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Comparative transcriptomic studies identify specific expression patterns of virulence factors under the control of the master regulator PhcA in the *Ralstonia solanacearum* species complex

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ABSTRACT

The global regulator PhcA controls numerous traits associated to virulence and bacterial proliferation in strains of the plant pathogen *Ralstonia solanacearum* species complex. Here, we conducted a genome-wide RNA sequencing study of the GMI1000 wild-type strain and a derived *phcA* mutant grown in complete medium. The PhcA regulon we identified is the largest regulon described to date in the *R. solanacearum* species complex with 1581 regulated genes, representing about 30% of the bacterial genome. Among these genes, 166 transcription regulators were identified including known regulators controlling major cellular functions such as the Type 3 secretion system and 27 novel regulators that were not identified in previous transcriptomic studies. This study highlights that PhcA controls other functions beside pathogenicity *stricto sensu* which participate to the global cell homeostasis (metabolism, energy storage). We then compared the PhcA regulon identified in complete medium to the recently published PhcA regulon obtained *in planta*. This comparison of the set of GMI1000 genes subjected to PhcA regulation in both conditions revealed 383 common genes. Among them, 326 (85%) had a similar PhcA dependent regulation pattern in complete medium and *in planta*, and 57 (15%) displayed an opposite regulation pattern. A large majority of the genes repressed by PhcA in complete medium but activated *in planta* belong to the HrpG-HrpB regulon, which represents a set of key genes required for *R. solanacearum* pathogenesis. This latter class of genes appears to be specifically induced by PhcA in the plant environment whereas PhcA represses their expression in complete medium. The large set of direct and indirect targets identified in this study will contribute to enrich our knowledge of the intricate regulatory network coordinating the expression of virulence and metabolic functions in the model plant pathogen *R. solanacearum*.

1. Introduction

Strains from the *Ralstonia solanacearum* species complex (RSSC) cause wilt disease of more than 250 plant species distributed around the world, mainly under tropical climates. Bacteria enter susceptible plants through the roots, invade the xylem vessels and spread rapidly to aerial parts of the plants. Extensive colonization of the vascular system and production of large amounts of exopolysaccharides (EPS) alter water fluxes in the plant, causing wilting symptoms and eventual plant death.

RSSC pathogenesis is regulated by a complex regulatory network that responds to the soil and plant environments, the presence of plant cells and the bacterial cell density [1]. At the center of this network is the global regulator PhcA, a LysR-type transcriptional regulator that controls directly or indirectly expression of many virulence factors such as EPS production, plant cell wall degrading enzymes, bacterial motility and the Type 3 secretion system (T3SS) that allows the translocation of effector proteins into plant cells [2,3].

Levels of functional PhcA are regulated in response to bacterial cell density by a quorum-sensing (QS) mechanism that involves methyl 3-hydroxypalmitate (3-OH PAME) or methyl 3-hydroxymyristate (3-OH MAME) as a QS signal [4,5]. Therefore, at low cell density, for example during saprophytic life in soil or during early plant colonization steps, levels of functional PhcA are low, leading to a low virulence phenotype and expression of both twitching and swimming motility optimized for survival in soil or water, and invasion of plant tissues. In contrast, at high cell density, such as during plant xylem colonization, the accumulation of the QS signal leads to production of abundant functional PhcA and, subsequently, production of multiple virulence factors while suppressing production of survival/invasion factors. Recently, Peyraud et al. [6] reported that PhcA controls a trade-off between virulence

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Abbreviations

FDR	False Discovery Rate
RNA-seq	RNA sequencing
WT	wild-type
DEGs	Differentially Expressed Genes
T3E	Type 3 Effector
T3SS	Type 3 Secretion System
T6SS	Type 6 Secretion System
QS	Quorum sensing
HSL	Homo-Serine Lactone
RSSC	*Ralstonia solanacearum* species complex

factor production and bacterial proliferation in a RSSC strain. Indeed, a *phcA* mutant is avirulent but, as a consequence of not paying the cost for virulence, has a better growth rate and is able to metabolize 17 substrates more than the wild-type strain [6,7].

Genetic studies identified numerous traits known to be positively or negatively regulated by PhcA [1,2]. However, the whole PhcA regulon was only characterized recently through global transcriptome studies in strains GMI1000 and OE1-1 (a strain phylogenetically close to GMI1000) [7,8]. These genome-wide RNA sequencing (RNA-seq) studies were conducted using either total RNA extracted during tomato colonization (strain GMI1000) [7] or after growth in minimal medium (strain OE1-1) [8]. Here, we conducted a RNA-seq profiling study for the GMI1000 wild-type strain and a derived *phcA* mutant grown in complete medium. In order to evaluate the relevance of plant-derived signals in the activation of virulence determinants, we also compared the PhcA regulon in complete medium to the recently published PhcA regulon obtained *in planta*. Our analysis revealed that PhcA controls an extremely large set of genes corresponding to biological functions that extend beyond pathogenicity in the strictest sense. It also pinpoints a class of pathogenicity factors (including T3SS) which are subjected to a specific PhcA dependent regulation pattern, being repressed when bacteria are grown in complete medium but induced once inside the plant.

2. Material and methods

2.1. Bacterial strains and growth conditions

R. pseudosolanacearum strains used in this study are the wild-type (WT) strain GMI1000 [9] and the *phcA* disruption mutant derivative GMI1605 (*phcA*::Ω) [3]. Strains were grown in complete BG medium [10]. For agar plates, BG medium was supplemented with D-Glucose (5 g liter-1) and triphenyl-tetrazolium chloride (0.05 g liter-1). When needed, spectinomycin was added to the media at a final concentration of 40 mg liter-1.

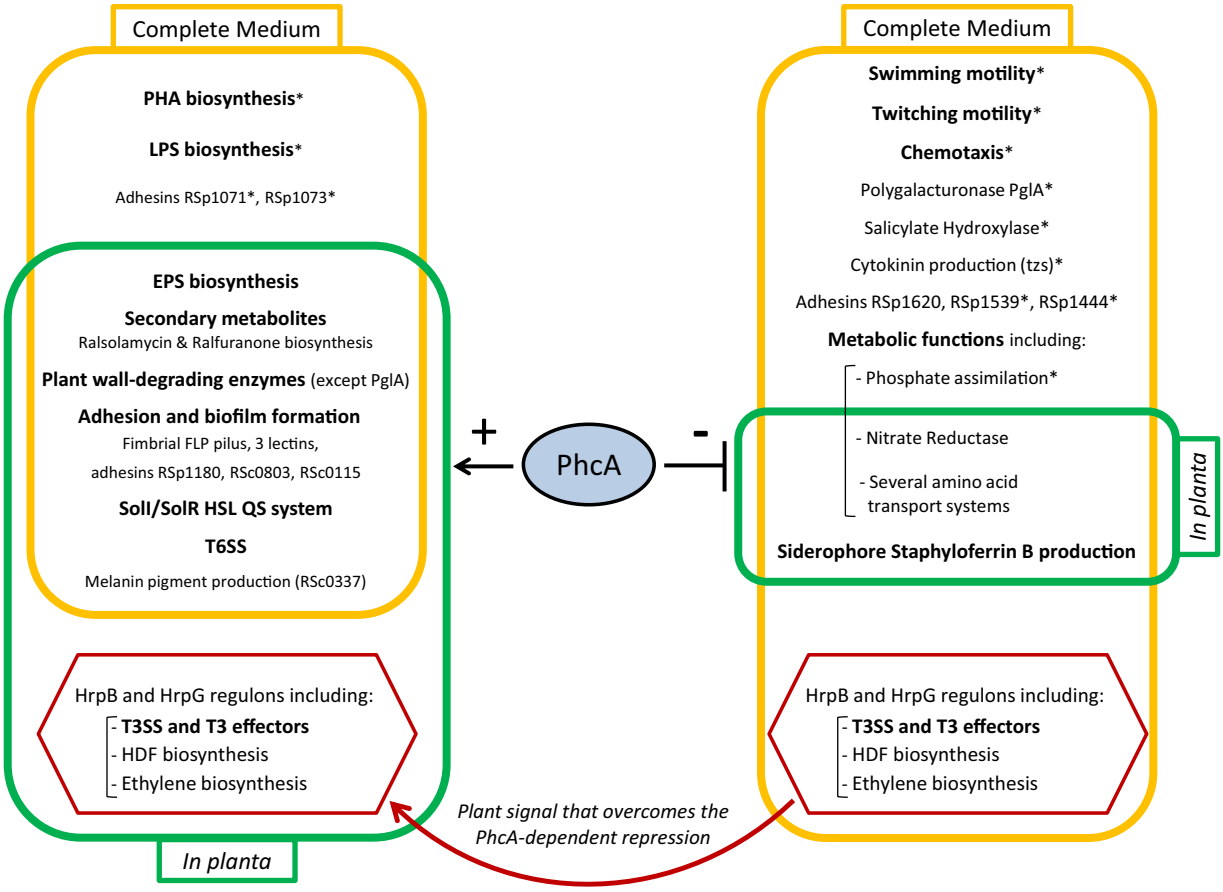

Fig. 1. Overview of the *R. solanacearum* traits regulated by PhcA based on transcriptomic analyses.
The genes and associated functions are separated as positively regulated by PhcA (left part) or negatively regulated (right part). The gold boxes highlight the traits controlled in complete medium while green boxes indicate *in planta* regulated traits. Traits showing a similar regulation pattern in complete medium and *in planta* appear in overlapping boxes. The asterisk * indicates functions controlled by PhcA in complete medium but information is missing about their regulation *in planta*. Virulence functions corresponding to the HrpB and HrpG regulons (red boxes) are repressed by PhcA in complete medium but are specifically activated *in planta* (red arrow). PHA: poly-*b*-hydroxybutyrate; LPS: lipopolysaccharide; EPS: exopolysaccharide; HSL QS: acyl homoserine lactone quorum sensing system; T6SS: Type 6 Secretion System; T3SS: Type 3 Secretion System; HDF: HrpB-dependent diffusible factor (3-hydroxy-indolin-2-one). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. RNA extraction, depletion of rRNA and sequencing

Total RNA was extracted from the wild-type (WT) GMI1000 strain and the *phcA*::Ω mutant strain growing in BG medium at comparable cell densities (OD_{600nm}–0.5). Three biologically independent experiments were conducted for each strain. Before RNA extraction, the bacterial culture was first stopped by mixing 1 ml ethanol/phenol (95:5) to 20 ml of culture during 3 min using a vortex. The culture was then centrifuged at 4000 × *g* for 10 min at 4 °C and the pellet was re-suspended in 200 µl H2O RNase free for total RNA extraction. Total RNA was isolated and depleted of ribosomal RNAs as previously described [11].

Oriented paired-end RNA sequencing (2x125 bp) was carried out by Fasteris (Fasteris SA, Plan-les-Ouates, Switzerland), using an Illumina HiSeq 2500 instrument and the procedures recommended by Illumina, with adaptors and amplification primers designed by Fasteris. The size of selected inserts was 150–250 bp. Libraries were sequenced in paired-end.

2.3. Mapping and statistical analysis of RNAseq data

Read pairs were mapped using the glint software (http://lipm-bioinfo.toulouse.inra.fr/download/glint/) with parameters set as follows: matches ≥ 40 nucleotides, with ≤ 3 mismatches, only best-scoring hits taken into account. Ambiguous matches (same best score for several read-pairs) were removed. Finally, between 16.8 and 29.6 M non ambiguous read-pairs were obtained. Mapped reads were imported into R environment. The package HTSFilter was used to eliminate very low-expressed genes from the analysis. A total of 4990 genes out the 5307 predicted genes were thus kept in. R package DESeq2 was used to normalize and complete the differential analysis by conducting the built-in Wald test [12]. The *p*-values thus obtained were adjusted for multiple comparisons using the false-discovery rate (FDR) method [13]. Genes with a FDR-adjusted *p*-value (padj,FDR) < 0.005 and an absolute Fold Change |FC| > 2 between strains were taken into further consideration in this work.

3. Results and discussion

3.1. The PhcA regulon is wider in complete medium than in planta

We analyzed the *in vitro* transcriptome of the GMI1000 and the *phcA*::Ω strains grown in complete medium. Differentially expressed genes (DEGs) (> 2-fold differentially expressed; *p*-value (padj,FDR) < 0.005) between the two strains confirmed that PhcA is a major master regulator since the comparative analysis identified 1581 DEGs, including 787 up-regulated and 794 down-regulated genes, representing about 30% of the GMI1000 strain coding sequences (Table S1). The PhcA regulon is therefore the largest regulon described to date in the *R. solanacearum* species complex.

The central position of PhcA in the RSSC regulatory network is illustrated by the fact that 166 genes annotated as transcription regulators are under the direct or indirect control of PhcA in complete medium (positive regulation for 42 transcription regulators, negative regulation for 122 transcription regulators). Many of these target transcription regulator genes are themselves known regulators of major cellular functions such as *hrpB/hrpG* (T3SS and other pathogenicity functions), *oxyR* (antioxidant response), *flhC* (flagellar motility), *cheY* (chemotaxis), *ntrC* and *phoB* (nitrogen and phosphate assimilation, respectively), *epsR* and *xpsR* (EPS biosynthesis) or *solR* (acyl-HSL responsive regulon) (Table S1) [1,2]. In agreement with previous studies [7,8], the cellular processes regulated by PhcA in complete medium also include Type 6-dependent secretion system (T6SS), twitching motility, siderophore production, adhesins and lectins presumably involved in biofilm formation. Among the PhcA regulated traits identified in our study are chemotaxis, Flp pili required for biofilm formation [14], some plant cell wall degrading enzymes (*pglA, pehC* and *pme*), biosynthesis of the core lipopolysaccharide [15], poly-β-hydroxybutyrate storage genes [16], the *tzs* gene (cytokinin biosynthesis) or the secondary metabolites ralstonins/ralsolamycin A & B [17,18] (Fig. 1).

In addition, we identified novel target genes negatively regulated by PhcA that encode for metabolite transporters such as phosphate utilization genes (RSc1529-RSc1535) and several ABC transport systems of unknown function (e.g. RSc1791-RSc1794, RSc2039-RSc2040, RSc3407-RSc3410, RSp1447). On the 199 genes with measured transcripts and annotated to be part of an ABC transporters, we found only 15 to be negatively regulated by PhcA in complete medium. These observations further support the recent finding that the enlarged metabolic versatility of a *phcA* mutant compared to the wild-type strain at high cell density is due to a resource allocation trade-off between growth and virulence instead of a transcriptional control of substrate usage [6]. The RSc0337 gene encoding a tyrosine hydroxylase responsible for the melanin pigment produced by GMI1000 [19] was found to be positively regulated by PhcA, in agreement with the observed phenotype of pigment production at high cell density when the strain is grown on complete medium plate. Finally, our analysis unraveled 27 novel target genes coding for transcription regulators that were positively regulated by PhcA in complete medium and were not identified in previous transcriptomic studies.

The recently published *in planta* transcriptome of the GMI1000 and *phcA* mutant strains revealed a total of 620 DEGs (> 2-fold differentially expressed; *p*-value (padj,FDR) ≤ 0.005) [7]. Using the same cutoff values, the number of DEGs in complete medium was more than twice the number of DEGs obtained *in planta*. It is worth mentioning that many genes or functions, such as flagellar and twitching motility or metabolic genes known to be regulated by PhcA according to phenotypic analyses [1,6,20,21], were present in the DEGs from the *in vitro* transcriptome studies performed in complete and minimal medium [8] but absent in the DEGs from the *in planta* transcriptome study [7]. Surprisingly, several genes previously described as PhcA-regulated and expressed *in planta* (e.g. *hrpG*, *vsrC*, numerous T3E and T3SS genes) [2] were also absent in the DEGs from the *in planta* transcriptome study [7]. This discrepancy could be the consequence of a technical bias either in the RNA sampling or RNA sequencing, thus leading to an under-representation of expressed genes *in planta*. If this is the case, it is therefore likely that some of the novel PhcA regulated genes identified in our study are also controlled in a similar fashion under *in planta* conditions.

3.2. The PhcA-dependent regulation pattern in rich medium is mostly conserved in planta

In order to compare the set of GMI1000 genes subjected to PhcA regulation in complete medium and *in planta*, we took the list of 787 and 794 genes up-regulated and down-regulated, respectively, in the *phcA* mutant in complete medium and looked for their expression pattern *in planta* using the Khokhani et al. dataset [7]. First, we observed that a large majority of the PhcA regulated genes in complete medium could not be compared (687 repressed and 511 activated genes) since these genes were absent (*i.e* no value provided) in the Khokhani et al. [7] dataset (Fig. 2). The PhcA dependent genes expressed in both conditions comprised 383 genes with 100 genes up-regulated and 283 genes down-regulated in complete medium (Fig. 2). Among these 383 genes, 326 (70 + 256), *i.e* 85%, had a similar PhcA dependent regulation pattern in complete medium and *in planta*. This set of genes included the two previously described direct targets of PhcA, XpsR [22] and PrhIR [23], which are respectively activated and repressed by PhcA. It also included many other virulence-related genes positively regulated by PhcA in both environmental conditions such as EPS (*epsABCDEFPR*), lectins (*rsl, rsl2* and RSp0569), glucanases (*eglA*, *cbhA*), T6SS (*tssABDEHJKL*), adhesins (RSc0115, RSp1180, RSp1605),

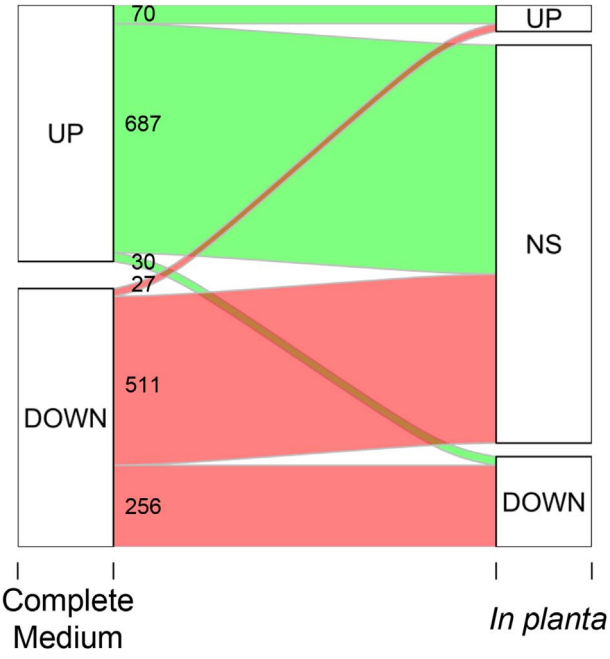

Fig. 2. Alluvial plot showing the distribution of PhcA-dependent genes *in planta* among the PhcA-dependent genes in complete medium.
In a *R. solanacearum* GMI1000 *phcA* mutant strain, (i) 70 up-regulated genes in complete medium are also up-regulated *in planta*, while 30 genes are down-regulated and (ii) 256 down-regulated genes in complete medium are also down-regulated *in planta*, while 27 are up-regulated. A large set of genes (687 + 511) is annotated NS *in planta*, indicating that they are either not differentially expressed or absent from the *in planta* dataset [7].

ralfuranone biosynthesis (*ralA* and *ralD*) and the acyl-homoserine lactone quorum sensing transcriptional activator (*solR*). On the other hand, the nitrate reductase (*narGHJI*) and siderophore biosynthesis genes (RSp0416-RSp0424) were found to be negatively regulated by PhcA in both conditions.

3.3. A subclass of pathogenicity genes is specifically activated by a signal perceived in plant that overcomes the PhcA-dependent repression

Among the 383 PhcA dependent genes that were differentially expressed both in complete medium and *in planta*, 57 (30 + 27) genes (15%) displayed an opposite PhcA regulation pattern (Fig. 2). In the *phcA* mutant, 30 genes were down-regulated *in planta* but up-regulated in complete medium (Fig. 2, Table 1) and 27 genes were up-regulated *in planta* but down-regulated in complete medium (Fig. 2, Table 2). The 27 genes up-regulated *in planta* and down-regulated in complete medium comprised a majority of ABC-type transporters for unidentified metabolites, except for one annotated as sugar transporter (RSc0547) (Table 2). This class of transporters specifically induced *in planta* in a *phcA* mutant highlights a probable specific metabolic adaptation of the *phcA* mutant in the plant xylem environment.

Among the 30 PhcA regulated genes that were up-regulated in rich medium but down-regulated *in planta* (Fig. 2), seven genes were also down-regulated in a *phcA* mutant grown in minimal medium conditions [8], thus suggesting that regulation for these genes is not plant-specific. Among the 23 remaining genes, 16 genes were reported to be HrpB-dependent [24,25] and included five T3E genes (*ripAB*, *ripX*, *ripAD*, *ripF1_1* and *ripAC*), two structural genes of the T3SS (*hrpY* and *hrcC*), three genes belonging to the *hdf* locus (*hdfA*, *hdfB*, *hdfF* [26]) and other *hrpB*-regulated genes (RSc0616, RSc0617, RSp0840, RSp0882, RSp1461) [25] (Table 1). The last seven genes included four HrpG-dependent but HrpB-independent genes (RSc2285, RSc2286, RSc3101, RSp0883) and three genes of unknown function (RSp0817, RSp0818, RSp0819) (Table 1). It thus appears that a large majority (23 out of 30) of the genes repressed by PhcA in complete medium but PhcA-activated *in planta* belong to the HrpG-HrpB regulon which represent a set of key genes required for *R. solanacearum* pathogenesis [27]. Because the HrpB-HrpG regulon contains more than a hundred of genes [25,27], the relatively low number identified in our comparative analysis is probably due to the under-representation of expressed genes in the *in planta* transcriptomic study [7]. Genes controlled by HrpG and HrpB therefore appear to be subjected to a specific expression pattern, being activated by PhcA *in planta* as essential actors of the pathogenicity program (*e.g* T3SS) but repressed in replete (nutrient rich) conditions such as during

Table 1
List of genes up-regulated in complete medium and down-regulated *in planta* in a *R. solanacearum* strain GMI1000 *phcA* mutant.

Gene ID	Gene Name	Description	CM		PLT[(a)]	
			log2 Fold Change	padj,FDR	log2 Fold Change	padj,FDR
RSc0616		CONSERVED HYPOTHETICAL PROTEIN	2.37	8.50E-07	-3.26	2.79E-04
RSc0617		SIGNAL PEPTIDE, CONSERVED HYPOTHETICAL PROTEIN	1.71	3.86E-06	-2.63	8.46E-04
RSc1729		TRANSCRIPTION REGULATOR	2.07	2.14E-05	-3.74	3.91E-03
RSc2285		SIGNAL PEPTIDE, CONSERVED HYPOTHETICAL PROTEIN	1.96	3.30E-06	-3.73	1.72E-05
RSc2286		CONSERVED HYPOTHETICAL PROTEIN	1.02	1.31E-03	-3.94	1.75E-04
RSc2510		SIGNAL PEPTIDE COG1638, TRAP-type C4-dicarboxylate transport system, periplasmic component	2.21	1.09E-04	-2.10	3.32E-04
RSc3101		SERINE PROTEASE	2.86	1.39E-41	-2.72	3.64E-05
RSp0200		OXIDOREDUCTASE PROTEIN WITH COG5322, Predicted dehydrogenase AND COG4992, Ornithine/acetylornithine aminotransferase DOMAINS	1.23	6.22E-04	-3.96	6.38E-05
RSp0201		CONSERVED HYPOTHETICAL PROTEIN	1.18	2.45E-05	-2.49	3.76E-04
RSp0693	hdfA	HDF BIOSYNTHESIS, COG0346, Lactoylglutathione lyase and related lyases	4.85	1.58E-11	-3.09	1.69E-03
RSp0694	hdfB	HDF BIOSYNTHESIS, TRYPTOPHAN-2,3-DIOXYGENASE	3.84	4.76E-17	-3.05	3.80E-04
RSp0698	hdfF	HDF BIOSYNTHESIS, AMINOTRANSFERASE	2.91	2.81E-10	-2.46	1.41E-03
RSp0817		OUTER MEMBRANE DRUG EFFLUX LIPOPROTEIN	1.64	1.63E-04	-2.29	2.17E-03
RSp0818		TRANSMEMBRANE DRUG EFFLUX PROTEIN	3.04	8.11E-41	-1.93	2.77E-04
RSp0819		DRUG EFFLUX LIPOPROTEIN	5.29	2.02E-42	-1.93	3.37E-03
RSp0840		TRANSMEMBRANE METHYL-ACCEPTING CHEMOTAXIS TRANSDUCER	5.78	1.25E-65	-2.63	1.02E-03
RSp0855	hrpY	TYPE 3 SECRETION SYSTEM PILUS SUBUNIT HRPY	2.68	7.87E-08	-1.26	1.85E-03
RSp0873	hrpB	TYPE 3 SECRETION SYSTEM REGULATORY PROTEIN HRPB	3.92	2.39E-27	-2.23	3.88E-03
RSp0874	hrcC	TYPE 3 SECRETION SYSTEM CONSERVED PROTEIN HRCC	4.16	6.29E-21	-2.00	2.83E-03
RSp0875	ripAC	TYPE 3 EFFECTOR PROTEIN RIPAC	1.29	2.97E-03	-1.20	2.64E-03
RSp0876	ripAB	TYPE 3 EFFECTOR PROTEIN RIPAB	4.53	1.41E-41	-1.32	4.68E-04
RSp0877	ripX	TYPE 3 EFFECTOR PROTEIN RIPX	5.51	3.96E-32	-1.20	8.83E-04
RSp0882		HYPOTHETICAL PROTEIN (HrpB regulon)	3.69	1.47E-07	-2.83	1.09E-03
RSp0883		HYPOTHETICAL PROTEIN	4.83	5.73E-30	-3.45	1.38E-03
RSp0958		COG2846, Regulator of cell morphogenesis and NO signaling	1.51	9.62E-04	-1.72	1.01E-03
RSp1416		TRANSMEMBRANE,CONSERVED HYPOTHETICAL PROTEIN	3.23	4.73E-17	-4.27	3.92E-05
RSp1461		SIGNAL PEPTIDE,CONSERVED HYPOTHETICAL PROTEIN	4.55	6.08E-66	-2.20	2.94E-03
RSp1555	ripF1_1	TYPE 3 SECRETION SYSTEM TRANSLOCON PROTEIN RIPF1_1	2.16	9.86E-10	-1.68	1.28E-04
RSp1601	ripAD	TYPE 3 EFFECTOR PROTEIN RIPAD	2.50	1.45E-21	-2.43	8.56E-04
RSp1652		COG1409, Phosphohydrolase	1.61	2.97E-03	-3.88	6.56E-04

In blue are highlighted the *hrpB*-regulated genes; in grey the *hrpG*-regulated genes. CM (complete medium); PLT (*in planta*); padj,FDR (p-value adjusted FDR); (a) RNAseq data from Khokhani *et al.* [7]. Genes highlighted in red correspond to the down-regulated genes in a *phcA* mutant whereas the up-regulated genes are highlighted in green.

Table 2
List of genes down-regulated in complete medium and up-regulated *in planta* in a *R. solanacearum* strain GMI1000 *phcA* mutant.

Gene ID	Gene Name	Description	CM		PLT[(a)]	
			log2 Fold Change	padj,FDR	log2 Fold Change	padj,FDR
RSc0474		OXIDOREDUCTASE 3-HYDROXYACYL-COA DEHYDROGENASE	-1.11	6.85E-10	1.43	1.38E-04
RSc0481		ABC TRANSPORTER AMINO-ACID-BINDING PERIPLASMIC PROTEIN (PBP)	-1.38	1.10E-03	1.63	2.41E-04
RSc0482	gltJ	ABC TRANSPORTER GLUTAMATE/ASPARTATE TRANSMEMBRANE PROTEIN	-1.05	8.57E-04	1.32	3.04E-04
RSc0484	gltL	ABC TRANSPORTER GLUTAMATE/ASPARTATE TRANSPORT ATP-BINDING PROTEIN	-1.27	1.28E-03	1.68	3.61E-05
RSc0547		TRANSMEMBRANE SUGAR TRANSPORTER	-2.29	8.45E-08	2.51	1.51E-05
RSc1014		TRANSCRIPTION REGULATOR	-1.38	1.28E-04	1.14	7.63E-04
RSc1293	emrA	TRANSMEMBRANE MULTIDRUG RESISTANCE PROTEIN A (TRANSLOCASE)	-2.25	5.88E-17	1.26	3.86E-03
RSc1342	ssuF	ORGANOSULFONATE UTILIZATION PROTEIN, MOLYBDOPTERIN-BINDING PROTEIN	-1.46	4.66E-15	1.20	3.04E-03
RSc1380		ABC TRANSPORTER SUBSTATE-BINDING PERIPLASMIC PROTEIN (PBP)	-1.39	8.36E-07	1.89	1.36E-04
RSc1381		ABC TRANSPORTER TRANSMEMBRANE PROTEIN	-1.47	3.49E-07	1.72	2.64E-03
RSc1766		NADP-DEPENDENT OXIDOREDUCTASE	-1.02	6.00E-10	1.07	3.21E-03
RSc1972	phaP3	POLYHYDROXYBUTYRATE GRANULE-ASSOCIATED PROTEIN (PHASIN) PHAP3	-1.67	8.99E-11	2.07	5.46E-05
RSc2353		SIGNAL PEPTIDE, CONSERVED HYPOTHETICAL PROTEIN	-1.39	1.13E-03	1.32	2.68E-03
RSc2938		ABC TRANSPORTER TRANSMEMBRANE PROTEIN	-1.42	1.74E-06	4.39	3.15E-09
RSc3047		ABC TRANSPORTER ATP-BINDING PROTEIN	-1.27	3.33E-14	2.10	1.63E-04
RSc3048		ABC TRANSPORTER SUGAR ABC TRANSPORTER, PERMEASE PROTEIN	-1.92	8.58E-11	1.63	2.13E-03
RSc3051		ABC TRANSPORTER COG1653, ABC-type sugar transport system, periplasmic component	-2.30	4.92E-11	2.29	4.86E-06
RSc3128	exaC	OXIDOREDUCTASE NAD+ DEPENDENT ACETALDEHYDE DEHYDROGENASE	-1.34	1.72E-07	1.77	6.64E-05
RSc3270		TRANSMEMBRANE, CONSERVED HYPOTHETICAL PROTEIN	-2.03	5.81E-15	1.58	4.73E-03
RSc3341		ABC TRANSPORTER TRANSMEMBRANE PROTEIN	-1.84	2.03E-07	2.26	3.17E-05
RSc3342		ABC TRANSPORTER SUBSTRATE-BINDING PERIPLASMIC PROTEIN (PBP)	-2.13	3.76E-09	2.26	6.67E-06
RSc3344		ABC TRANSPORTER ATP-BINDING PROTEIN	-1.07	3.40E-06	2.04	3.86E-05
RSp0053	fdhA	OXIDOREDUCTASE GLUTATHIONE-INDEPENDENT FORMALDEHYDE DEHYDROGENASE	-1.16	1.82E-04	1.96	7.89E-04
RSp1113		TRANSMEMBRANE MULTIDRUG-EFFLUX SYSTEM LIPOPROTEIN	-4.17	1.07E-129	1.32	9.14E-04
RSp1590		OXIDOREDUCTASE COG0665, Glycine/D-amino acid oxidases (deaminating)	-2.05	5.84E-11	1.32	1.24E-03
RSp1591		OXIDOREDUCTASE ALDEHYDE DEHYDROGENASE	-2.13	4.38E-14	1.58	1.28E-03
RSp1592		ABC TRANSPORTER ABC-type branched-chain amino acid transport systems, periplasmic component	-2.06	5.30E-14	1.85	5.71E-05

CM (complete medium); PLT (*in planta*); padj,FDR (p-value adjusted FDR); (a) RNAseq data from Khokhani *et al.* [7]. Genes highlighted in red correspond to the down-regulated genes in a *phcA* mutant whereas the up-regulated genes are highlighted in green.

the late stages of infection when the plant dies (Fig. 1). Expression of *R. solanacearum* T3SS genes is still effective when bacteria colonize xylem tissues [28,29] and results of transcriptomic analyses indicate that PhcA plays a key role in the fine-tuning of T3SS expression. These results also implies that a specific signal is perceived in plant xylem tissues by bacteria in order to relieve the PhcA-dependent repression that is exerted on *hrpG/hrpB*, and/or to specifically convert PhcA from a repressor to an activator of this class of genes through an unknown mechanism. It remains to be determined whether this signal is independent of the plant cell contact activation pathway known to activate HrpG/HrpB [30].

4. Conclusion

PhcA is a central regulatory node in RSSC strains, which controls a broad array of biological functions with a transcriptional control extending up to 30% of the bacterial genome. As such, PhcA controls other functions beside pathogenicity *stricto sensu* which participate to the global cell homeostasis (metabolism, energy storage). The comparison of the transcriptomic profiles of *R. pseudosolanacearum* grown in complete medium *in vitro* or after *in planta* growth revealed that a large majority of the PhcA controlled genes followed the same regulation pattern in both conditions, except for a set of HrpG-HrpB regulated genes including the T3SS and T3Es. This latter class of genes appears to be specifically induced by PhcA in the plant environment whereas this regulator represses their expression in complete medium. The large set of direct and indirect targets identified in this study will contribute to enrich our knowledge of the intricate regulatory network coordinating the expression of virulence and metabolic functions in this model plant pathogen [31].

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Conflicts of interest

The authors report no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.micpath.2018.01.028.

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