25
pGMYC	*plac-rhlR* cloned on pUCP20	24
pSB1075	*lasRlasI'* (*P. aeruginosa* PAO1)*::luxCDABE* (*Photorhabdus luminescens* [ATCC 29999]) fusion in pUC18 AmpR, AHL biosensor producing bioluminescence	18
pBAD18-*lacZ*481	Translational *lacZ* fusion vector, Apr	26
pBO-*rhlA*	*P. aeruginosa rhlA* small ROSE element (nt 74 to 109) fusion in pBAD18lacZ481	This study
pBO-*rhlA*[ΔG78]	*P. aeruginosa rhlA*ΔG78 ROSE element (nt 74 to 109) fusion in pBAD18lacZ481	This study
pBO-*rhlA*[G92A]	*P. aeruginosa rhlA*G92A ROSE element (nt 74 to 109) fusion in pBAD18lacZ481	This study
pBOC-*rhlA*	*P. aeruginosa rhlA* ROSE element (nt 1 to 109) fusion in pBAD18lacZ481	This study
pBOC-*rhlA[*ΔG78]	*P. aeruginosa rhlA* ΔG78 ROSE element (nt 1 to 109) fusion in pBAD18lacZ481	This study
pBOC-*rhlA*[G92A]	*P. aeruginosa rhlA* G92A ROSE element (nt 1 to 109) fusion in pBAD18lacZ481	This study
pBO-*rhlA,rhlB*	*P. aeruginosa rhlA* coding region with small version of the ROSE element cloned in frame with *lacZ.* Fusion starts with the AUG codon of *rhlB* in pBAD18lacZ481	This study
pBO-Δ*rhlA,rhlB*	pBO-*rhlA,rhlB* with a deletion of an internal *rhlA* fragment (nt +548 to +874)	This study
pBOC-*lasI*	*P. aeruginosa lasI* ROSE-like element (nt 1 to 109) fusion in pBAD18lacZ481	This study
pBOC-*lasI* [C84A]	*P. aeruginosa lasI* C84A ROSE-like element (nt 1 to 109) fusion in pBAD18lacZ481	This study
pBOC-*gyrA*	*E. coli gyrA* fusion in pBAD18lacZ481	9
pBOC-*lasR*	*P. aeruginosa lasR* 5' UTR (nt 1 to 110) fusion in pBAD18lacZ481	This study

Table S2. Transcripts which were differentially regulated at 25°C and 37°C in PAO1, grown in PPGAS media (>1.4-fold).

ORF	Gene	Gene product [a]	p-value (37 vs. 25)	Fold-Change (37 vs. 25)
PA0672	*hemO*	heme oxygenase	1.84E-06	1.55
PA4221	*fptA*	Fe(III)-pyochelin outer membrane receptor precursor	3.46E-06	4.90
PA4225	*pchF*	pyochelin synthetase	3.84E-06	3.40
PA4222		probable ATP-binding component of ABC transporter	4.33E-06	3.15
PA4224	*pchG*	pyochelin biosynthetic protein PchG	7.05E-06	4.20
PA3815	*iscR*	iron-sulfur cluster assembly transcription factor IscR	8.79E-06	2.88
PA4843		probable two-component response regulator	9.34E-06	1.66
PA2588		probable transcriptional regulator	1.03E-05	1.95
PA4223		probable ATP-binding component of ABC transporter	1.32E-05	2.71
PA4156		probable TonB-dependent receptor	1.38E-05	2.45
PA4218	*ampP*	siderophore transporter	1.70E-05	3.50
PA4220		hypothetical protein	1.88E-05	4.40
PA3572		hypothetical protein	2.20E-05	-1.56
PA0614		hypothetical protein	2.57E-05	-1.61
PA2483		conserved hypothetical protein	3.98E-05	1.50
PA1911	*femR*	sigma factor regulator, FemR	4.18E-05	1.41
PA0044	*exoT*	exoenzyme T	4.27E-05	1.55
PA1300		probable sigma-70 factor, ECF subfamily	5.62E-05	1.84
PA4726	*cbrB*	two-component response regulator CbrB	7.75E-05	1.42
PA4226	*pchE*	dihydroaeruginoic acid synthetase	8.79E-05	3.33
PA3009		hypothetical protein	9.70E-05	1.47
PA1912	*femI*	ECF sigma factor, FemI	1.06E-04	1.73
PA1711	*exsE*	hypothetical protein	1.21E-04	1.57
PA1151	*imm2*	pyocin S2 immunity protein	1.43E-04	1.83
PA3530	*bfd*	conserved hypothetical protein	1.46E-04	1.62

Table S2. Cont.

ORF	Gene	Gene product [a]	p-value (37 vs. 25)	Fold-Change (37 vs. 25)
PA4219	*ampO*	hypothetical protein	1.46E-04	1.65
PA3813	*iscU*	probable iron-binding protein IscU	1.59E-04	2.06
PA2030		hypothetical protein	1.59E-04	2.01
PA5531	*tonB*	TonB protein	1.61E-04	2.34
PA0471		conserved hypothetical protein	1.65E-04	1.50
PA4896		probable sigma-70 factor, ECF subfamily	1.89E-04	1.45
PA0665		conserved hypothetical protein	2.57E-04	2.49
PA4217	***phzS***	**flavin-containing monooxygenase**	**2.81E-04**	**3.59**
PA1847	*nfuA*	hypothetical protein	3.31E-04	2.56
PA0810		probable haloacid dehalogenase	3.54E-04	-1.44
PA3814	*iscS*	L-cysteine desulfurase (pyridoxal phosphate-dependent)	3.73E-04	1.79
PA4231	*pchA*	salicylate biosynthesis isochorismate synthase	4.06E-04	3.24
PA0270		hypothetical protein	4.70E-04	1.53
PA0941		hypothetical protein	4.77E-04	1.40
PA1432	***lasI***	**autoinducer synthesis protein LasI**	**5.26E-04**	**2.62**
PA3662		hypothetical protein	5.65E-04	1.96
PA5346	*sadB*	Predicted signal transduction protein	6.19E-04	1.75
PA3600		conserved hypothetical protein	6.29E-04	1.70
PA1169		probable lipoxygenase	6.95E-04	-1.54
PA4142	*rpmJ*	50S ribosomal protein L36	7.64E-04	1.65
PA4324		hypothetical protein	9.08E-04	1.85
PA1708	*popB*	translocator protein PopB	9.53E-04	2.01
PA3617	*recA*	RecA protein	9.78E-04	1.42
PA3529		alkylhydroperoxide reductase C	1.05E-03	3.23
PA0436		probable transcriptional regulator	1.05E-03	1.51
PA2034		hypothetical protein	1.06E-03	1.86

Table S2. Cont.

ORF	Gene	Gene product [a]	p-value (37 vs. 25)	Fold-Change (37 vs. 25)
PA5481		hypothetical protein	1.10E-03	-5.16
PA3972		probable acyl-CoA dehydrogenase	1.17E-03	1.90
PA3183	*zwf*	glucose-6-phosphate 1-dehydrogenase	1.21E-03	1.50
PA3601		conserved hypothetical protein	1.42E-03	3.96
PA0505		hypothetical protein	1.58E-03	1.83
PA5499	*np20*	transcriptional regulator np20	1.61E-03	1.59
PA4230	*pchB*	salicylate biosynthesis protein PchB	1.63E-03	3.62
PA0269		conserved hypothetical protein	1.66E-03	2.07
PA5276	*lppL*	Lipopeptide LppL precursor	1.72E-03	1.42
PA2401	*pvdJ*	Non-ribosomal peptide synthetase	1.80E-03	1.69
PA4570		hypothetical protein	1.91E-03	2.08
PA4525	*pilA*	type 4 fimbrial precursor PilA	2.38E-03	2.14
PA4470	*fumC1*	fumarate hydratase	2.40E-03	2.23
PA1905	***phzG2***	**probable pyridoxamine 5'-phosphate oxidase**	**2.59E-03**	**3.00**
PA4141		hypothetical protein	2.63E-03	3.06
PA3841	*exoS*	exoenzyme S	2.77E-03	1.53
PA4251	*rplE*	50S ribosomal protein L5	2.86E-03	1.66
PA0336	*ygdP*	Nudix hydrolase YgdP	2.96E-03	1.52
PA2031		hypothetical protein	3.33E-03	2.12
PA0050		hypothetical protein	3.52E-03	-1.53
PA0200		hypothetical protein	3.71E-03	-2.35
PA5339		conserved hypothetical protein	3.90E-03	1.55
PA3441		probable molybdopterin-binding protein	3.97E-03	1.61
PA3973		probable transcriptional regulator	4.09E-03	2.53
PA1168		hypothetical protein	4.19E-03	-2.82
PA0456		probable cold-shock protein	4.47E-03	1.85

Table S2. Cont.

ORF	Gene	Gene product [a]	p-value (37 vs. 25)	Fold-Change (37 vs. 25)
PA2575		hypothetical protein	4.54E-03	1.67
PA3182	pgl	6-phosphogluconolactonase	4.62E-03	1.75
PA4155		hypothetical protein	4.88E-03	1.92
PA2400	pvdJ	Non-ribosomal peptide synthetase	5.10E-03	2.02
PA1747		hypothetical protein	5.19E-03	1.40
PA1749		hypothetical protein	5.74E-03	1.43
PA2742	*rpmI*	50S ribosomal protein L35	5.86E-03	1.52
PA3014	*faoA*	fatty-acid oxidation complex alpha-subunit	6.81E-03	-1.44
PA0263	*hcpA*	secreted protein Hcp	7.19E-03	-2.25
PA0872t	*phhA*	phenylalanine-4-hydroxylase	7.26E-03	2.62
PA5170	*arcD*	arginine/ornithine antiporter	7.26E-03	-2.09
PA2009	*hmgA*	homogentisate 1,2-dioxygenase	7.39E-03	1.58
PA4615		probable oxidoreductase	7.45E-03	1.67
PA0870	*phhC*	aromatic amino acid aminotransferase	8.06E-03	1.48
PA3880		conserved hypothetical protein	8.24E-03	-1.69
PA0296	*spuI*	Glutamylpolyamine synthetase	8.39E-03	1.45
PA4209	***phzM***	**probable phenazine-specific methyltransferase**	**8.95E-03**	**1.47**
PA2411		probable thioesterase	9.06E-03	1.74
PA5429	*aspA*	aspartate ammonia-lyase	9.26E-03	1.46
PA1677		conserved hypothetical protein	9.30E-03	1.62
PA3812	*iscA*	probable iron-binding protein IscA	9.54E-03	1.83
PA2033		hypothetical protein	1.01E-02	2.07
PA1148	*toxA*	exotoxin A precursor	1.04E-02	-1.45
PA2399	*pvdD*	pyoverdine synthetase D	1.06E-02	1.77
PA4229	*pchC*	pyochelin biosynthetic protein PchC	1.08E-02	2.26
PA1802	*clpX*	ATP-dependent Clp protease ATP-binding subunit ClpX	1.11E-02	1.64

Table S2. Cont.

ORF	Gene	Gene product [a]	p-value (37 vs. 25)	Fold-Change (37 vs. 25)
PA4471	lldD	L-lactate dehydrogenase	1.12E-02	2.27
PA3361	lecB	fucose-binding lectin PA-IIL	1.12E-02	2.20
PA2405		hypothetical protein	1.13E-02	1.52
PA2402		probable non-ribosomal peptide synthetase	1.14E-02	1.92
PA3181		2-keto-3-deoxy-6-phosphogluconate aldolase	1.15E-02	1.47
PA3195	gapA	glyceraldehyde 3-phosphate dehydrogenase	1.20E-02	1.79
PA4250	*rpsN*	30S ribosomal protein S14	1.27E-02	1.57
PA2412		conserved hypothetical protein	1.31E-02	2.21
PA2377		hypothetical protein	1.40E-02	-2.91
PA4254	*rpsQ*	30S ribosomal protein S17	1.49E-02	1.60
PA1244		hypothetical protein	1.56E-02	2.08
PA2015	*gnyD*	Citronelloyl-CoA dehydrogenase, GnyD	1.58E-02	1.45
PA4139		hypothetical protein	1.61E-02	-2.53
PA2880		hypothetical protein	1.61E-02	1.63
PA1914		conserved hypothetical protein	1.61E-02	-2.32
PA1895		hypothetical protein	1.68E-02	-1.56
PA2427		hypothetical protein	1.68E-02	1.75
PA1875		probable outer membrane protein precursor	1.71E-02	-1.44
PA4264	*rpsJ*	30S ribosomal protein S10	1.77E-02	1.50
PA4253	*rplN*	50S ribosomal protein L14	1.77E-02	1.47
PA4751	*ftsH*	cell division protein FtsH	1.78E-02	1.45
PA2743	*infC*	translation initiation factor IF-3	1.79E-02	1.71
PA0713		hypothetical protein	1.80E-02	1.84
PA5100	*hutU*	urocanase	1.82E-02	1.60
PA2016	*gnyR*	Regulatory gene of gnyRDBHAL cluster, GnyR	1.85E-02	1.83
PA3407	*hasAp*	heme acquisition protein HasAp	1.86E-02	1.54

Table S2. Cont.

ORF	Gene	Gene product [a]	p-value (37 vs. 25)	Fold-Change (37 vs. 25)
PA4917		hypothetical protein	2.32E-02	1.44
PA2604		conserved hypothetical protein	2.31E-02	1.56
PA1431	***rsaL***	**regulatory protein RsaL**	**2.42E-02**	**1.74**
PA1793	*ppiB*	peptidyl-prolyl cis-trans isomerase B	2.45E-02	1.41
PA2501		hypothetical protein	2.61E-02	1.53
PA4228	*pchD*	pyochelin biosynthesis protein PchD	2.65E-02	1.83
PA3049	*rmf*	ribosome modulation factor	2.69E-02	1.90
PA3385	*tpbA*	protein tyrosine phosphatase TpbA	2.77E-02	1.90
PA4130		probable sulfite or nitrite reductase	2.87E-02	1.55
PA5018	*msrA*	peptide methionine sulfoxide reductase	3.03E-02	1.47
PA5351	*rubA1*	Rubredoxin 1	3.36E-02	-1.71
PA0423	*pasP*	Uncharacterized conserved protein	3.37E-02	1.42
PA4467		hypothetical protein	3.41E-02	1.41
PA0980		hypothetical protein	3.63E-02	-1.53
PA2404		hypothetical protein	1.30E-02	1.44

[a] The genes regulated by QS are shown in boldface.

Table S3. Transcripts of RhlR regulon which were differentially regulated at 25°C and 37°C in PAO1, grown in PPGAS media (>1.4-fold).

ORF	Gene	Gene	p-value (37 vs. 25)	Fold-Change (37 vs. 25)
PA3479	*rhlA*	rhamnosyltransferase chain A	0.274746	1.49
PA3477	*rhlR*	transcriptional regulator RhlR	0.116055	1.36
PA3478	*rhlB*	rhamnosyltransferase chain B	0.12511	1.72

Table S4. Oligonucleotides used in this study.

Name	Oligonucleotide sequence 5´→3 [plasmid constructed]´[a,b]
lecAF	ACCC**GGTACC**GGTTCGACCC [pLECA70]
lecAR	TAAAATCC**CTCGAC**GGTAACCCCG [pLECA70]
phzA1 EcoRI	GTGGGG**GAATTC**GCGTAGCCGTCGCGGG [pHZA1-20]
phzA1 KpnI	CATTGCCA**GGTACC**GCTCCACCGTGGCG [pHZA1-20]
rhlA-pBADSc	**CTAGC**CTGTTCGAAAATTTTTGGGAGGTGTGAAATGCGGG [pBO-*rhlA*]
rhlA-pBADaSc	**AATTC**CCGCATTTCACACCTCCCAAAAATTTTCGAACAGG [pBO-*rhlA*]
rhlA-Sc-G78	**CTAGC**CTTTCGAAAATTTTTGGGAGGTGTGAAATGCGGG [pBO-*rhlA*(ΔG78)]
rhlA-aSc-G78	**AATTC**CCGCATTTCACACCTCCCAAAAATTTTCGAAAGG [pBO-*rhlA*(ΔG78)]
rhlA-S-G92A	**CTAGC**CTGTTCGAAAATTTTTAGGAGGTGTGAAATGCGGG [pBO-*rhlA*(G92A)]
rhlA-aS-G92A	**AATTC**CCGCATTTCACACCTCCTAAAAATTTTCGAACAGG [pBO-*rhlA*(G92A)]
rhlA-FL-WT	GATGGCCG**GCTAGC**TACTTGTCTGCCGT [pBOC-*rhlA* and pBOC-*rhlA*(G92A)]
rhlA-RLc-WT	CCAACAGACT**GAATTC**CCGCATTTCACACCTCC [pBOC-*rhlA*]
rhlA-RL-G92Ac	CT**GAATTC**CCGCATTTCACACCTCCTAAAAATTTTCGAACAGG [pBOC-*rhlA*(G92A)]
rhlA- TS pBAD18	GCAGCGATAGCT**GCTAGC**CTGTTCG [pBO-*rhlA*,*rhlB*]
rhlB-TR pBAD18	CGATGAG**GAATTC**GTGCATGGGGCT [pBO-*rhlA*,*rhlB*]
rhlA-lΔG78F	GCGATAGCTGTTTGCCTGTTCGAAAATTTTTGGGAG [deletion of G78 in pBOC-*rhlA*]
rhlA-lΔG78R	CTCCCAAAAATTTTCGAACAGGCAAACAGCTATCGC [deletion of G78 in pBOC-*rhlA*]
lasI-Ter-Fw	CGGACGTTTCT**GCTAGC**CTAG [pBO-*lasI*]
lasI-Ter-Rv	GCCGACCAAT**GAATTC**GATCATC [pBO-*lasI*]

lasI-C84A-Fw	AATTTGCATAAATTCTTCAGCTTACTATTTGGAGGAAGTGAAGATGA [pBOC-*lasI* (C84A)]
lasI-C84A-Rv	TCATCTTCACTTCCTCCAAATAGTAAGCTGAAGAATTTATGCAAATT [pBOC-*lasI* (C84A)]
gyrAEC-FW	**CTAGC**GGGATACAGTAGAGGGATAGCGGTTAGATGAGCG [pBOC-*gyrA*]
gyrAEC-Rv	**AATTC**GCTCATCTAACCGCTATCCCTCTACTGTATCCCG [pBOC-*gyrA*]
lasR -Ter-Fw	CGCATG**GCTAGC**GGCAGG [pBOC-*lasR*]
lasR-Ter-Rv	CCGTC**GAATTC**GGCCATAGCG [pBOC-*lasR*]

[a] Introduced restriction sites are in boldface.

[b] Nucleotides mutated and exchanged in the oligonucleotides are underlined and shaded.

Table S5. Primers used for qRT-PCR.

Amplicon	Primer	Sequence 5´→3´	Position[a]	Length
rhlA	rt_rhlA-F	AACATTTCAACGTGGTGCTG	155	100
	rt_rhlA-R	ATTTCCACCTCGTCGTCCTT	254	
rhlR	rt_rhlR-F2	CTGGGCTTCGATTACTACGC	112	124
	rt_rhlR-R2	CCCGTAGTTCTGCATCTGGT	215	
rhlI	rt_rhlI-F	GGAGCGCTATTTCGTTCG	429	100
	rt_rhlI-R	GTAGGCCGGGAAGCTGAT	528	
lasR	rt_lasR-F	CGGTTTTCTTGAGCTGGAAC	15	100
	rt_lasR-F	GCCGAACAGGATCTTCGAG	114	
lasI	rt_lasI-F	GGCTGGGACGTTAGTGTCAT	94	100
	rt_lasI-F	CCTGGGCTTCAGGAGTATCTT	193	
lasB	rt_lasB-F	AACCGTGCGTTCTACCTGTT	1279	100
	rt_lasB-F	TGGTGGCGGTCCAGTAGTA	1378	
phzA1	rt_phzA1-F	AACCACTACATCCATTCCTTCG	355	100
	rt_phzA1-R	CGGCTATTCCCAATGCAC	454	

[a] Position in the coding-region

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