SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE

Table S1. Oligonucleotide sequences used

Oligonucleotide	Sequence[a]
	Cloning
P120brlR	GCGCGCGCGCAAGCTTGATGGGTTCACTGTTG
P250brlR	GCGCGCGCAAGCTTTGCTTGTCGCTGTACTTGC
P500brlR	GCGCGCGCAAGCTTCCAGCTGCATCAATTCCTC
PrevbrlR	GCGCGCGCGCGGATCCTACCGGCAGACTACGGATTG
PetbrlRF	CACCATGCTCACCATCGGC
PetbrlRR	ATAGATGGGGATATACAGGTCGAC
BrlRhisexcisF	GCGCGCGCGCGCTAGCATGCTCACCATCGGCC
BrlRhisexcisR	GCGCGCGCACTAGTTCAATGGTGATGGTGATG
V5-fleQ-NheI-for	GCGCGCGCGCTAGCATGggtaagcctatccctaaccctctcctcggtctcgattctacgATGTGGCGCGAAA CCAAACTC
fleQ-SacI-rev	GCGCGCGCGAGCTCTCAATCATCCGACAGGTCGTC
	Chromatin immunoprecipitation (ChIP enrichment)
PrevbrlR	GCGCGCGCGCGGATCCTACCGGCAGACTACGGATTG
P120brlR	GCGCGCGCGCAAGCTTGATGGGTTCACTGTTG
pscF_GS_R	CCACGGTATCGAGGGTATTC
pscE_GS_F	AAGGCGGTCTCGGCATTCTTTC
	5'RACE
5'RACE abridged anchor primer	GGCCACGCGTCGACTAGTACGGGGGGGGGG
GSP1	CAGGGTGTGCTGGTGTCGTTGCAG
GSP2	GAACGTCCAGCCGGCGCAG
GSP3	GAGTATGCGCGAGAGCTGCTCGATC
	EMSA/Streptavidin bead assays
PbrlR-120F[a]	ATGGGTTCACTGTTGGGAC
PbrlR-120R	GGTTCCTGCTGCGGGATAC
PbrlR-7-108bp-F	CGGGAGGCGACGGCGGAC
PbrlR-7-108bp-R	TTCCTGCTGCGGGATACC
PbrlR-regionC-F	ACTCGCTCACACCCTTGG
PbrlR-regionC-R	GTCCGCCGTCGCCTCCCG
PbrlR-regionB-F	GGGTCATCTCGCTCACCG
PbrlR-regionB -R	CCAAGGGTGTGAGCGAGT
PbrlR-regionA-F	GCTTCCTTACGGAGGCGG
PbrlR-regionA-R	CGGTGAGCGAGATGACCC
pscE_GS_F	AAGGCGGTCTCGGCATTCTTTC

pscF_GS_R	CCACGGTATCGAGGGTATTC
19bp-promF	TTGCCCCAGGGGCAATCCG
19bp-promR [a]	CGGATTGCCCCTGGGGCAA
prom-mut1-F	TTGCCCCATTTTCAATCCG
Prom-mut1-R [a]	CGGATTGAAAATGGGGCAA
prom-mut2-F	GGCAATCCGCGATGGGTT
prom-mut2-R [a]	AACCCATCGCGGATTGCC
-10-promF	TTGCCCCAGGGGCAATCCGTAGTCTGC
-10-promR [a]	GCAGACTACGGATTGCCCCTGGGGCAA
-35-promF	CCTTGACCTTGCCCCAGGGGCAATCCG
-35-promR [a]	CGGATTGCCCCTGGGGCAAGGTCAAGG
	RT-PCR/PCR
mreBf	CTGTCGATCGACCTGGG
mreBr	CAGCCATCGGCTCTTCG
brlR_RT-for	GCAACGACACCAGCACAC
brlR_RT-rev	GAAGCGTTCCCAGAGCTG
mexE-for	GTCATCGAACAACCGCTG
mexE-re	GTCGAAGTAGGCGTAGACC
Pser-up	CGAGTGGTTTAAGGCAACGGTCTTGA
Pser-down	AGTTCGGCCTGGTGGAGCAACTCG

[a], Primers are biotinylated. Non-biotinylated primers were used for the generation of non-biotinylated competitor DNA. Sequence for V5-tag is indicated by lower case letters.

SUPPLEMENTARY FIGURES.

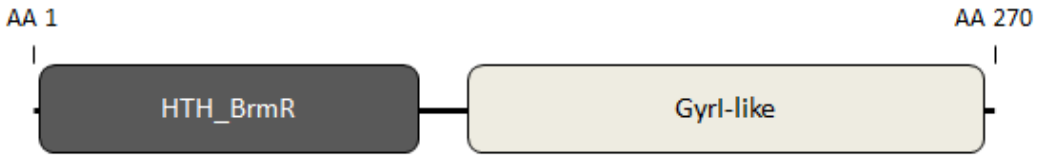

Figure S1. BrlR domain organization. HTH_BmrR, Helix-Turn-Helix DNA binding domain of the MerR BmrR transcription regulator; GyrI-like, GyrI-like small molecule binding domain. AA, indicates amino acid position.

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brlR          CCCAGGGGCAATCCGTAGTCTGCCGGTATCCCGCAGCAGGAACCGGAAATGCTCACCATC
RACE1         TCGACTAGTACGGGGGGGGGGGGGTGTATCCCGCAGCAGGAACCGGAAATGCTCACCATC
RACE2         TCGACTAGTACGGGGGGGGGGGGGTGTATCCCGCAGCAGGAACCGGAAATGCTCACCATC
              ````````````````````````````````````````**********************************
brlR   GGCCAACTGGCGCGAATCTTCGAGATCTCCACCAAGACGCTTCGCCACTACGATGCCATC
RACE1   GGCCAACTGGCGCGAATCTTCGAGATCTCCACCAAGACGCTTCGCCACTACGATGCCATC
RACE2   GGCCAACTGGCGCGAATCTTCGAGATCTCCACCAAGACGCTTCGCCANTACGATGCCATC
              ````````````**********************************************  ************
brlR          GGCCTGTTCGTCCCCGCGCGCACCGGTAGCGACAACGGCTACCGCTACTA-CCAGCCGGA
RACE1         GGCCTGTTCGTCCCCGCGCGCACCGGTAGCGACAACGGCTACCGCTACTA-CCAGCCGGA
RACE2         GGCCTGTTCGTCCCCGCGCGCACCGGTAGCGACAACGGCTACCGCTACTATCCAGCCGG-
              ************************************************** ********
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**Figure S2. Alignment of DNA sequences obtained from the 5'RACE experiment with DNA sequences upstream and downstream of the ATG start codon of *brlR*.** The DNA sequences RACE1 and RACE2 represent biological replica clones obtained by anchored PCR. The sequence GGCCACGCGTCGACTAGTACGGGGGGGGGG originates from the 5'RACE abridged anchor primer (Table S1) which was added during PCR amplification, following homopolymeric dC-tailing of the 5' end of the cDNA. *, denotes identical nucleotides.

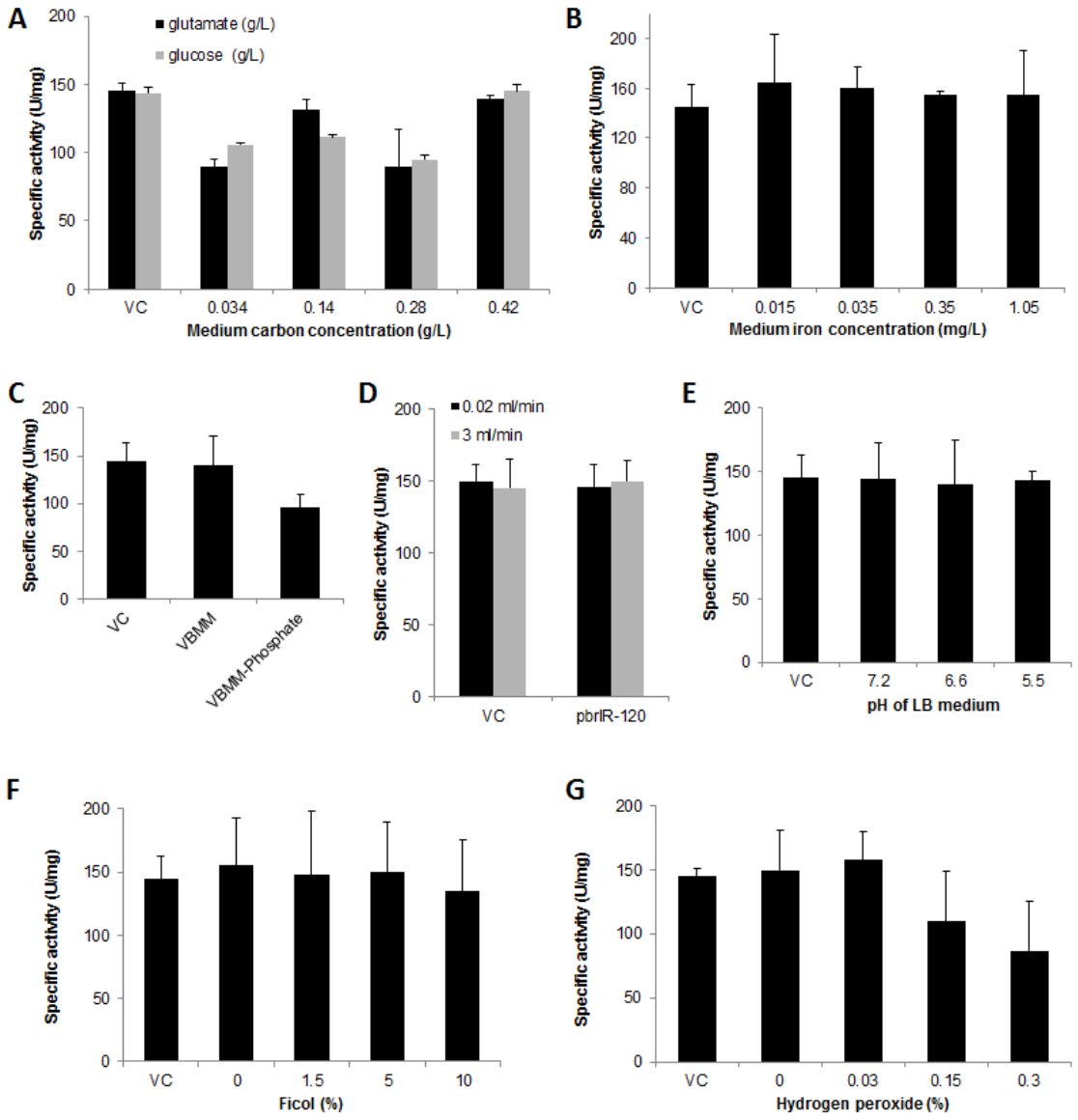


**Figure S3. Environmental factors that do not induce *brlR* expression.** Specific β-galactosidase activity was determined from cells obtained from the promoter reporter strain PAO1::P*brlR*-120-lacZ grown to exponential phase (A) in Vogel-Bonner minimal medium (VBMM) (Schweizer, 1991) with increasing concentrations of glutamate or glucose, (B) iron, or (C) in the absence or presence of phosphate. An iron concentration of 0.035 mg/L is normally present in VBMM. (D) Specific β-galactosidase activity was determined from cells grown in LB medium in a chemostat for 16 hr at a flow rate of 0.02 or 3 ml/min. (E) Specific β-galactosidase activity was determined using cells grown planktonically to exponential phase in MOPS-buffered LB medium in which the pH was adjusted as indicated using KOH, (F) or in LB medium containing 0-10% ficol to vary the

viscosity of the medium. (G). Specific β-galactosidase activity in cells grown planktonically in LB medium for 16 hr to stationary phase prior to and after the addition of 0-0.3% hydrogen peroxide for 30 min. Sodium thiosulfate was used to neutralize hydrogen peroxide following the 30 min exposure time. PAO1 harboring an empty pMini-CTX-*lacZ* vector was used as vector control (VC). pbrlR-120, *brlR* promoter reporter strain PAO1::P*brlR*-120-lacZ. All experiments were carried out at least in triplicate. Error bars denote standard deviation.

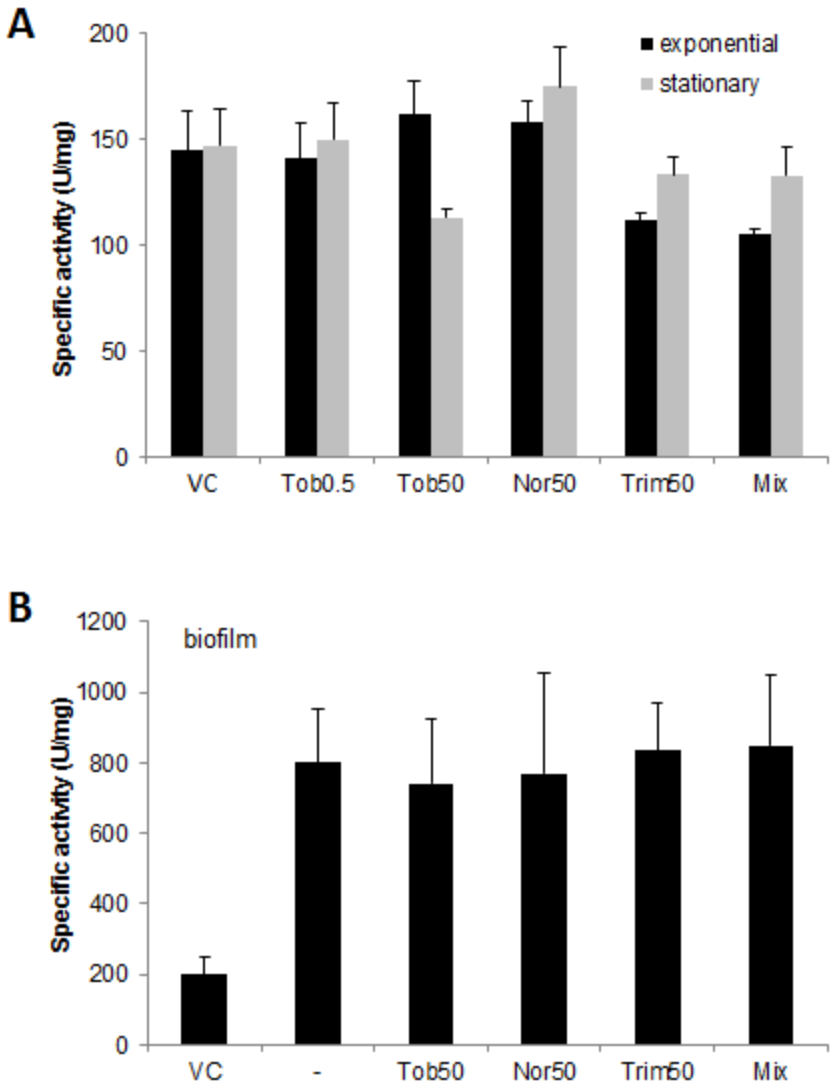


**Figure S4. *brlR* expression is not induced upon exposure to antimicrobial agents.** Specific β-galactosidase activity was determined from cells obtained from the reporter strain PAO1::P*brlR*-120-lacZ (A) grown planktonically to exponential and stationary phase or (B) grown as biofilms for 5 days. Biofilm cells subsequently exposed for 1 hr under flowing conditions to 50 ug/ml of tobramycin (Tob50), norfloxacin (Nor50), trimethoprim (Trim50), or a mixture of the three antibiotics (Mix). Planktonic cells were exposed for 30 min to the antibiotics. All experiments were carried out at least in triplicate. Error bars denote standard deviation. PAO1 harboring an empty pMini-CTX vector was used as vector control (VC).

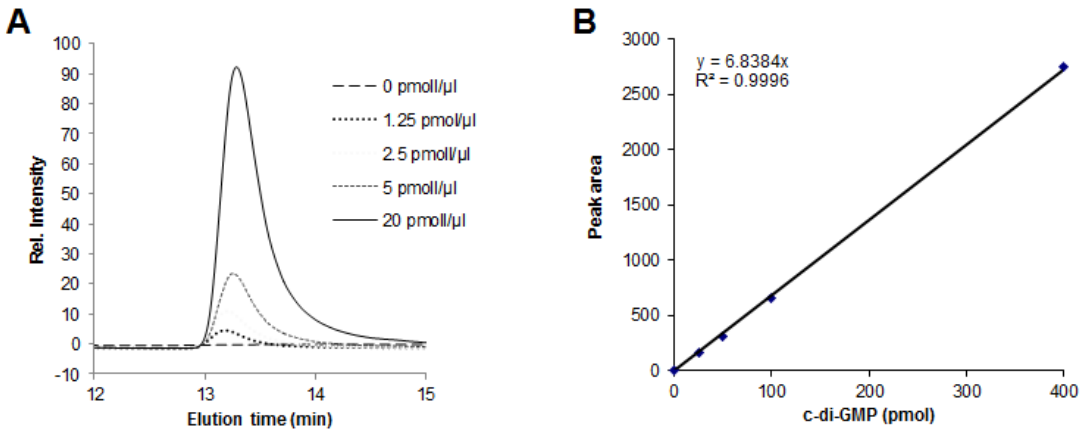


**Figure S5. Detection and quantitation of c-di-GMP.** (A) Detection of increasing concentrations of commercially available c-di-GMP by HPLC. (B) Standard curve based on HPLC-derived peak areas of c-di-GMP. A total of 20 µl of the respective c-di-GMP solutions were analyzed. C-di-GMP detection and quantitation was performed as described in detail by Basu Roy et al. (Basu Roy *et al.*, 2013).

**Supplementary References**

Basu Roy, A., O.E. Petrova & K. Sauer, (2013) Extraction and Quantification of Cyclic Di-GMP from Pseudomonas aeruginosa. *bio-protocol*: http://www.bio-protocol.org/wenzhang.aspx?id=828.

Schweizer, H.P., (1991) The *agmR* gene, an environmentally responsive gene, complements defective *glpR*, which encodes the putative activator for glycerol metabolism in *Pseudomonas aeruginosa*. *J. Bacteriol.* **173**: 6798-6806.