Proteomic Analysis of the GacA Response Regulator in *Pseudomonas chlororaphis* O6

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The GacS/GacA system in the root colonizer *Pseudomonas chlororaphis* O6 is a key regulatory system of many traits relevant to the plant probiotic nature of this bacterium. The work in this paper elucidates proteins using proteomics approach in *P. chlororaphis* O6 under the control of the cytoplasmic regulatory protein, GacA. A *gacA* mutant of *P. chlororaphis* O6 showed loss in production of phenazines, acyl homoserine lactones, hydrogen cyanide, and protease, changes that were associated with reduced *in vitro* antifungal activity against plant fungal pathogens. Production of iron-chelating siderophore was significantly enhanced in the *gacA* mutant, also paralleling changes in a *gacS* mutant. However, proteomic analysis revealed proteins (13 down-regulated and 7 upregulated proteins in the mutant compared to parental strain) under GacA control that were not apparent by a proteomic study of a *gacS* mutant. The putative identity of the downregulated proteins suggested that a *gacA* mutant would have altered transport potentials. Notable would be a predicted loss of type-VI secretion and PEP-dependent transport. Study of mutants of these GacA-regulated proteins will indicate further the features required for probiotic potential in this rhizobacterium.

Keywords: GacA response regulator, Proteomic analysis, Type 6 secretion system, Siderophore

Received June 3, 2018

Revised June 6, 2018

Accepted June 22, 2018

Fluorescent pseudomonads have been studied and developed as biocontrol agents promoting plant health (Kim and Anderson, 2018; Kim et al., 2011). Biological control-active *Pseudomonas* strains possess many beneficial traits such that they can be considered as examples of plant probiotics (Kim and Anderson, 2018). Recent comparative genomic analysis of ten pseudomonads strains connects their biocontrol potential in part to the production of an array of metabolites active across kingdoms (Loper et al., 2012). The root colonizer, *Pseudomonas chlororaphis* O6, produces several compounds with antifungal, nematocidal and insecticidal activities, as well as metabolites influencing plant growth that include indole acetic acid and the volatile, 2R,3R-butanediol (Anderson

and Kim, 2018; Kim and Anderson, 2018).

The production of many of these biocontrol products is regulated by a two-component global regulatory system. This system is composed of a membrane-bound sensor kinase, GacS, that under environmental stimulus activates the cytoplasmic regulator, GacA (Heeb and Haas, 2001). Transcriptome analyses of *gacS* or *gacA* mutants find 10% of the genes in *P. fluorescens* Pf-5 are under control of the GacS/GacA regulon (Hassan et al., 2010). Findings with *P. chlororaphis* isolates and other pseudomonads reveal that Gac-regulated traits differ between strains (Li et al., 2015). In *P. chlororaphis* G5, swimming and indole acetic acid production were decreased, but there was increased biofilm in biofilm formation and survival under oxidative stresses in *gacS* mutant (Li et al., 2015). In contrast, for *P. chlororaphis* O6 the *gacS* mutation increases swimming and swarming, and indole acetic acid formation (Anderson et al., 2017; Kang et

Research in Plant Disease

pISSN 1598-2262, eISSN 2233-9191

www.online-rpd.org

al., 2006; Kim et al., 2014b; Kim et al., 2014c; Oh et al., 2013a). Our recent phenotype analysis of the *gacS* mutant of *P. chlororaphis* O6 reveals the importance of Gac regulation of polyamine production as part of the regulatory cascade involved in expression of biocontrol-related traits (Park et al., 2018).

Proteomic analysis of mutants defective in the environmental sensor, GacS, or the stress-related sigma factor, RpoS, reveals novel functional proteins that are related to biocontrol (Kim et al., 2014a; Oh et al., 2013b). These include enzymes that function in relief of oxidative stress and in the synthesis of antibiotics. In this study, we constructed a *P. chlororaphis* O6 mutant defective in *gacA* to augment our knowledge of the array of beneficial genes controlled by the Gac system. Phenotypic changes in the mutant were examined and proteins markedly changed by elimination of GacA function were identified by a proteomic approach.

Microbial strains and growth conditions. Bacteria were stored at -80°C in 25% glycerol. The *P. chlororaphis* O6 parental and mutant strains were grown at 28°C with shaking at 200 rpm in King's medium B broth with supplemented with appropriate antibiotics. Cultures of *Escherichia coli* DH5 α were grown at 37°C on Luria-Bertani (LB) broth. Antibiotics, streptomycin (50 $\mu\text{g/ml}$), kanamycin (50 $\mu\text{g/ml}$), and tetracycline (25 $\mu\text{g/ml}$) were added where appropriate. To examine *in vitro* antagonism of growth of fungal pathogens, the wilt pathogen of tomato *Fusarium oxysporum* (KACC 44452) and the cause of tomato gray mold, *Botrytis cinerea* (KACC 40574), obtained from the Korean Agricultural Culture Collection (KACC, National Agrobiodiversity Center, Wanju, South Korea), were selected as test targets. The fungal isolates were grown on potato dextrose agar (PDA; Difco Inc., Detroit, MI, USA).

Construction of *P. chlororaphis* O6 *gacA* mutant. General DNA manipulations, including gene cloning and plasmid isolation were performed using standard protocols (Russell and Sambrook, 2001). All restriction enzymes and modified enzymes were purchased from FastDigest, Thermo Scientific Korea Ltd. (Seoul, Korea). Specific primer set with *EcoR*I recognition site at 5'-end was designed to amplify the full *gacA* gene, the forward primer, 5'-CGGAATTCCTTAAGCTTGCTGAAGCGCC-3' *uvrC* gene encoded the excinuclease C unit (PchlO6_3798) and the reverse primer, 5'-CGGAATTCCTT-CAGTTATCGGGCCTTGT-3' encoded the hypothetical gene

Fig. 1. Gene arrangement of the amplified 2.3 kb PCR product from *Pseudomonas chlororaphis* O6 genomic DNA with the specific primer set to amplify the intact *gacA* gene sequence. Arrows indicate the open reading frames and orientation of *gacA* and adjacent genes. Vertical arrows indicated the insertion site of the *Xba*I-digested *nptII* gene encoding kanamycin resistance into *StyI* site of open reading frame of *gacA* gene to interrupt the gene.

(PchlO6_3800) in *P. chlororaphis* O6 genome (Loper et al., 2012). The 2.3 kb PCR product, containing a partial sequence of *uvrC*, the intact sequence of *gacA*, and partial sequence of a hypothetical gene (Fig. 1), was digested with *EcoR*I and cloned into the pUC19 cloning vector. The *gacA* gene was interrupted by digestion with *StyI* and insertion of *Xba*I digested kanamycin resistance gene (*nptII*) from pRL648 (Elhai and Wolk, 1988). The 3.2 kb *EcoR*I fragment contained the disrupted *gacA::nptII* was subcloned into *EcoR*I digested pC-R111 vector (ThermoFisher Scientific, Carlsbad, CA, USA). The mutated *gacA* gene was digested with *Nsi*I and ligated into *Pst*I-digested pCPP54 marker exchange vector, and transferred into *P. chlororaphis* O6 through tri-parental mating using pRK2075 as a helper plasmid. The *gacA* mutant of *P. chlororaphis* O6 was selected on LB agar (Difco Inc.) supplemented with 5% sucrose and kanamycin (Miller et al., 1997). To confirm the mutation in *gacA*, PCR products were amplified from the mutant using *gacA* specific primers. The PCR product showed an increase of about 1 kb in product size as anticipated due to insertion of the kanamycin-resistance gene (data not shown). Sequencing revealed the presence of the *nptII* gene inserted into the *gacA* gene between the 103th amino acid, threonine, and the 104th amino acid, lysine, from the start amino acid (Fig. 1).

Phenotypic assays related to biocontrol traits. Biocontrol-related phenotypes of the *gacA* mutant were assayed by following published procedures (Fig. 2). Briefly, production of acyl homoserine lactones (AHLs) and phenazines were assayed on TLC plates with cell-free ethyl acetate extracts from *P. chlororaphis* O6 strains grown in LB

Fig. 2. Phenotypes associated with biocontrol-related traits of the parent strain of *P. chlororaphis* O6 and its *gacA* mutant. Phenazine production is denoted in parent strain by orange pigmentation when grown on LB and as fluorescent spots for 2-OH phenazine carboxylic acid (PCA) or 2-OH phenazine when metabolites are separated by TLC. Siderophore production causes a halo where the blue coloration of CAS medium is removed. Acyl homoserine lactones (C6 and C8 derivatives) are detected after TLC of extracellular metabolites by violet coloration with the *C. violaceum* CV026 indicator strain. HCN production causes a red color to develop with Cyantesmo indicator paper. Inhibition of fungal growth is demonstrated on PDA after co-inoculation of bacteria and either of two fungal plant pathogens. Each trait was positive for the parental strain but absent in the *gacA* mutant. The images are representative of two independent experiments for each bioassay.

broth (Oh et al., 2013a). Fluorescence under UV irradiation was used to detect the phenazines after TLC separation. The *Chromobacterium violaceum* CV026 bioindicator was used to detect AHLs on the TLC plate (McClean et al., 1997). Production of AHLs on the TLC plate (McClean et al., 1997). Production of hydrogen cyanide was detected on King's medium B agar amended with glycine (4.5 g/l) using Cyantesmo paper (Machery-Nagel GmbH & Co., Duren, Germany) (Kang et al., 2018; Lee et al., 2011). Production of siderophores were detected on chromazol-S agar containing 0.1 mM FeCl₃ (Schwyn and Neilands, 1987). For antifungal assessment, the strains were co-cultured for 5 d with the fungal pathogens *F. oxysporum* and *B. cinerea* on PDA and growth effects measured. All the phenotypic analysis was repeated at least twice.

Production of HCN, phenazines, and AHLs were significantly decreased by disruption of the *gacA* gene in *P. chlororaphis* O6 (Fig. 2). No phenazines were detected in extracts from the *gacA* mutant although they were produced by the parental strain (Fig. 2). The *gacA* mutation in *P. chlororaphis* G5 also caused the same phenotypic changes (Li et al., 2015).

Mycelial growth of *F. oxysporum* and *B. cinerea* was inhibited by wild type *P. chlororaphis* O6 but these fungi overgrew the *gacA* mutant colonies. Production of an extracellular protease (data not shown) was also reduced by the mutation whereas siderophore was overproduced, as observed with a *gacS* mutant. These phenotypes were similar to those of the *gacS* mutant of *P. chlororaphis* O6 (Anderson et al., 2017; Kang et al., 2004, 2007; Kim et al., 2014a, 2014b, 2014c; Oh et al., 2013a; Park et al., 2018; Ryu et al., 2007). These findings indicate that GacA is a functional regulator for these traits, working through regulation by GacS (Kim and Anderson, 2018).

2-D PAGE analysis. To determine some of the proteins that are under GacA in *P. chlororaphis* O6, soluble peptides were obtained from stationary phase cells and separated by 2D polyacrylamide gel electrophoresis. Comparisons of the peptide spots generated from extracts of the parental and mutant strains revealed many differences. The 2-D gels were prepared by Genomine (Genomine Inc., Pohang,

Korea) in Korea (Oh et al., 2013b). Briefly, cell-free soluble protein extracts were obtained by sonication in sample buffer of pelleted cells harvested from 2 d cultures grown in LB broth. Proteins were first separated by isoelectric focusing at 20°C with a pH gradient (4–10) using a Multiphor II electrophoresis unit (Amersham Biosciences, Piscataway, NJ, USA). Separation by size involved loading onto 10–16% gradient sodium dodecyl sulfate using a Hoefer DALT 2D system (Amersham Biosciences). Peptides in these gels were stained with alkaline silver and their intensities analyzed using PDQuest (BioRad, Hercules, CA, USA) software of the digitized images. Three independent 2-D gel analyses were performed, and the peptides that were significantly up or down-regulated by the *gacA* mutation were selected, and excised from the gel for matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) peptide mass fingerprinting (Kim et al., 2014a; Oh et al., 2013a). The selected protein spots were digested enzymatically in-gel with trypsin (Promega, Madison, WI, USA) for 45 min on ice (Shevchenko et al., 1996) and were desalted on columns. Mass spectra were recorded using an Ettan MALDI-TOF/Pro instrument (Amersham Biosciences, Bucks, UK). Peptide mass fingerprinting was employed to identify proteins of interest. Protein identification was performed using the ProFound search engine, which matches the observed peaks against theoretical peaks in NCBI nr and SWISSPROT in nr databases.

For Q-TOF analysis, the remaining trypsin-digested peptides were eluted with 1.5 µl of 50% methanol/49% water/1% formic acid into a pre-coated borosilicate nano-electrospray needle (Micromass, Manchester, UK), and the MS/MS data obtained with a nano-ESI on a Q-TOF2 mass spectrometer (Micromass). The MS/MS spectra were searched against the protein sequences from the NCBI databases using the MASCOT search program (www.matrixscience.com).

Three independent 2-D PAGE analyses showed more than 1,700 protein spots were reproducibly detected in each gel in the extracts from the parental or mutant cells. More than 100 protein spots were differentially expressed in the *gacA* mutant compared to the patterns from the parental strain (Fig. 3). From the gels, we selected 20 peptides which changed in intensity more than two-fold as illustrated in Fig. 3. Nine peptides were identified by ProFound search (Table 1) and 11 peptides were classified after LC-MSMS analysis (Table 2).

The putative identities of the proteins that were de-

Fig. 3. Intensities of 20 selected peptides differentially expressed in the parental and *gacA* mutant of *Pseudomonas chlororaphis* O6. Peptides were detected after two-dimensional polyacrylamide gel electrophoresis. The figures show the peptide intensities for each of three independent analyses. The peptides are denoted by arrows and their putative identities are shown in Tables 2 and 3. The peptides labelled in red increased in the mutant whereas those in black were decreased in intensity by the *gacA* mutation.

creased in intensity with the *gacA* mutation indicate that the transport properties of the bacterial cell are altered in multifunctional ways. GacA is required for production of proteins concerned with a type-VI secretion system, with a phosphoenolpyruvate-protein phosphotransferase (PtpS) involved in sugar import into the cell, with efflux involving

Table 1. Identification of the differentially expressed protein spots in *Pseudomonas chlororaphis* O6 wild type and *gacA* mutant by ProFound search program with data obtained from MALDI-TOF peptide fingerprinting of in-gel tryptic digested protein spots

Spot number	Observed migration ^a			Identified protein by ProFound Search			Fold change		
	Mr (kDa)	pI	Est's Z ^b	Identified gene and protein in <i>P. chlororaphis</i> O6 (locus_tag) ^c	Mr (kDa)	pI	Mean ^d	SE ^d	P-value ^e
113	28	4.45	1.50	<i>pppA</i> , serine/threonine phosphatase (PchlO6_3739)	26	4.95	-2.6	0.5	0.020
1123	27	4.74	2.26	phage portal protein, HK97 family (PchlO6_1242)	45	5.84	>-100		0.008
1827	87	4.79	2.35	<i>ptsP</i> , phosphoenolpyruvate-protein phosphotransferase (PchlO6_5904)	84	5.44	>-100		0.018
2503	52	4.96	1.71	<i>emhC</i> , efflux transporter, outer membrane factor (PchlO6_1402)	52	5.69	>-100		0.025
2628	59	5.09	2.40	<i>tssC2</i> , type VI secretion protein TssC2 (PchlO6_6187)	56	5.24	>-100		0.007
3519	54	5.16	2.32	<i>arcA</i> , arginine deiminase (PchlO6_4747)	47	5.49	>-100		0.010
5013	20	5.76	1.71	type VI secretion system effector, Hcp1 family (PchlO6_0238)	19	5.63	>-100		0.016
6713	81	6.02	2.35	<i>parE</i> , DNA topoisomerase IV, B subunit (PchlO6_0539)	70	5.73	>100		0.005
8902	139	7.37	2.37	<i>narG</i> , nitrate reductase, alpha subunit (PchlO6_3628)	140	6.31	>100		0.001

^aThe Mr and pI values were estimated from 2-dimensional gels obtained in three independent experiments.

^bEstimated Z score by ProFound program. Z scores of 1.281, 1.645, 2.326, and 3.090 mean that the search are in the 90th, 95th, 99th and 99.9th percentiles.

^cAnnotation from NCBI databases and SWISSPRO-nr using the ProFound program.

^dThe mean and standard error (SE) of fold change of the selected spot was calculated by comparing spot intensities between wild type and *gacA* mutant of three independent gels using quantitative image analysis (PDQuest 2-D analysis Software).

^eStudent's t-test.

an outer membrane transport factor (EmhC) and with the protein-export membrane protein (SecD) that functions in protein translocation across the cytoplasmic membrane. A portal protein of the HK97 family also decreased in intensity in the *gacA* mutant. The significance of these changes to the function of the bacterial cell in the rhizosphere awaits full investigation. Naturally occurring *gac*⁺ and *gac*⁻ pseudomonad strains are found in rhizospheres and soil (Seaton et al., 2013). They discuss that both contribute functions that are mutually valuable and suggest that the impact of a functional Gac system would vary with the complexity of the environment (Seaton et al., 2013). Indeed Natsch et al. (1994) found that although a *gacA* mutant of *P. fluorescens* colonized plant roots, it was less persistent whereas neither colonization nor persistence were affected in the *P. fluorescens* isolate used in the studies of Seaton et al. (2013).

In *P. aeruginosa*, loss of proteins of the type VI, TssC1, TssC2, and Hcp1, was observed in the *gacA* mutant. Additionally the detected decrease in serine/threonine phosphatase (PppA) may lead to altered post translational regulation of type VI secretion (Chen et al., 2015). Type-VI secretion appears to be multifunctional (Records, 2011). In plant beneficial pseudomonads it is important in biofilm formation as well as in inter-bacterial competition (Bernal et al., 2018). For instance, *tssC*

mutants in *P. fluorescens* did not form a biofilm and caused inhibition of biofilm formation of prey target cells in inter-bacterial competition (Gallique et al., 2017). A T6SS is required for control of the plant bacterial pathogen, *Xanthomonas campestris* by *P. putida* (Bernal et al., 2017). Similarly a type-VI secretion mechanism is involved in release of toxic effectors, Hcp1 and Hcp2, by *P. fluorescens* to challenge the soft rot pathogen, *Pectobacterium carotovorum* (Decoin et al., 2014). Our findings that formation of the type-VI secretion system is dependent on GacA agreed with earlier observations for the *gacA* mutant of *P. fluorescens* Pf5 (Hassan et al., 2010). Gac regulation of type VI secretion also is evident in the bean pathogen, *Pseudomonas syringae* (Records and Gross, 2010).

There was strong down regulation of a variety of proteins involved in general metabolism in the *gacA* mutant. Changes involved arginine deiminase (ArcA) required for producing citrulline in the urea cycle, a lipase active in lipid catabolism and an oxidoreductase, zinc-binding dehydrogenase catalyzing the oxidation of alcohols. A putative ATP-dependent helicase involved DNA replication could affect cell division. Transcripts of an oxidoreductase, zinc-binding dehydrogenase, and lipase also were reduced in the *gacA* mutant of *P. fluorescens* (Hassan et al., 2010). These findings exemplify similarities in GacA regulation between pseudomonads.

Table 2. Identification of the protein spots on 2-dimensional analysis regulated by GacA from *Pseudomonas chlororaphis* O6

Spot number	Observed migration ^a			Identified protein		Fold change			
	Mr (kDa)	pI	Identified protein in <i>P. chlororaphis</i> O6 (locus_tag) ^b	Ion Score or Matching sequence ^d	Mr (kDa)	pI	Mean ^c	SE ^c	P-value ^d
1318	39	4.71	oxidoreductase, zinc-binding dehydrogenase (PchlO6_0868)	DYGDYVRNFWGGGERKP	15	6.14	>-100		0.001
1719	72	4.82	protein-export membrane protein SecD (PchlO6_5012)	AIYHFFR NGGAFVNPIVALAR	68	5.22	>-100		0.001
2315	41	5.13	lipase, class 3 (PchlO6_3236)	KISITHAYVWNNSAR RTSVPASISPGTAIRKK	36	5.31	>-100		0.002
2621	67	5.11	type VI secretion protein TssC1 (PchlO6_3748)	LPYHGHFNR IFTTPEHPAVSR	55	5.37	>-100		0.016
2721	72	4.96	putative ATP-dependent helicase (PchlO6_5564)	CAALLNCYFPGAR	158	6.05	>-100		0.016
316	39	4.57	extracellular solute-binding protein (PchlO6_3412)	R.AVDAEFWVDHGEELEQR.F(80) R.FNAWAAR.(21)	38	5.10	>100		0.025
1014	15	4.91	MaoC-like protein (PchlO6_5497)	R.INLFAEATGDFQFIHVPVK.A(87) K.KPGQWLLK.A(43) K.MVVNYGLDSVR.F(39)	17	5.20	>100		0.018
2209	38	5.05	TonB-dependent outer membrane receptor (PchlO6_3670)	CAAVSLDLENHFP EGAHDPPFALDER	86	5.57	2.8	0.6	0.021
3001	24	5.14	antioxidant, AhpC/TSA family (PchlO6_4904)	KAYDVESEGGVAFRG (66) R.SQIVNDLPLGR.N (32) R.NVEIVASIDSHFTHNAWR.N (14)	22	5.18	>100		0.013
6703	80	5.89	monovalent cation/proton antiporter, MnhAB/PhaAB subunit (PchlO6_2634)	SSLAKHYWLP	105	9.55	>100		0.014
6706	73	5.92	hypothetical protein (PchlO6_2333)	ESLLMEADPSSH KLDGVADSADVENGLSPPG	57	6.25	>100		0.021

^aThe Mr and pI values were estimated from 2-dimensional gels obtained in three independent experiments. Ions score is $-10 \times \log(P)$, where P is the probability that the observed match is a random event. Individual ions scores > 49 indicate identity or extensive homology ($p < 0.05$). Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits. Amino acid sequences without ion scores were determined by Q-TOF analysis.

^bAnnotation from NCBI databases using the MASCOT search program (www.matrixscience.com).

^cThe mean and standard error (SE) of fold change of the selected spot was calculated by comparing spot intensities between wild type and *gacA* mutant of three independent gels using quantitative image analysis (PDQuest 2-D analysis Software).

^dStudent's t-test.

In our previous proteomic analysis with a *gacS* mutant, we identified reduced levels of proteins with major functions in reducing oxidative stress, a catalase/peroxidase (KatG) and glutathione peroxidase. The failure of the *gacS* mutant to produce the antibiotic, pyrrolnitrin, also correlated with loss of a functional GacS sensor eliminating tryptophan synthesis (TrpE) and tryptophan halogenase (PrnA), catalyzing the first step in synthesis of this antibiotic (Kim et al., 2014a). However, none of these previously identified proteins were identified as being changed with loss of GacA. Reduced expression of genes for the Fe-superoxide dismutase, a catalase and a

peroxidase were also evident from transcriptome analysis of the effects of a *gacA* mutation in *P. fluorescens* (Hassan et al., 2010) and this difference may reflect variability among isolates.

Expression of eight protein spots was significantly enhanced by loss of the GacA function. These proteins have diverse but little understood functions such as the B subunit of DNA topoisomerase IV, an extracellular solute-binding protein, a MaoC-like protein, a monovalent cation/proton antiporter and an hypothetical protein, as well as proteins with more characterized roles: nitrate reductase, a TonB-

dependent outer membrane receptor, and an antioxidant of the AhpC/TSA family. The increased expression of nitrate reductase contrasts with the findings in *P. fluorescens* Pf5 where the expression from a gene involved in the enzyme's structure was decreased in by *gacA* mutation (Hassan et al., 2010), again illustrating the uniqueness of regulatory pathways between isolates in the pseudomonad genus. Increased expression of nitrate reductase could be related to the need of the cell to use denitrification to supplement altered electron flow possibly due to cell membrane disturbance.

Higher levels of the TonB-dependent outer membrane receptor may be related to the enhanced secretion of siderophore in the *gacA* mutants of *P. chlororaphis* strains (Liu et al., 2018; Oh et al., 2013b). Several siderophore-related transcripts also increased in the transcriptome analysis of the *gacA* mutant of *P. fluorescens* (Hassan et al., 2010). The proteomic analysis of the GacS function in *P. chlororaphis* O6 showed GacS dependency for production of isozymes of catalase and peroxidase involved in protection against oxidative stress (Kang et al., 2004). In this study, levels of an antioxidant, AhpC/TSA family protein, were significantly increased in the *gacA* mutant of *P. chlororaphis* O6. Whether this change promotes tolerance of the *gacA* mutant to oxidative stress requires examination.

In summary, mutational analysis of *gacA* confirmed the key role of the GacS/GacA global regulator in expression of many traits in *P. chlororaphis* O6 involved with biocontrol of microbes. The proteomics analysis confirmed a role of GacA as a key regulator of proteins with varied cellular function, especially in production of a type VI secretion system. These findings indicate that future research into how the Gac regulatory system affects biofilm formation and interactions with other microbes should aid in understanding the probiotic potential for isolates such as *P. chlororaphis* O6 to improve plant health.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry

and Fisheries (IPET) through the Agri-Bio Industry Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (grant no. 316032-5).

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