Investigation of the quorum-sensing regulon of the biocontrol bacterium *Pseudomonas chlororaphis* strain PA23

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Running title: Quorum sensing regulon of *Pseudomonas chlororaphis* PA23

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Abstract

Pseudomonas chlororaphis strain PA23 is a biocontrol agent capable of protecting canola from stem rot disease caused by the fungal pathogen *Sclerotinia sclerotiorum*. PA23 produces several of inhibitory compounds that are under control of a complex regulatory network. Included in this cascade is the PhzRI quorum sensing (QS) system, which plays an essential role in PA23 biocontrol. The focus of the current study was to employ RNA sequencing to explore the spectrum of PA23 genes under QS control. Transcriptomic profiling revealed 545 differentially expressed genes (365 downregulated; 180 upregulated) in the *phzR* mutant and 534 genes (382 downregulated; 152 upregulated) in the AHL-deficient PA23-6863. In both strains, decreased expression of phenazine, pyrrolnitrin, and exoprotease biosynthetic genes was observed. We have previously reported that QS activates expression of these genes and their encoded products. In addition, elevated siderophore and decreased chitinase gene expression was observed in the QS-deficient stains, which was confirmed by phenotypic analysis. Inspection of the promoter regions revealed the presence of "*phz*-box" sequences in only 58 of the 807 differentially expressed genes, suggesting that much of the QS regulon is indirectly regulated. Consistent with this notion, 41 transcriptional regulators displayed altered expression in one or both of the QS-deficient strains. Collectively, our findings indicate that QS governs expression of approximately 13% of the PA23 genome affecting diverse functions ranging from secondary metabolite production to general metabolism. To the best of our knowledge, this represents the first global transcriptomic analysis of the QS regulon of a biocontrol pseudomonad.

47 **Introduction**

48 Certain pseudomonads are able to inhibit fungal pathogens via the production of
49 secondary metabolites through a process known as biological control. *Pseudomonas*
50 *chlororaphis* strain PA23 is one such organism that suppresses canola stem rot caused by the
51 fungal pathogen *Sclerotinia sclerotiorum* (1,2). We have established that biocontrol by this
52 bacterium occurs through direct and indirect mechanisms. Direct pathogen inhibition results
53 from exposure to secreted bacterial products including the antibiotics pyrrolnitrin (PRN) and
54 phenazine (PHZ), together with HCN, chitinases, proteases, lipases and siderophores (1,3). PA23
55 also exerts its effects indirectly through priming the plant defense response, enabling the plant to
56 more effectively fend off pathogen attack (4).

57 A complex regulatory network governs expression of PA23 antifungal (AF) compounds.
58 At the top of this hierarchy sits the GacS-GacA two component signal transduction system that is
59 essential for PA23 biocontrol (5,6). Working in concert with Gac is the Rsm system, which
60 consists of RsmA-like translational repressor proteins and small regulatory RNAs (7). Additional
61 regulators overseeing production of PA23 biocontrol metabolites include the stationary phase
62 sigma factor RpoS (8), PsrA (Pseudomonas Sigma Regulator A) (6), the stringent response (SR)
63 (8), the anaerobic regulator ANR (9), and a novel LysR-type regulator called PtrA (10).

64 Adding to this complexity, PA23 AF compounds are expressed in a population-density-
65 dependent fashion through quorum sensing (QS) (11). Like other gram-negative bacteria, PA23
66 uses N-acylhomoserine lactones (AHLs) as indices of population density (11–13). The first QS
67 system identified in PA23 consists of the transcriptional regulator PhzR and the AHL synthase
68 PhzI. The genes encoding these elements, *phzR* and *phzI,* are situated upstream of the
69 *phzABCDEFG* biosynthetic locus responsible for PHZ production (11). Characterization of a

70 *phzR* mutant (PA23*phzR*) and an AHL-deficient strain (PA23-6863) revealed a lack of fungal
71 inhibition, which was attributed to reduced PHZ, PRN and protease production (11). A second
72 QS system called CsaRI (Cell Surface Alteration) has been identified in the closely related *P.*
73 *chlororaphis* 30-84 (14). The Csa system is not involved in the regulation of secondary
74 metabolites or biocontrol genes, instead, it controls cell surface properties and biofilm formation
75 (14). While homologs of *csaI* and *csaR* are present in the PA23 genome, the role of this QS
76 system in PA23 remains unknown. Another QS system, called AurRI has been reported in *P.*
77 *chlororaphis* subsp. *aurantiaca* PB-St2; however, it has not been characterized (15).
78 In *Pseudomonas aeruginosa*, global transcriptomic analysis using microarrays revealed
79 that over 300 genes are under QS control (16,17), greatly exceeding the number identified
80 through more targeted approaches. For the first time, the magnitude of QS control was
81 recognized to extend well beyond virulence, regulating diverse aspects of *P. aeruginosa*
82 physiology. In organisms that employ AHL-based QS, control is mediated in one of two ways:
83 directly through interaction with the promoter regions of target genes, or indirectly through other
84 regulators. For several genes in the former category, consensus sequences have been identified
85 that are required for LuxR-AHL complex binding (18). These "lux box-like" sequences are
86 located in different positions depending on the gene in question (17,18). In strain PA23, a "*phz*-
87 box" sequence was identified upstream of the PHZ- biosynthetic locus as well as other genes
88 under QS control (6,11). We have previously established that the Phz QS system is deeply
89 enmeshed in the complex hierarchy of gene regulation in strain PA23. Analysis of *gac* mutants
90 revealed a lack of AHL production; consequently QS appears to be under control of this global
91 regulatory system (5). The PhzRI QS system is also interconnected with RpoS (11), ANR (9),

92 and the transcriptional regulator PtrA (19). As such, a large number of QS-regulated genes are
93 expected to be indirectly regulated in this bacterium.

94 The focus of the current study was to explore the scope of genes under QS control in
95 strain PA23 through RNA sequencing (RNA-seq). Analysis of a *phzR* mutant and an AHL-
96 deficient derivative revealed that QS regulates approximately 13% of the PA23 genome,
97 impacting diverse aspects of physiology ranging from secreted exoproducts to central
98 metabolism. Moreover the vast majority of genes in the QS regulon appear to be indirectly
99 controlled as very few contained *phz* boxes in their promoter regions. To the best of our
100 knowledge, this is the first study to define the spectrum of genes under QS regulation in a
101 biocontrol pseudomonad.

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107 **Materials and Methods**

108 **Bacterial strains and growth conditions**

109 Bacterial strains used in this study are outlined in Table 1. *E. coli* strains were cultured on
110 Lennox Luria Bertani (LB) media (Difco Laboratories, Detroit, MI) at 37°C. *P. chlororaphis*
111 PA23 was cultured and maintained on LB media (Difco) at 28°C. *S. sclerotiorum* was cultured
112 and maintained on Potato Dextrose Agar (PDA; Difco) at 22°C. Antibiotics were purchased from
113 Research Products International Corp. (Prospect, IL) and supplemented at the following
114 concentrations: gentamicin (Gm; 20 µg mL^{-1}), tetracycline (Tc; 15 µg mL^{-1}) for *P. chlororaphis*
115 PA23 and Gm (15 µg mL^{-1}) and Tc (15 µg mL^{-1}) for *E. coli*. For phenotypic assays and cDNA
116 library synthesis, strains were cultured in M9 Minimal Salts Media (M9; Difco) supplemented
117 with 1 mM $MgSO_4$ and 0.2% glucose, from here on referred to as M9-Glc.

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129 **Table 1.** Bacterial strains, plasmids and oligonucleotides sequences

Strains	Relevant genotypes	Source or reference
Pseudomonas chlororaphis		
PA23	PHZ$^+$, PRN$^+$; Rif^R; WT (soy bean root tip isolate)	(2)
PA23*phzR*	Gm^R marker inserted into *phzR* gene	(11)
PA23-6863	PA23 carrying pME6863 (AHL-deficient)	(11)
Oligonucleotide sequences		
phzA RT-PCR FWD	5′- GACTGGCAATGGCACAAC-3′	(18)
phzA RT-PCR REV	5′- GCAATAACCTTCGGGATAACC-3′	(18)
prnA RT-PCR FWD	5′- CTGTCGTCGTGCTTTCTG-3′	(18)
prnA RT-PCR REV	5′- GATCTCGGCGTTGAATGC-3′	(18)
hcnC-FOR	5′- AACTGCTCAACGGTTGCCTG-3′	(9)
hcnC-REV	5′- TGCTGATGTCGAAGCCCTTG-3′	(9)
phzI RT-PCR FWD	5′- GCGATGCCGTTGTTCTGG-3′	(18)
phzI RT-PCR REV	5′- AGCCGTTCGTAGTGGACTC-3′	(18)
phzR RT-PCR FWD	5′- GAATCCTTGGCTTCAGACC-3′	(18)
phzR RT-PCR REV	5′- ATCAGGCGGCTAACTACG-3′	(18)
rpoB RT-PCR FWD	5′- CGTGTTCCTGTCCGCTATC-3′	(18)
rpoB RT-PCR REV	5′- GCCGCAACCGAAACTACC- 3′	(18)
csaR-qpcr-F	5′- GAGATTGCCCTGATCCTGGG-3′	This study.
csaR-qpcr-R	5′- GCCAGAGTCTTGTTGGCTGC-3′	This study.
csaI-qpcr-F	5′- CTTGCTGGCCCTGGACTTTG-3′	This study.
csaI-qpcr-R	5′- GCCTGATTCAGCGAAGCCAC-3′	This study.
aurR-qpcr-F	5’- TTGGCGTTGCAGACCTG-3’	This study.
aurR-qpcr-R	5’- AGTGGGTGATGATGGGC-3’	This study.
aurI-qpcr-F	5’- TGGTCAGCAACGCCATG-3’	This study.
aurI-qpcr-R	5’- ATCGGCATGTTCAGCAGC-3’	This study.

130 Rif; rifampicin; Gm; gentamicin.

131 **Nucleic acid manipulations**

132 Isolation, purification, endonuclease digestion and all other manipulation of DNA was
133 performed according to the protocols described in Sambrook *et al.* (20). Polymerase Chain
134 Reaction (PCR) was conducted following standard conditions outlined by New England Biolabs
135 (NEB) (Ipswich, USA) data sheets supplied with their buffer system and *Taq* polymerase.

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138 **RNA extraction and cDNA library synthesis**

139 cDNA libraries were generated for PA23, PA23*phzR,* and PA23-6863. Three biological
140 replicates of each strain were cultured in 30 ml of M9-Glc. Cells were harvested at early
141 stationary phase (OD_{600}1.20-1.50) by pelleting for 10 min at 6000 rpm at 4°C followed by flash
142 freezing in liquid nitrogen. Pellets were stored at -80°C for up to one week. Total RNA was
143 extracted using the Fermentas Plant RNA extraction kit (Waltham, USA) per manufacturer's
144 instructions. Residual genomic DNA was removed by treatment with TURBO RNAase-free
145 DNAse I (Ambion, Carlsbad, USA) according to manufacturer's instructions. RNA
146 concentration was verified using a NanoVue spectrophotometer (GE Healthcare), and quality
147 was measured using an Agilent 2100 Bioanalyzer with Agilent RNA 6000 Pico and Nano Chips
148 (Agilent Technologies; Santa Clara, CA, USA). cDNA libraries were constructed using the
149 alternative HTR protocol described by Kumar et al. (21) adapted for bacterial RNA. Ribosomal
150 RNA was depleted using the MicrobExpress kit (Ambion) as per manufacturer's instructions.
151 Fragmentation time was reduced to 10 min and the number of cycles for final PCR amplification
152 of the libraries was adjusted to 12 (21). Final cDNA libraries with ligated adaptors were size-
153 selected to fall between 250 and 500 bp using the E-Gel® electrophoresis system (Invitrogen).
154 cDNA quantity was measured using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Thermo
155 Fisher, Rockford, USA) with a Nanodrop 3300 (Thermo Fisher). cDNA was validated using
156 Agilent Bioanalyser High Sensitivity DNA Chips (Agilent Technologies) at three points: i) after
157 first and second strand cDNA synthesis; ii) after the final PCR amplification of the libraries; and
158 iii) after size selection with the E-Gel® electrophoresis system. 100-bp single-end RNA-
159 sequencing was carried out at Génome Québec (Montreal, Canada) on the Illumina HiSeq 2000
160 platform with a multiplex value of 9.

161 **Data analysis**

162 Sequenced reads were analysed by FastQC to determine quality
163 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and the Trimmomatic tool (22)
164 enabled removal of low quality reads (Q < 30) and barcode adapters. Reads mapping to each
165 gene was counted after mapping raw reads to the genome via the Rockhopper program (23)
166 using the *P. chlororaphis* PA23 reference annotation from NCBI (gi: accession no.
167 NZ_CP008696). Of total reads, 93-96% mapped to the *P. chlororaphis* PA23 genome across
168 samples (S1Table). The R Bioconductor package DESeq2 (24) was employed to normalise raw
169 read counts from each replicate and to determine significantly differentially expressed genes
170 (adjusted p-value ≤ 0.01). This output enabled generation of gene expression profiles for each
171 strain. The $\log_2$ fold change values ≥1.5 or ≤ -1.5 were selected based on previous analysis
172 carried out by Shemesh *et al.*, (25) on the *Streptococcus mutans* transcriptome. Functional
173 analysis was carried out via Cluster of Orthologous Groups (COG) analysis. COG categories
174 were assigned to the translated transcript sequences through the Conserved Domain Database
175 (CDD) and the batch web-CD search tool (26,27).

176

177 **Autoinducer assay**

178 The amount of AHL produced by each strain was approximated by spotting 5 µl of an M9-
179 Glc culture, grown in for 24 h and adjusted to an OD_{600nm} of 1, onto *Chromobacterium violaceum*
180 CVO26 seeded LB agar plates. The radius of the purple zone surrounding each colony was
181 measured after 24 h of incubation at 28°C.

182 **Siderophore assay**

183 To analyze siderophore production, a 5µl volume of overnight bacterial culture grown in
184 M9-Glc was spotted onto Chrome Azurol S (CAS) media (28). The diameter of the yellow zone
185 surrounding the colonies, indicative of siderophore production, was measured following 24 hours
186 of incubation at 28°C.

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188 **Chitinase assay**

189 Strains were tested for their ability to produce chitinase according to the protocol outlined
190 by Wirth & Wolf (29). Strains were cultured in M9-Glc broth until they reached early stationary
191 phase (OD_{600} 1.20-1.50). A 250-µl aliquot of cell free supernatant was incubated with equal parts
192 of 0.1M NaOAc, pH 5.2 (250 µL) and carboxymethyl-chitin-Remazol brilliant violet aqueous
193 solution (250 µL) (Blue Substrates, Göttingen, Germany). After incubation for 1 hour at either
194 28°C or 37°C, reactions were stopped by the addition of 250 µl of 1M HCl. Mixtures were
195 cooled on ice for 10 min, spun at 20,000xg for 10 min and absorbance readings were taken at
196 550nm. Three replicates were analysed for each strain and experiments were performed in
197 triplicate.

198

199 **Motility assays**

200 Swimming motility assays were carried out by growing cultures of PA23 and derivative
201 strains were to early stationary phase (24 h) in 3 ml of M9-Glc at 28°C. The cultures were then
202 standardized to an OD_{600nm} of 1 and stab-inoculated (half-way down) into the centre of M9-Glc
203 media solidified with 0.3% agar. Plates were incubated for 72 h at 28°C and the diameter of the

204 swim zone was measured every 24 h. Five replicates were inoculated for each strain and three
205 independent experiments were performed.

206 To assess swarming motility, fresh colonies of PA23 and derivative strains were gently
207 inoculated using an applicator stick onto the surface of a swarm media plate (0.5% peptone, 0.3%
208 yeast extract solidified with 0.8% agar). Plates were incubated for 96 h at 28°C, pictures of each
209 plate were taken every 24 h and the area of the swarming colony was measured using ImageJ
210 software (30). Five plates were analyzed for each strain and three independent experiments were
211 performed.

212

213 **Quantitative PCR (qPCR)**

214 Quantitative PCR (qPCR) was used to monitor expression of metabolite and regulatory
215 genes involved in biocontrol. Triplicate cultures of PA23 and derivative strains were grown to
216 early stationary phase in a 3ml volume of M9-Glc. Cells were harvested by incubating 500µl of
217 culture with 2x volume of RNAprotect reagent (QIAGEN, Valencia, USA) for 5 min followed
218 by centrifugation for 10 min at 6000 rpm. Pellets were stored at -20° for up to one week. Total
219 RNA was extracted using the RNeasy Mini Kit (QIAGEN). Residual genomic DNA was
220 removed by treatment with TURBO RNAase-free DNAse I (Ambion). RNA concentrations were
221 measured at absorbance 260 and 280 nm; only RNA samples with A260/A280 between 1.8 and
222 2.0 were used in subsequent steps. cDNA was generated by reverse transcription using the
223 Maxima First Strand cDNA Synthesis Kit (Thermo Scientific) and random hexamer primers in a
224 20-µL total reaction volume. The following conditions were employed: initial heating at 25°C
225 for 10 min, reverse transcription at 50°C for 15 min, and enzyme denaturation at 85°C for 5 min.
226 Sequences for the PA23 genes of interest were obtained from GenBank (gi: NZ_CP008696) and

227 the primer sequences are listed in Table 1. PCR was performed using a CFX96 Connect™ Real-
228 Time PCR Detection System (Bio-rad, Hercules, USA) and SsoFast™ EvaGreen® Supermix
229 (Bio-rad). The final 10-µL volume mixture in each well contained 0.4 µL of both forward and
230 reverse primers (12µM), 1 µL of 1:20 diluted cDNA, 5 µL of SsoFast™ EvaGreen® Supermix
231 and 3.4 µL of nuclease-free water. PCR reaction conditions included an initial denaturation at
232 98°C for 2 min, followed by 39 cycles of 98°C for 5 s, 60°C for 30 s, and 60◦C for 5 s. Melt-
233 curve analysis was performed to evaluate the formation of primer dimers and other artefacts to
234 validate results. Each reaction was performed in triplicate and experiments were repeated three
235 times with three biological replicates. Relative gene expression was calculated using the ΔΔCt
236 method as described by Livak & Schmittgen (31), with *rpoB* as the reference gene and the
237 CFXManager™ software (Bio-rad).
238
239 ***phz*-box identification**
240 The presence of putative *phz*-boxes upstream of differentially expressed genes
241 determined through RNAseq were identified using the MEME suite Motif Alignment and Search
242 Tool (MAST) algorithm (http://meme-suite.org/tools/mast) (32,33). A *phz*-box consensus
243 sequence VCKRCHWGHKYKBSHWRK, (where V= A, C or G; K= G, T; R= A, G; H= C, T or
244 A; W= A, T; Y=C, T; B= C, G or T; S= G, C; N= A, T, G or C) was generated using previously
245 identified *phz*-boxes in PA23 (9,11). Only consensus sequences located within 500 basepairs
246 upstream of the translational start site were considered significant.

247 **Results and Discussion**

248 **Identification of genes under QS control**

249 In PA23, the Phz QS system is essential for biocontrol as strains lacking this regulatory
250 circuitry no longer produce the antibiotics and degradative enzymes required for fungal
251 antagonism (11). To fully appreciate the global effect of QS on PA23 gene expression, RNAseq
252 was conducted on two QS-deficient strains. The *phzR* mutant, PA23*phzR*, was generated
253 through allelic exchange and exhibits reduced AHL levels compared to wild type (S1 Fig.) (11).
254 Conversely, PA23-6863 contains the *B. subtilis* AHL lactonase-encoding *aiiA* gene on a plasmid
255 and is AHL-deficient (S1 Fig.) (11). Because the PA23 genome encodes three QS systems
256 including PhzRI, CsaRI and AurRI, PA23-6863 is equivalent to a triple mutant. This is relevant
257 because in the closely related *P. chlororaphis* subsp. *aurantiaca* strain PB-St2, the three AHL
258 synthases, PhzI, CsaI and AurI, produce structurally similar molecules (15). The presence of the
259 lactonase ensures that there is no cross-activation by non-cognate AHLs.

260 We chose M9-Glc media to simulate the nutrient-limiting conditions present in the
261 environment and cells were harvested at early stationary phase (OD_{600} 1.2-1.5) when secondary
262 metabolite production occurs. Five hundred and forty-five differentially expressed genes were
263 identified in the *phzR* mutant background (365 downregulated; 180 upregulated), corresponding
264 to 8.8% of the genome (S2 Table). In the AHL-deficient PA23-6863, a total of 534 genes
265 showed altered expression (382 downregulated; 152 upregulated) representing 8.6% of the
266 genome (S3 Table). In total, 807 of the 6,179 coding sequences in the PA23 genome showed
267 differential expression in one or both of the QS-deficient strains.

268 The degree of overlap between differentially expressed genes in the *phzR* mutant and in
269 the AHL-deficient strain is quite low; 36.8% of downregulated and 26.2% of upregulated genes

270 were conserved between them (Fig. 1). Genes showing altered expression exclusively in PA23-
271 6863 is likely due to the fact that in this strain, all signal molecules are degraded leading to a
272 total loss of QS; conversely in PA23*phzR*, only the Phz system is affected. Intriguingly, 275
273 genes showed differential regulation in the *phzR* mutant but not the AHL-deficient strain. There
274 are examples of LuxR proteins that regulate gene expression in the absence of AHL; for instance
275 in *Pectobacterium atrosepticum*, VirR binds to the promoter of the *rsmA* gene activating
276 transcription. Upon binding to 3-oxo-C6-HSL, VirR undergoes a conformation change that
277 results in dissociation from the promoter and *rsmA* is no longer expressed (34–36). The *P.*
278 *aeruginosa* transcriptional regulator RhlR was recently reported to regulate gene expression in a
279 C4-HSL-dependent and C4-HSL-independent manner (37). In the absence of the AHL synthase
280 (RhlI), RhlR directs expression of genes involved in biofilm formation and virulence factors
281 (37). Finally, AHL-deficient strains of *Pseudomonas corrugata* and *Pseudomonas*
282 *brassicacearum* are phenotypically similar to wild type; whereas loss of the LuxR homolog
283 results in dramatically altered traits (38) (Saikai and de Kievit, unpublished data). Collectively,
284 these findings support a regulatory role for LuxR proteins in the absence of AHL binding.
285
286 **Figure 1:** Comparison of the PA23*phzR* and PA23-6863 regulons. Venn diagrams were
287 generated using Venny 2.1 (39) to determine the overlap of up (A) and down (B) regulated genes
288 between PA23*phzR* and PA23-6863.
289
290 While the global impact of QS in a biocontrol pseudomonad has yet to be undertaken,
291 microarray studies have been performed on two pathogenic pseudomonads, namely *P. aeruginosa*
292 PAO1 and *P. syringae* B728a (16,17,40,41) Results from the current study show that expression

293 of 13.06% of the PA23 genome is governed by QS. In *P. aeruginosa* PAO1, QS-modulated genes
294 were reported to account for 6-10% of the genome and the majority of genes were positively
295 regulated (16,17). We discovered that in PA23*phzR,* 67% of the differentially regulated genes
296 showed decreased expression, while 33% were upregulated. Similarly, in PA23-6863, 71.5% and
297 28.5% of genes displayed decreased and increased expression, respectively. Thus, for both PAO1
298 and PA23, QS is largely serving as a positive regulator of gene expression. In stark contrast to
299 these large regulons, only 9 genes were found to be controlled by the *P. syringae* QS circuitry (41).
300 All nine genes were located near the *ahlR* locus and all were positively regulated by QS.
301
302 **Functional characterisation of differentially expressed genes in PA23*phzR* and PA23-6863**
303 Next, we sought to predict the functional role of the PA23 QS-controlled genes through
304 Cluster of Orthologous Group (COG) analysis. COG clusters are constructed using functional
305 characterization based on prokaryotic genomes (42). According to their predicted function, the
306 545 differentially expressed genes in the *phzR* mutant could be divided into 21 COG categories
307 (Fig. 2a, S2a Fig., S2 Table). Similarly, the 534 genes identified in PA23-6863 could be grouped
308 into 22 COG categories (Fig. 2b, S2b Fig., S3 Table). Several of the more relevant categories are
309 discussed in detail in the following sections.
310
311 **Figure 2.** Functional analysis of differentially expressed genes in (A) PA23*phzR* and (B) PA23-
312 6863 in comparison to wild type using Cluster of Orthologous Group (COG) analysis. The red bars
313 indicate percent of differentially regulated genes that are downregulated, and the blue bars indicate
314 percentage of genes that are upregulated in each category.
315

316 **Role of QS in regulation of PA23 secondary metabolites**

317 In both QS-deficient strains, a number of genes involved in secondary metabolite
318 production were significantly downregulated. For example, genes required for synthesis of PHZ
319 (EY04_RS25715-45), hydrogen cyanide (EY04_RS11540-50), and exoprotease
320 (EY04_RS11085) exhibited ≥3.42 log_2fold decreased expression compared to the WT. Similarly,
321 expression of the *prn* biosynthetic genes (EY04_RS17650-35) was reduced 1.93- and 4.17-
322 log_2fold in PA23*phzR* and PA23-6863, respectively. In a previous study, PA23 QS-deficient
323 strains exhibited decreased *phzA-lacZ* and *prnA-lacZ* transcription and reduced PHZ, PRN and
324 protease production (11), consistent with our RNAseq data. When qPCR was used to validate our
325 global transcriptomic findings, expression of the *phz, prn* and *hcn* genes in PA23*phzR* and PA23-
326 6863 was decreased (Fig. 3).

327

328 **Figure 3.** qPCR fold change in gene expression in PA23*phzR* and PA23-6863 compared to PA23
329 wild type. Analyzed genes were compared against *rpoB* as a reference gene. Gene expression in
330 the wild type was normalized to 1.0. For strains that differ significantly from the wild type,
331 columns have been marked with an asterisk (* < 0.01; **p < 0.001).

332

333 Other exoproduct genes that were differentially expressed in both QS derivatives include
334 chitinase-encoding genes (EY04_RS16020, EY04_RS09705; downregulated ≥6.91- log_2fold)
335 and siderophore biosynthetic genes (EY04_RS15355-410; upregulated ≥ 2.61-log_2fold). When
336 we employed end-product analysis to support these findings, chitinase activity was completely
337 abolished in both PA23*phzR* and PA23-6863 (0.00 ± 0.001) compared to the wild type (0.20 ±
338 0.006). On CAS agar, orange halos indicative of siderophore production, were significantly

339 larger around colonies of PA23*phzR* (8.0±0.3 mm) and PA23-6863 (7.5±0.1 mm) compared to
340 the PA23 parent (3.5±0.2 mm). Collectively, the RNAseq findings are in keeping with
341 phenotypic characteristics of the QS-deficient strains, validating the robustness of this approach
342 for defining the PA23 QS regulon (3,11).
343
344 **Role of QS in motility**
345 Several motility genes were significantly upregulated in the QS-deficient strains (S3 Fig.,
346 S2 Table and S3 Table). In addition to a number of chemotaxis signal transduction genes being
347 induced, genes predicted to modulate cellular levels of cyclic di-GMP (EY04_RS05905,
348 EY04_RS09010, EY04_RS10080, EY04_RS15460, EY04_RS24510) were upregulated in one or
349 both of the QS-deficient strains. Notably, flagellin (EY04_RS07580), a diguanylate
350 phosphodiesterase (EY04_RS05905), as well as the alternative stators, MotD (EY04_RS07755)
351 and MotY (EY04_RS24205) were uniquely upregulated in PA23*phzR* (S3 Fig.). The MotCDY
352 stators along with low levels of c-di-GMP are required to provide higher torque for flagellar
353 movement through high agar concentrations in *P. aeruginosa* (43,44). Such findings suggest that
354 PA23*phzR* and PA23-6863 are more motile. Consistent with this, the swim zone of PA23*phzR* was
355 significantly larger than the WT and PA23-6863, while PA23-6863 was more motile than the WT
356 strain in 0.3% agar (Table 2, S3 Fig.). Since the swarming pattern of PA23 is irregular (5), the area
357 of the swarming colony on 0.8% agar was measured using ImageJ software (30). After four days,
358 PA23*phzR* swarmed significantly more than the WT strain (Table 2, S3 Fig.). PA23-6863 showed
359 more variation between replicates and the area of swarming was not significantly different from
360 the WT, but was less than PA23*phzR* (Table 2, S3 Fig.). These results are consistent with findings
361 that QS suppresses motility in other rhizobacteria, such as *P. syringae* and *Sinorhizobium meliloti*

362 (45–47). Since the switch from a sessile to a motile cell is energetically costly and flagella can
363 induce plant defenses, it is beneficial for bacteria to tightly regulate motility until a lack of nutrients
364 necessitate movement to a more nutritive environment (48).

365 **Table 2.** Motility of PA23 and derivative strains.

Strain	**Motility**			
	Swim 0.3% agar*			**Swarm 0.8% agar**
	24 h	**48 h**	**72 h**	**96 h†**
PA23 WT	7.2 (0.8)	10.4 (1.1)	12.4 (1.3)	162.8 (18.6)
PA23*phzR*	23.0 (2.1)§	46.6 (2.6)§	69.8 (3.6)§	815.2 (304.4)‖
PA23 -6863	11.3 (1.5)‖	24.0 (1.0)§	35.1 (2.9)§	83.2 (50.1)#

366 *Mean (SD) diameter (mm) of the swim zone obtained from three independent experiments.
367 †Mean (SD) area (mm^2) of swarming colony obtained from three independent experiments.
368 §Significantly different from WT ($P<0.001$).
369 ‖Significantly different from WT ($P<0.05$).
370 #Not significantly different from WT.

371

372 **Characterization of QS-regulated genes involved in other physiological processes**

373 The largest percentage of QS regulated transcripts encoded proteins of unknown function
374 (35.8% AHL^-; 29.7% *phzR*$^-$), which was also observed by Wagner et al. (17). Other major
375 categories found to be QS regulated include those associated with cellular energetics and
376 metabolism, such as energy production and conversion (category C; 3.4% AHL^-; 5.5% *phzR*$^-$),
377 amino acid transport and metabolism (category E; 5.4% AHL^-; 6.2% *phzR*$^-$), carbohydrate
378 transport and metabolism (category G; 5.4% AHL^-; 4.6% *phzR*$^-$), and lipid transport and
379 metabolism (category I; 3.6% AHL^-; 4.4% *phzR*$^-$). Consistent with these findings, PAO1 genes
380 linked to amino acid biosynthesis and metabolism, carbon compound catabolism, energy
381 metabolism, and fatty acid and phospholipid metabolism were differentially expressed in the
382 absence of QS (17). Genes involved in translation, ribosomal structure and biogenesis were also
383 found to be under QS control (category J; 3.0% AHL^-; 9.4% *phzR*$^-$), including a large number of

384 50S and 30S ribosomal proteins as well as the 16S rRNA processing protein, RimM. Similar results
385 were demonstrated for the PfsI/R QS regulon of the rice pathogen *Pseudomonas fuscovaginae,*
386 where the majority of genes positively regulated by PfsI/R are involved in translation, ribosomal
387 structure and biogenesis, including 20 ribosomal proteins and RimM (49).

388 In *P. aeruginosa*, QS controls expression of not only virulence factors but the secretion
389 systems required for export (17). Our transcriptomic analysis revealed decreased expression of
390 genes encoding type IV (EY04_RS00545) and VI (EY04_RS29490, EY04_RS29495,
391 EY04_RS29520) secretion systems in one or both of the PA23 QS-deficient strains. Moreover,
392 expression of resistance-nodulation-cell division (RND) efflux transporters including MexE
393 homologues (EY04_RS19235, EY04_RS17230, EY04_RS17225, EY04_RS00160) was reduced
394 in the absence of QS. Similarly in strain PAO1, three RND efflux systems were found to be under
395 QS control (17). At present, the mechanism by which PA23 biocontrol metabolites are transported
396 outside of cells has not been elucidated. We hypothesize that at least some of these compounds are
397 exported via these secretion systems and/or active efflux.

398

399 **QS directly and indirectly regulates gene expression in PA23**

400 For genes under QS control, regulation may occur in one of two ways: directly through
401 PhzR-AI binding to the promoter or indirectly through control of other regulators. For those in
402 the former category, an activated PhzR dimer is believed to bind to a highly-conserved
403 consensus sequence known as the *phz*-box, previously identified upstream of the PA23 *phzA* and
404 *phzI* genes (11). In *P. aeruginosa,* 7% of QS-regulated genes reportedly contain *las*- or *rhl*-boxes
405 in the promoter region (16,17). To determine if a similar trend is observed in PA23, we analysed
406 the genome for *phz*-boxes using the Motif Alignment and Search Tool (MAST) algorithm

407 (32,33). In PA23, 545 and 534 genes were differentially regulated in the PhzR- and AHL-
408 deficient backgrounds, respectively. However, only 58 genes contained *phz*-boxes within the
409 500-bp region upstream of the translational start (Table 3). A large number of transcriptional
410 regulators display altered expression in the QS-deficient strains (Table 4). In total, 41 genes
411 encoding TS regulators showed differential expression in one or both of the QS-deficient strains.
412 The expression of 11 genes encoding regulators was altered in both strains, while 18 and 12
413 genes exhibited altered expression in only PA23-6863 and PA23*phzR,* respectively (Table 4).
414 These findings support the hypothesis that a substantial proportion of the QS regulon is subject
415 to indirect control. While a detailed discussion of all 41 regulators is not feasible, examples of
416 prominent genes classified into each of these categories are provided below.
417
418
419
420

421 **Table 3.** Genes containing a *phz*-box sequence within 500 bp of the ATG start

Putative phz-boz sequence[1]	Function	COG category		Locus tag	log2 fold change		Distance between *phz*-box and ATG start
					PA23 -6863	PA23*phzR*	
gccgCCGGCCTGGTCGCGCAATcggc	Hypothetical protein	-	-	EY04_RS00390	-5.96	-2.73	386
ggttGCGGCCTGTGCGGCGTGGaagc	CbbBc protein	COG0243	C	EY04_RS00880	-	-2.11	451
tgcgCCTACAAGCTTTCGACGGcgac	AraC family transcriptional regulator	COG2207	K	EY04_RS01040	-2.70	-	97
tatgCCCGCAAGATCTGGCTGGcagg	Poly(3-hydroxyalkanoate) granule-associated protein PhaF	-	I	EY04_RS01500	-3.52	-2.57	346
gaagACGGCCTGATCGCCATGGgctc	ATP-dependent protease ATP-binding subunit HslU	COG1220	O	EY04_RS01530	3.93	-	218
gagtTCGGCAAGCGCGGGCAGGctca	Pyruvate dehydrogenase	COG2609	C	EY04_RS01840	-	-1.81	386
tccaCCGACAAGCTCAGGCAGGtcat	Histidine kinase	cl27674	K	EY04_RS02720	-	1.91	82
cgaaCCGGCCTGAGCGGCCAGAacca	Hypothetical protein	cl01077	-	EY04_RS03745	-	-2.16	474
ttctCCGACCTGTTCGGCAAGGgcct	Carbon-nitrogen hydrolase	COG0388	R	EY04_RS03865	3.14	1.90	397
ttcgGCTGCCTGACCGCCATGGcggg	N-acetylmuramoyl-L-alanine amidase	COG3023	M	EY04_RS05345	-2.52	-3.81	265
caggGCGGCATGGGCGGCCAAGtcgt	Hypothetical protein	-	-	EY04_RS05505	-4.80	-4.52	260
tacaACGGCCAGCGCTGGGTATcggt	Hypothetical protein	-	-	EY04_RS09900	-5.83	-3.03	327
cctgGCGGCCAGTTCGCGGTGGgtcg	Transporter	COG0697	GER	EY04_RS10775	-6.27	-6.50	216
caaaCCTACTAGATTGGCCTGGtgtt	(2Fe-2S)-binding protein HcnA	cl09928	Q	EY04_RS11540	-4.31	-3.42	115
gacaGCGCCCTGAGCGTGTTGTcggt	Acyl-homoserine-lactone synthase CsaI	cl17182	T	EY04_RS11850	-6.06	-5.74	353
catcGCGCCCTGCTCGCCCAGTtgct	LuxR family transcriptional regulator CsaR	COG2771	K	EY04_RS11855	-3.24	-2.64	422
atcgCCGCCTTGTTCGGGAAGTattg	Methyltransferase	cl28097	S	EY04_RS14345	-4.17	-4.39	313
ttcaACTCCATGAGTGTCTTGTtatt	Hypothetical protein	-	-	EY04_RS14655	-4.64	-	440
tgccACTACTTCAGTTTGCAAGgagc	Membrane protein	-	-	EY04_RS15430	-1.69	-	9
gaccCCGGCCAGATCTTCAAGGtgac	Chitinase	COG3469	G	EY04_RS16020	-7.67	-6.91	333
cgccGCGACCTGCGCTGGATGCactg	50S ribosomal protein L21	cl09109	J	EY04_RS16195	-4.88	-2.71	431
aagaTCGACCAGAGCTTCGTGGccat	Transcriptional regulator MvaV	-	K	EY04_RS16500	-2.92	-	458
atcgGCTACCTGTTCTCGCTGTtcgt	Tryptophan halogenase PrnA	cl26176	Q	EY04_RS17650	-4.17	-1.93	378
tacgGCTACCAGCGCTTCCAGGaata	Histidine kinase	cl27674	K	EY04_RS18900	-3.35	-	350
ctgcACGACCAGAGCGGCCCGGtgct	Multidrug resistance protein B	COG2814	G	EY04_RS19225	-3.31	-2.62	202
tacaACGACAAGCGTGCCCAATgggc	Hypothetical protein	-	-	EY04_RS20040	-4.28	-	75

aggaCCTACCAGAGTTGGTAGGttta	Dialkylrecorsinol condensing enzyme	-	-	EY04_RS20370	-5.81	-4.07	321
atggTCTGCCTGATCTGGCAGTtcac	Sugar ABC transporter ATPase	COG3839	G	EY04_RS22875	-4.48	-3.63	230
tgtgGCGACCTGTTTGTCTATGctga	Glyceraldehyde-3-phosphate dehydrogenase	COG0057	G	EY04_RS22920	-3.03	-4.91	179
catgCCTACCTCTGCGTGCAGTccaa	Amino acid APC transporter	COG0531	E	EY04_RS22975	-3.82	-	430
ctggGCGGCCAGATTGGCCAGTgcgc	Hypothetical protein	-	-	EY04_RS25435	-	3.00	316
agccCCTACCAGATCTTGCAGGtgcc	Acyl-homoserine-lactone synthase PhzI	cl17182	T	EY04_RS25705	-5.53	-5.15	59
tggaACTACCAGATCTTGTAGTgatt	Transcriptional regulator PhzR	COG2771	K	EY04_RS25710	-4.12	-2.71	261
atccACTACAAGATCTGGTAGTtcca	Phenazine biosynthesis protein PhzA	cl09109	-	EY04_RS25715	-10.14	-6.68	150
ctggACGACCAGCGTGCCCTGCgccg	Gamma-glutamyl kinase	COG0263	E	EY04_RS26495	-	1.52	254
gacaCCGGCGTGGGTTTCAAGGacaa	Lipoate-protein ligase	COG0321	H	EY04_RS27530	-	1.71	181
ttccGCGGCCTGAATGCCAAGTcctt	30S ribosomal protein S14	COG0199	J	EY04_RS28075	-	-2.00	199
agccCCGGCCTGCTCGGCATCGaggc	Hypothetical protein	cl01888	-	EY04_RS28670	-3.29	-	446
gactGGTGCCTGAGCGGCCTGGaaca	Zinc ABC transporter permease	COG1108	P	EY04_RS29155	6.22	6.52	310
gtggGCGGCAAGAGAGGGTTGGctta	Molecular chaperone DnaK	COG1734	J	EY04_RS29180	7.05	8.16	70
gcgcGCGACCAGCTCGCGAGGGgaga	Hypothetical protein	cl27250	-	EY04_RS29455	-2.26	-	478
tgcaCCAACCAGAACCTGCCGCgccg	Type VI secretion protein	COG3520	U	EY04_RS29525	-3.13	-1.78	92
gccgTCGACCTGATCGTCATGGccgg	Cystathionine gamma-synthase	COG0672	P	EY04_RS30070	1.55	2.10	402
ttaaGCGACATGTTCGTAAAAGaaca	sn-glycerol-3-phosphate transporter	COG2271	G	EY04_RS30160	-	-2.86	113
gcgcCCGACCTGCGTGGCCAGGtggt	Polysaccharide deacetylase	COG0726	GM	EY04_RS15615	4.97	-	447
atggGCTGCCAGCGCTGGATGTtacg	GTPase CgtA	COG0536	DL	EY04_RS26500	-	-1.60	94
cgtcCCGGCCAGGTCGCGCTGGaagt	Kinase	COG1947	I	EY04_RS25560	-	-1.58	317
tacaCCGACCAGAGCGTGCTCGactg	ATPase	COG4962	UW	EY04_RS02750	-	2.22	331
gtgaCCAGCCAGAGCTTCCAGGtcag	Molecular chaperone	COG3121	W	EY04_RS19375	-1.83	-	437
cctgGCGGCAGGATCTGCCAGGcggc	RNA polymerase sigma factor	COG1595	K	EY04_RS20250	2.66	-	406
ggatCCGACCTGCGATTGCTATacct	Preprotein translocase subunit YajC	COG1862	U	EY04_RS24760	-1.71	-	254
ccacACCACCTGCGTGGCCTGTcacc	Cytochrome oxidase subunit I	COG0843	C	EY04_RS28555	-1.71	-	343
ttgaACGGCTGGCTTGTGCAATattc	Hypothetical protein	-	-	EY04_RS30255	-6.08	-1.99	349
ctggGCGGCAAGAGCGGCCATTacca	Molybdenum cofactor biosynthesis protein MoaE	COG0314	H	EY04_RS04490	-	2.30	261
gcctGCTACCTGTTATGGCTGGccta	Lysine transporter LysE	COG1280	E	EY04_RS07965	-4.16	-	458
ctctACGGCCTGCTCGGGCATTgctg	Hypothetical protein	cl01215	-	EY04_RS12830	-	1.64	234
ctggGCGGCCTGCGCGCCAACTacct	Protein phosphatase	COG0631	T	EY04_RS29570	-3.23	-	80
gtcaACGGCAAGACCGTCAAAGgcag	Hypothetical protein	-	-	EY04_RS32550	-	-2.58	338

[1]Lower case letters denote sequences flanking the *phz*-box.

423 **Table 4.** Regulatory genes under quorum-sensing control in *Pseudomonas chlororaphis* PA23.

Strain	Regulator	Locus Tag	log2 fold change	
			PA23 -6863	**PA23*phzR***
PA23*phzR*	ArsR family transcriptional regulator	EY04_RS05450	-	-3.88
	MarR family transcriptional regulator	EY04_RS19240	-	-3.22
	Transcriptional regulator	EY04_RS22860	-	-2.60
	Transcriptional regulator	EY04_RS20895	-	-2.22
	AraC family transcriptional regulator	EY04_RS15230	-	-2.07
	GntR family transcriptional regulator	EY04_RS13280	-	-1.53
	MarR family transcriptional regulator	EY04_RS07295	-	-1.50
	Transcriptional regulator	EY04_RS28835	-	1.61
	DeoR family transcriptional regulator	EY04_RS24510	-	1.71
	AraC family transcriptional regulator	EY04_RS15465	-	1.91
	Transcriptional regulator	EY04_RS14845	-	2.60
	LysR family transcriptional regulator	EY04_RS14120	-	2.70
PA23-6863 & PA23*phzR*	Acyl-homoserine-lactone synthase CsaI	EY04_RS11850	-6.06	-5.74
	Acyl-homoserine-lactone synthase PhzI	EY04_RS25705	-5.53	-5.15
	Transcriptional regulator PhzR	EY04_RS25710	-4.12	-2.71
	Fis family transcriptional regulator	EY04_RS29535	-4.04	-1.87
	LysR family transcriptional regulator	EY04_RS27730	-3.78	-1.69
	PadR family transcriptional regulator	EY04_RS24465	-3.57	-4.12
	Cro/Cl family transcriptional regulator	EY04_RS18125	-3.52	-1.68
	RNA polymerase sigma factor RpoS	EY04_RS05305	-3.28	-2.13
	LuxR family transcriptional regulator CsaR	EY04_RS11855	-3.24	-2.64
	GntR family transcriptional regulator	EY04_RS23170	-2.98	-3.18
	Transcriptional regulator	EY04_RS29740	1.85	1.92
PA23-6863	LuxR family transcriptional regulator	EY04_RS06740	-4.47	-
	Transcriptional regulator	EY04_RS27335	-4.13	-
	TetR family transcriptional regulator	EY04_RS01505	-3.20	-
	AraC family transcriptional regulator	EY04_RS23115	-2.96	-
	Transcriptional regulator MvaV	EY04_RS16500	-2.92	-
	AraC family transcriptional regulator	EY04_RS01040	-2.70	-
	LuxR family transcriptional regulator	EY04_RS23625	-1.77	-
	H-NS histone MvaT	EY04_RS23865	-1.73	-
	LuxR family transcriptional regulator	EY04_RS02735	-1.71	-
	Crp/Fnr family transcriptional regulator	EY04_RS06720	-1.70	-
	GntR family transcriptional regulator	EY04_RS07920	-1.66	-
	AraC family transcriptional regulator	EY04_RS19450	1.60	-
	AraC family transcriptional regulator	EY04_RS04305	1.81	-
	Transcriptional regulator	EY04_RS01770	2.16	-
	RNA polymerase sigma factor	EY04_RS00050	2.19	-
	RNA polymerase subunit sigma-70	EY04_RS10675	2.22	-
	RNA polymerase sigma factor	EY04_RS20250	2.66	-
	GntR family transcriptional regulator	EY04_RS12060	3.76	-

424
425

426 **Regulatory genes showing differential expression in both QS-deficient strains**

427 *phzI* and *phzR* are among the 11 regulatory genes that showed differential expression in
428 PA23*phzR* and PA23-6863 (Table 4). As expected, both genes were positively regulated
429 consistent with the paradigm of autoinduction (Table 4). The gene encoding RpoS showed 2.13-
430 and 3.28-$\log_2$fold lower expression in PA23*phzR* and PA23-6863 respectively. Cross-regulation
431 between QS and RpoS has been previously demonstrated (11). *rpoS,* was found to be positively
432 regulated by the Phz QS system (11), in keeping with findings presented herein.

433 Our RNAseq analysis revealed a connection between the Csa and Phz QS systems. In
434 PA23*phzR* and PA23-6863, *csaR* was downregulated by a factor of 2.64 and 3.24, whereas *csaI*
435 was downregulated 5.74 and 6.06, respectively. Through qPCR analysis, we discovered that
436 *csaR* and *csaI* were both downregulated at least 10-fold in PA23-6863 and PA23*phzR* (Fig. 3). In
437 the case of *aurI* and *aurR*, differential expression could not be determined from the RNAseq data
438 due to low read coverage of this region. Nevertheless qPCR analysis revealed that *aurI* was
439 upregulated, while *aurR* was downregulated in PA23-6863 (Fig. 3). A similar trend was seen in
440 PA23*phzR*, however expression of *aurI* and *aurR* was not significantly different from PA23 (Fig
441 3). While the regulatory network governing *aurIR* and *csaIR* expression has not yet been
442 determined, this hierarchical arrangement is similar to that observed in *P. aeruginosa* which
443 employs two AHL-based QS systems called Las and Rhl. In this bacterium, both the
444 transcriptional activator (*rhlR*) and the AHL synthase (*rhlI*) genes are under control of the Las
445 system (50,51).

446

447 **Regulatory genes showing differential expression in PA23*phzR* or PA23-6863**

448 Twelve genes encoding regulators belonging to diverse families including AraC, ArsR,
449 DeoR, GntR, LysR and MarR, exhibited altered expression exclusively in PA23*phzR* (Table 4).
450 The AHL-deficient strain displays differential expression of 18 other regulatory genes (Table 4).
451 This latter group includes two homologues of MvaT and MvaV, which are functionally and
452 structurally similar to the H-NS family of regulators reported to play a role in exoproduct
453 secretion by biocontrol and pathogenic pseudomonads (12,52). In PA23-6863, *mvaT* and *mvaV*
454 exhibited a 1.73- and 2.92-$\log_2$ fold reduction in gene expression, respectively. In *P. protegens*
455 CHA0, biocontrol activity against *Pythium ultimum* was virtually abolished in *mvaV mvaT*
456 double mutants, and reduced in *mvaT* and *mvaV* single mutants (52). Surprisingly, MvaT and
457 MvaV are repressors of most genes encoding exoproducts such as DAPG, HCN and
458 exoproteases in CHA0, while positively modulating the production of PLT and siderophores. In
459 *P. aeruginosa* PAO1, MvaT is a global regulator of virulence factors and biofilm formation, and
460 is involved in transcriptional repression of QS (53). At present, the role of the aforementioned
461 regulators, including MvaT and MvaV, in PA23 physiology has yet to be defined.

462

463 **Summary**

464 Exploration of the QS regulon of biocontrol strain PA23 has revealed that approximately
465 13% of the genome is under QS control. This circuitry regulates diverse aspects of PA23
466 physiology that extend well beyond the secreted factors required for fungal antagonism. We
467 believe that much of the QS regulon is subject to indirect control as *phz*-box elements were
468 identified upstream of only a small percentage of QS-regulated genes. The fact that numerous
469 transcriptional regulators show altered expression in the absence of QS further supports this

470 notion. The number of differentially expressed genes that are unique to the AHL-deficient strain
471 compared to the *phzR* mutant suggests that the Csa and/or Aur regulons are quite expansive and
472 likely govern more than cell surface properties (14). Future transcriptomic analysis of *csaRI*- and
473 *aurRI*-mutants should be conducted to reveal the scope of genes under CsaRI and AurRI QS
474 control. Such studies will undoubtedly uncover interactions with other regulators, adding another
475 layer to the increasingly complex cascade governing expression of PA23 biocontrol factors.
476

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482
483

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638 **Supporting information**

639 **S1 Fig.** Autoinducer produced by PA23 and derivative strains, assessed using the AHL biosensor
640 strain, *Chromobacterium violaceum* CVO26. Picture is representative of five biological replicates
641 obtained from three independent experiments.

642 **S2 Fig.** Differentially expressed genes in (A) PA23*phzR* and (B) PA23-6863 when compared to
643 PA23 wild type. Significantly differentially expressed genes are divided into functional categories
644 based on Cluster of Orthologous Groups (COGs) (54). Genes important for the synthesis and
645 regulation of biocontrol products are marked.

646 **S3 Fig.** Motility of PA23, PA23*phzR* and PA23-6863. A) Motility genes upregulated in PA23*phzR*
647 and PA23-6863 compared to PA23. B) Swim plates (0.3% agar) after 24, 48, and 72 h of
648 incubation; Swarm plates (0.8% agar) after 96 h incubation. Pictures are representative of five
649 biological replicates obtained from three independent experiments.

650 **S1 Table.** RNA-sequencing library reads mapped to the *Pseudomonas chlororaphis* PA23
651 genome.

652 **S2 Table.** Differentially expressed genes in PA23*phzR* compared to PA23 wild type.

653 **S3 Table.** Differentially expressed genes in PA23-6863 compared to PA23 wild type.

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Figure 1

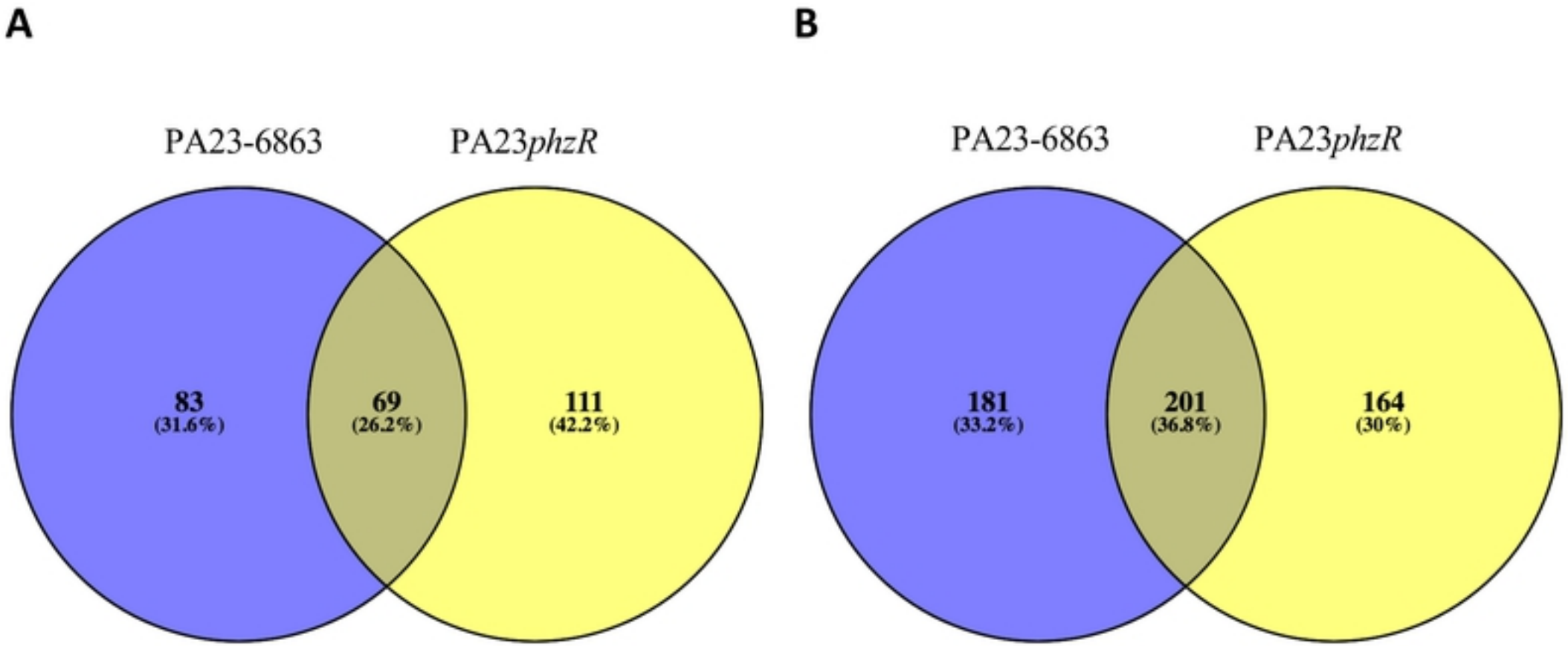

Figure 1

Figure 2A

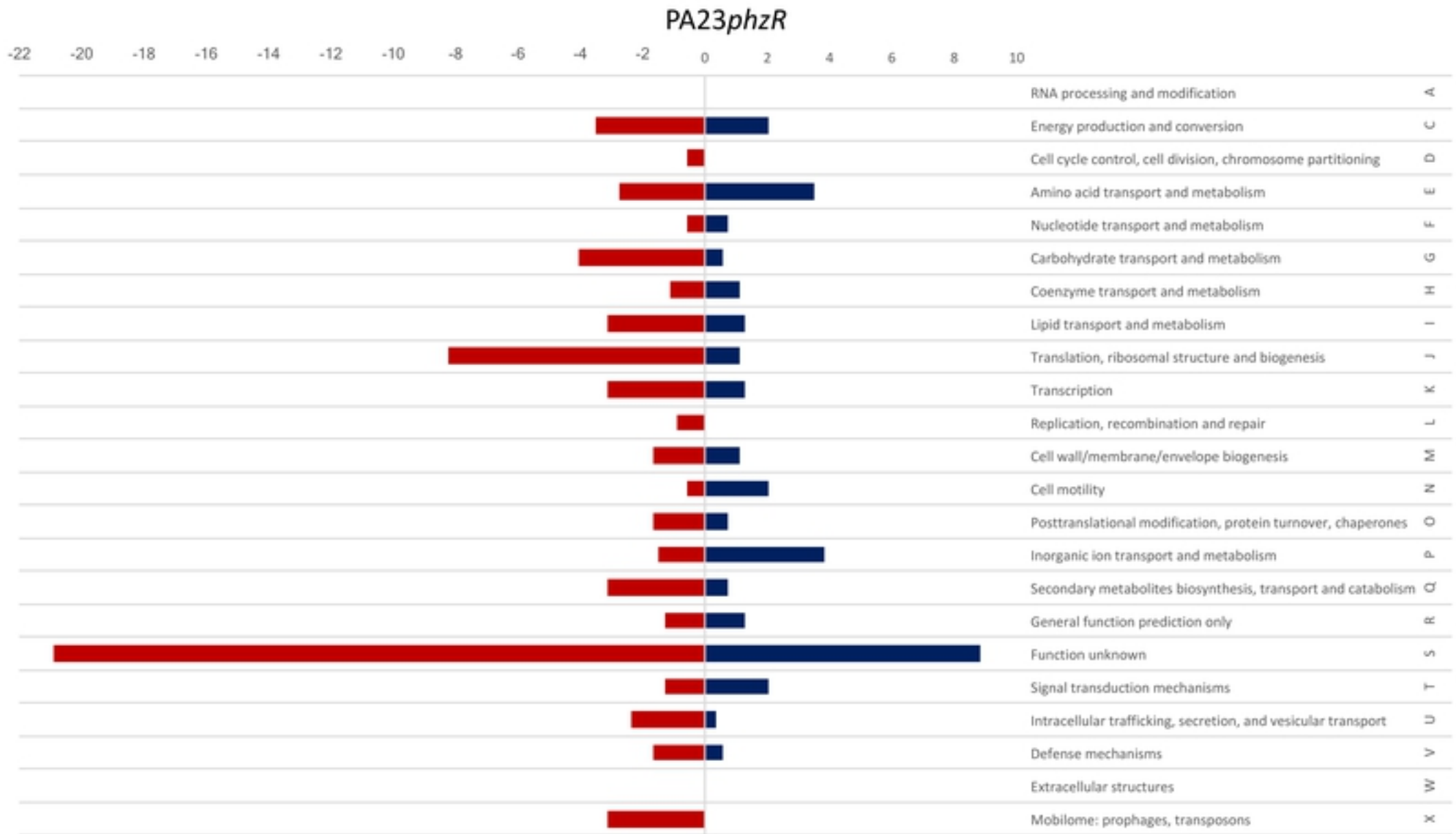

Figure 2A

Figure 2B

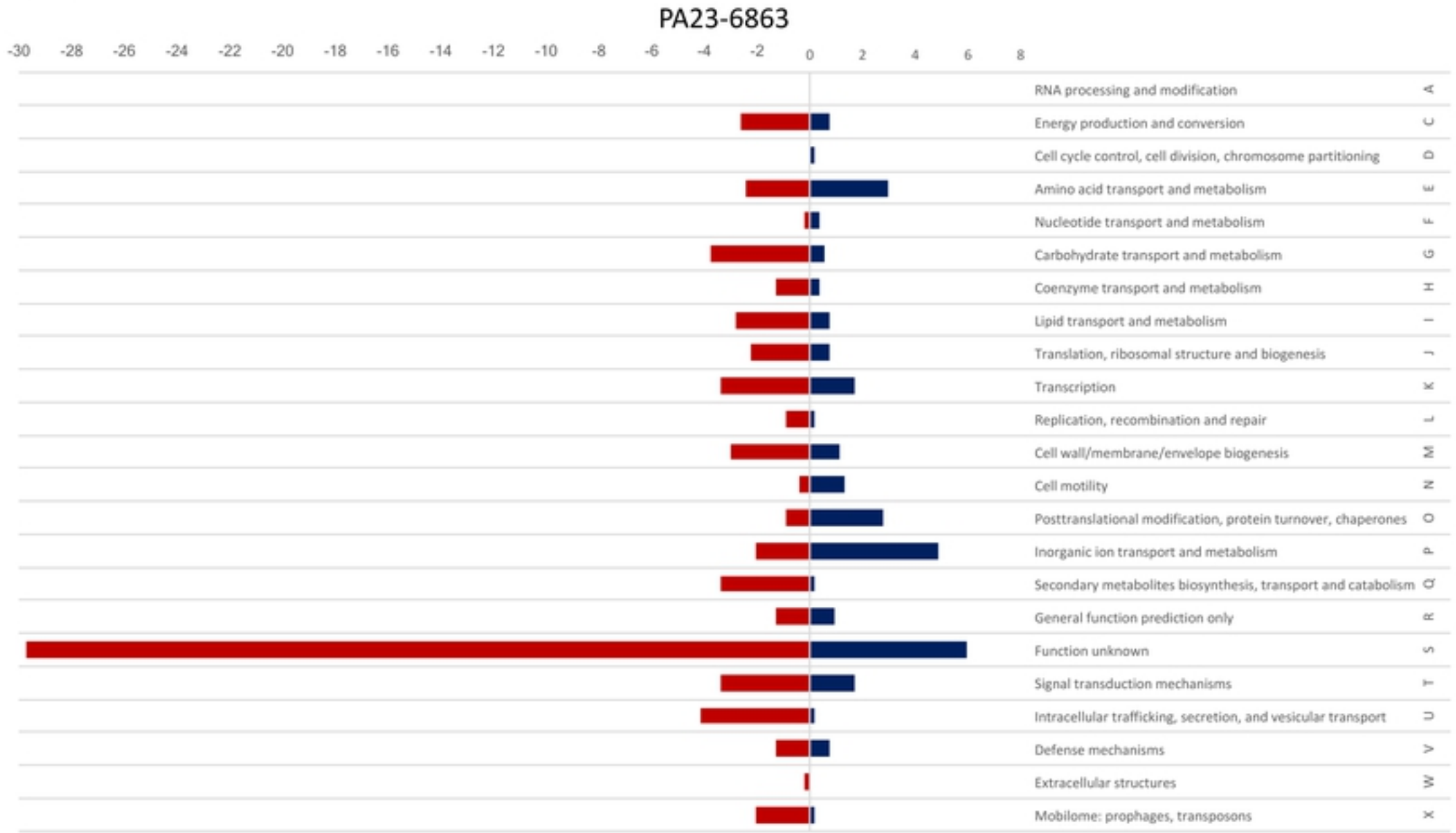

Figure 2B

Figure 3

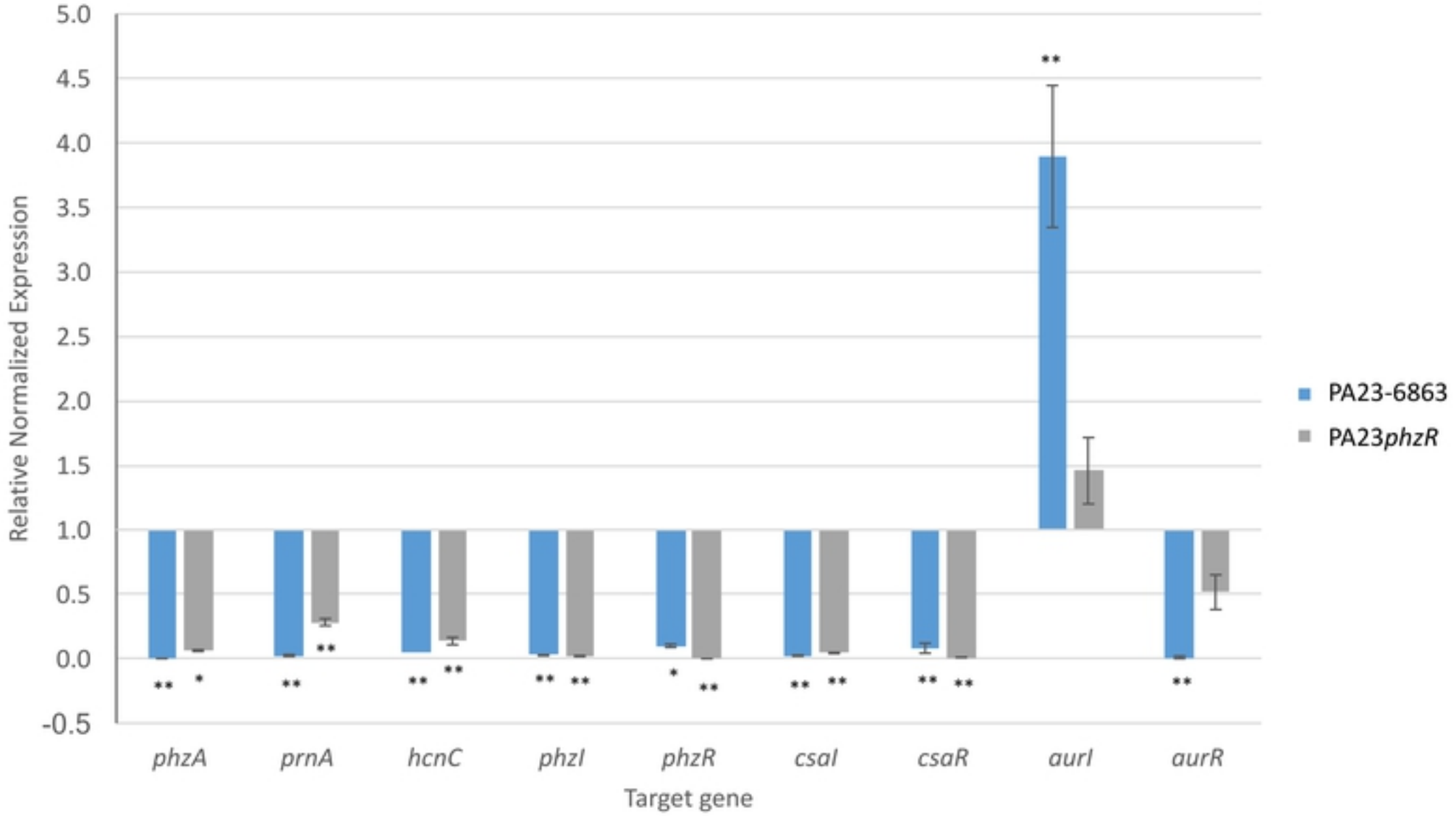

Figure 3