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Full Length Research Paper

A novel one-component system, XvgA involved in regulation of bacterial growth and virulence of *Xanthomonas campestris* pv. campestris

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One-component systems were the simplest and the most effective signaling mechanism in bacteria. Per-ARNT-Sim (PAS) domains were important modules that play crucial roles in light or oxygen or redox signaling in bacteria, and most characterized GGDEF proteins involved in cyclic di-GMP (c-di-GMP) synthesis played crucial roles in c-di-GMP signaling in bacteria. In Xanthomonas campestris pv. Campestris (Xcc), xvgA encoded a protein containing both a PAS and a GGDEF domains. Deletion of xvgA altered bacterial morphology and significantly reduced not only Xcc virulence but also bacterial growth, the growth assays under different light conditions indicated that XvgA involved in light (red or far-red) signaling in Xcc. XvgA had been shown to possess diguanylate cyclase (DGC) activity that converted GTP to c-di-GMP *in vitro*. Moreover, the PAS domain was necessary for its DGC activity, and glutamic acid 98 (E98) site in the PAS domain was an active site, which was verified with bioinformatics and biochemical assays *in vitro.* These findings indicated that XvgA was an important one-component system involved in c-di-GMP and light signaling, which contributed to regulate Xcc growth, motility and virulence.

Key words: Xcc, PAS domain, c-di-GMP, one-component system.

INTRODUCTION

One-component systems (OCS) are evolutionarily older, more widely distributed among bacteria, and display a greater diversity of domains than two-component systems (TCS) (Ulrich et al., 2005). Also, OCSs are predicated to be the simplest and the most effective regulating mechanisms in bacteria (Ulrich et al., 2005). A signal transducer of OCS is the direct fusion of an input domain to an output domain in a single protein molecule, and the input domain normally senses environmental signals such as oxygen or redox or light. The Aer (aerotaxis) receptor in *Escherichia coli* senses redox and oxygen inside the cell, enabling the bacteria to respond to changes in internal energy, and a Per-ARNT-Sim (PAS) domain is the sensor for Aer and a HAMP domain

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interacts with the PAS domain to form an input–output module for signal transduction (Taylor and Zhulin, 1999; Taylor, 2007). Dos, a PAS Protein in *E. coli*, is also a direct oxygen sensor containing a PAS domain and a phosphodiesterase domain in the protein molecule (Delgado-Nixon et al., 2000). FixL is an oxygen sensor regulating nifA and fixK Genes in Rhizobium meliloti (de Philip et al., 1990), in which, a heme-binding PAS domain and a functional C-terminal histidine kinase domain can be separated (Monson et al., 1992). Many PAS proteins have been shown to participate in light signaling as putative photoreceptors. For example, the structure of Bph was revealed composing two PAS domains which possibly performed functions in Pr and Pfr switching (Wagner et al., 2005; Wagner et al., 2007), the RsbP containing an N-terminal PAS domain and a C-terminal phosphatase domain possibly interact with red light with its PAS domain (Avila-Perez et al., 2010). PAS domains are the sensory input sites for these proteins, and are widespread signaling modules that sense light, redox potential, oxygen, small ligands and the overall energy level of a cell (Taylor and Zhulin, 1999).

Cyclic di-GMP (c-di-GMP) is a second messenger that is known to regulate a range of behaviors in diverse bacteria (Ross et al., 1987; D'Argenio and Miller, 2004; Jenal, 2004; Romling et al., 2005; Jenal and Malone, 2006; Romling and Amikam, 2006; Ryan et al., 2006; Ryan et al., 2007). Accumulating evidence demonstrates that the steady-state levels of c-di-GMP are a key determinant that affects the expression of many bacterial virulence genes (He and Zhang, 2008; He et al., 2009). The synthesis of c-di-GMP from two molecules of GTP is catalyzed by diguanylate cyclase (DGC), which is characterized by the presence of a GGDEF domain (Tal et al., 1998; Paul et al., 2004; Christen et al., 2005; Ryjenkov et al., 2005; Schmidt et al., 2005). The formation of linkage, that PAS domain as an potential input domain is indirectly connected to a GGDEF domain as output domain, was frequently observed in two components signal transduction system (TCSTS) (Qian et al., 2008). Nevertheless, functions of most PAS domains are still uncovered in these formations.

Xanthomonas campestris pv. campestris (Xcc) is the causative agent of crucifer black rot disease, which causes severe losses in world-wide agricultural yield, especially in Africa and Asia (Swings et al., 1993). Xcc generally invades and multiplies in cruciferous plant vascular tissues, resulting in the characteristic “black rot” symptoms of blackened veins and V-shaped necrotic lesions at the foliar margin (Alvarez, 2000). In Xcc, although a role for c-di-GMP signaling has been demonstrated in Xcc virulence (Ryan et al., 2007), the interaction or crosstalk of light signaling and c-di-GMP signaling remains unclear. To study the relationship between the both signalings, we have deleted all PAS-GGDEF proteins in Xcc (our unpublished data) and found that the XC_1036 mutant exhibited significant reductions in virulence and growth (thereafter designated as xvgA). Here, we report the detail functions and the involvement in light and c-di-GMP signaling of XvgA as a one component system.

MATERIALS AND METHODS

Bacterial strains, plasmids and growth conditions

All bacterial strains and plasmid constructions used in this study are listed in Table S1. *E. coli* DH10B was used in propagating plasmid constructs, *E. coli* S17-1 (Simon et al., 1983) in delivering plasmids to Xcc by conjugation, *E. coli* BL21-AI and BL21(DE3) in expressing His-tagging protein. *E. coli* was routinely grown in Luria-Bertani broth at 37°C. Xcc 8004 was grown in rich medium NYG or in minimal medium MMX at 28°C (Qian et al., 2008). Antibiotics were added to media, if required, at concentrations of kanamycin at 25 µg/ml for both Xcc and *E. coli*; spectinomycin at 150 µg/ml for both Xcc and *E. coli*; ampicillin at 100 µg/ml for E. coli; rifampin at 100 ug/ml for Xcc and *E. coli*; and tetracycline at 25ug/ml for Xcc and E. coli. For genetic transformation, *E. coli* competent cells were prepared by washing the exponential-phase cells (OD600 approximately 0.4 to 0.5) grown at 37°C in liquid SOB medium (peptone, 20 g/L; yeast extract, 5 g/L; NaCl, 0.5 g/L; 2.5 mM KCl, pH 7.0) with 10% ice-cold glycerol.

DNA manipulation

Molecular biological methods including purification of plasmid and genomic DNA, transformations, restriction endonuclease digestions, DNA extraction from agarose gels, ligation reactions, PCR and DNA transformation into *E. coli*, were carried out using standard protocols (Sambrook and Russell, 2001). PCR products were cleaned using the Axygen PCR purification kit (Axygen, China) and DNA fragments were recovered from agarose gels using Tiangen gel purification kit (Tiangen, China). Oligonucleotide primers were synthesized from Invitrogen (Invitrogen, China). Primer sequences are provided in Table S2. For construction of C-terminal His6-tag protein, xvgA and the truncated fragment (GGDEF) were amplified and ligated into the pGEM T-easy vector (Promega, USA) and then subcloned into pET23b vector (Novagen, Germany).

Construction of mutants

An in-frame deletion mutant of xvgA was created by a suicide vector based method (Schäfer et al., 1994). To generate a complementary strain of the mutant, a DNA fragment that contains the full gene and about 500 bp upstream sequence was ligated into the promoterless broad-host vector pLAFR6 (Huynh et al., 1989). The recombinant vectors were transformed into the mutant strain to create a complementary strain (pLCxvgA) as described previously (Qian et al., 2005). An over-expression strain of xvgA (pHMxvgA) was also constructed, containing the full-length xvgA gene ligated into pHM1 vector (Davis et al., 1985).

Virulence assays

Plant inoculation of Xcc infection was as described in previous studies (Dow et al., 1990; Qian et al., 2005). The virulence of Xcc to four-week-old cabbage cultivar *Brassica oleraceae* cv. Zhonggan 8 was estimated after bacteria were introduced into the leaves by leaf clipping. Xcc 8004 wild-type and sterile water were used as controls. Bacteria grown overnight in NYGB medium were washed and re-suspended in 1 mM $MgCl_2$ to an OD at 600 nm of 0.1 or

0.001. After inoculation, the plants were kept in a greenhouse at approximately 28 to 35°C, with an intensity of light illuminating, and with a relative humidity > 70%. Between 15 and 30 leaves were inoculated for each strain tested. Lesion length was measured 7 days after inoculation. Each strain was tested in at least three separate experiments.

Protein purification

Proteins were purified as C-terminal His6-tag fusions with Ni-NTA resin under native conditions according to the manufacturers' instructions. The cultures were grown under constant shaking at 37°C in LB medium supplemented with ampicillin (100 mg ml^{-1}) to an OD600 of 0.5. The protein was induced by adding arabinose (0.2%) and sustained for 3 h at 20°C. Cells were collected by centrifugation at 14,400 g for 12 min, then pelleted and stored at –80°C until needed. The cells were resuspended in lysis buffer (20 mM Tris-HCl, pH 8; 1 M NaCl, 10% glycerol, 10 mM imidazole, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mg/ml lysozyme, 10 units/ml benzonase nuclease (Novagen)), incubated at room temperature for 30 min, sonicated (5 times, 10 s) and centrifuged for 60 min at 25,000 g to generate a cleared lysate. The supernatant was loaded onto Ni-NTA resin (Qiagen), washed twice with 25 ml of wash buffer (20 mM Tris-HCl, pH 8; 0.5 M NaCl, 10% glycerol, 20 mM imidazole, 0.1% Triton X-100, 1 mM PMSF; 25 mM $MgCl_2$), and then eluted using 1 ml of elution buffer (50 mM Tris-HCl, pH 7.6; 0.5 M NaCl; 25 mM $MgCl_2$; 10% glycerol; 300 mM imidazole). All samples were filtered through an Ultrafree-MC (15KD) spin filter (Millipore) and then aliquoted for storage in storage buffer (50 mM Tris-HCl, pH 8; 0.5 mM EDTA; 2 mM dithiothreitol [DTT]; 25 mM MgCl2; 100 mM KCl; 30% glycerol) at –80°C. Protein concentrations were measured using bicinchoninic acid protein assay regent and a BSA standard. Protein preparations were examined by 12% SDS-PAGE to verify molecular weight and purity.

Enzymatic assays

The assay buffer and reaction conditions were described elsewhere (Ross et al., 1987; Ryjenkov et al., 2005). A standard reaction mixture contains purified 5 µM protein. 50 mM Tris-HCl (pH 7.6), 10 mM MgCl2, 0.5 mM EDTA, 50 mM NaCl and 10 mM MnCl2 in 0.6 ml volume. Reactions were started by the addition of 150 µM (final concentration) GTP (Sigma, USA) or c-di-GMP (Biolog, German). All experiments were performed in dark or light conditions. Aliquots of 100 µl were withdrawn to a sterile Eppendorf tube at regular time intervals and immediately placed in boiling water for 5 min. After centrifugation at 15,000 g for 2 min, the supernatant was filtered through an Ultrafree-MC (3 KD) spin filter (Millipore) and analyzed by High-performance liquid chromatography (HPLC).

Determination of c-di-GMP concentration with HPLC

HPLC: Samples (each, 10 µl) were injected into the 15 by 4.6 cm Supelcosil LC-18-T column (Supelco) and separated by reversed-phase HPLC (Waters HPLC system; USA). Buffer A (100 mM KH_2PO_4 [pH 5.9]) and buffer B (85% A, and 15% acetonitrile) were used in the gradient program. The following protocol was used for separation (the values are times in minutes and percentage of buffer B used): 0.0 min (m), 0%; 2.5 m, 0%; 5.0 m, 30%; 10.0 m, 60%; 14.0 m, 100%; 21.0 m, 100%; 22.0 m, 50.0%; 23.0 m, 0% and 28.0 m, 0% at a flow rate of 0.7 ml/min. Nucleotides were detected at a wavelength of 254 nm.

Growth assays of Xcc strains

Xcc strains were grown in MMXC medium with 100 rpm agitation at 28°C in red (4.30 mW/cm^2), far-red (3.36 mW/cm^2), white light (12000 lux) or in dark. In each condition, Xcc cell-number and viability were estimated at the fourth and fifth day by plating on NYG agar at 28°C, and the mean value in dark (T0) and light condition (T1) were used in growth analysis. Growth rate (GR) was calculated as the ratio of the mean value in (T1) to that in (T0), and relative growth rate (RGR) was the ratio of the mutated strain GR compared with that of wild-type Xcc 8004.

Motility assays

Strains grown overnight in NYGB medium were washed and resuspended in 10 mM MgCl2 (OD600 = 0.1). Motility was assayed on 0.3% (w/v) NYGB agar as previously described (DiLuzio et al., 2005). Xcc strains were inoculated into the center of the plate using a sterile needle. Visualization of the colony was improved by staining with Coomassie brilliant blue R250 for 1 min. To assay different oxygen cultures, plates were cultured in a Heraeus CO2 incubator (Thermo Scientific, Germany) at 28°C with 1 or 20% oxygen.

The colony diameters on motility plates with 20% oxygen (T0) or 1% oxygen (T1) were measured. The motility rate was calculated as the ratio of T1 to T0. The relative motility rate was the ratio of the motility rate of mutants to that of the wild type.

Bioinformatics analysis

Similarm searches were carried out using BLASTN and BLASTP (Altschul et al., 1990). Protein domains organizations were determined by searching in SMART (Letunic et al., 2009). The secondary structure of each PAS domain was predicted in three tools: Jpred3 (Cole et al., 2008), PSIPRED (Jones, 1999) and I-TASSER (Roy et al., 2010). The tree of topology similarity of secondary structures was built with ClustalX (Larkin et al., 2007) in profile-mode alignment, in which, gap opening and extension penalties were set as 4 and 0.2, and with a user defined matrix (C-C:1, H-H:4, E-E:4, the other pair with 0). The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). Neighbor-joining phylogenetic trees were built with MEGA4 (Tamura et al., 2007). The sequences were trimmed in VISSA (Li and Godzik, 2006). Sequence logos were generated using WebLogo (Crooks et al., 2004).

RESULTS

XvgA is a c-di-GMP synthase

SMART search shows that XvgA is a putative GGDEF characterized protein (Figure 1A). Since most GGDEF characterized proteins have been proved to act as DGCs (Tal et al., 1998; Paul et al., 2004; Christen et al., 2005; Ryjenkov et al., 2005; Schmidt et al., 2005), we predicted that XvgA could convert GTP to c-di-GMP in vitro. To test this, we expressed and purified full-length protein of XvgA (Figures 1A and B) and performed enzymatic assays with GTP and XvgA. HPLC results showed that the content of c-di-GMP in the reaction mixtures increased after a 60 min reaction (Figure 1C), implying that XvgA is a c-di-GMP synthase.

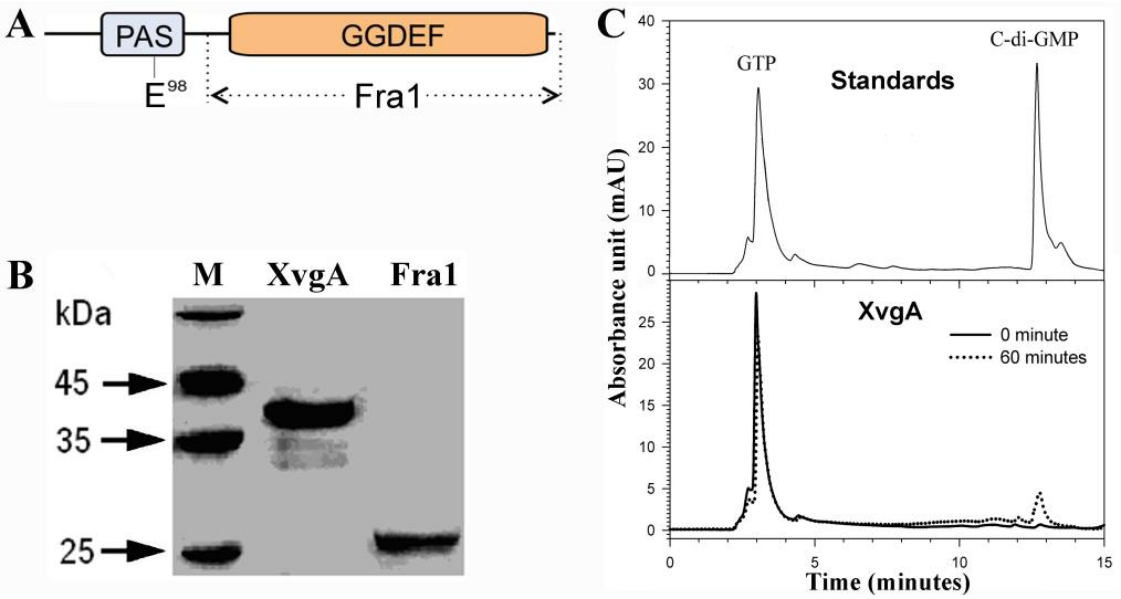

Figure 1. The DGC activity of XvgA. A. Motif structure of XvgA showed that it had one PAS and one GGDEF characterized domain; B. SDS-PAGE analysis of purified protein and fragment 1 of XvgA (Fra1) indicated that their molecular weights were approximate 38 kDa and 27 kDa respectively; C. HPLC analysis of the reaction mixture of XvgA and GTP indicated that XvgA had DGC activity.

Bioinformatics analysis on PAS domain

SMART analysis indicates that XvgA contains a PAS domain (Figure 1A) that might be involved in light, redox potential, oxygen, small ligands, or other stimuli (Taylor and Zhulin, 1999). For the purpose of functional clustering, 16 known PAS protein were collected, which were known involving in blue light, oxygen, redox or voltage signaling (Table S3). Bioinformatics analysis based on the secondary structure topology (SST) of the PAS domains showed that the XvgA cluster (circled with blue color in Figure 2A) is closely linked to O07014 (RsbP), which was deduced involving in red light response in Bacillus subtilis (Avila-Perez et al., 2010). The next linking node to the XvgA cluster was arose from a voltage signaling PAS domain (Q02280). The PAS domains of XvgA and RsbP were also linked together in aclustering analysis of our previous work, and several functional linkage of PAS domains were identified in that research or literatures searching. Therefore, PAS domains of the XvgA cluster were predicted having a potential role in red light signaling, but those still need to be validated with biochemistry and genetics methods.

Sequence alignments of the cluster (circled in blue colour in Figure 2A), which include 2 HKs, 2 GGDEF characterized proteins and 1 transcription regulator, showed that there was a conserved Glutamic acid (E98 of XvgA) in Fa region of PAS domain among the homologues of XvgA (Figure 2B), which indicates that E98 site is possibly an important site for the functions of these PAS domains.

PAS domain is indispensable and E98 is an active site for XvgA DGC function

To determine whether the PAS domain of XvgA is actually involved in diguanylate cyclase (DGC), we deleted the PAS domain (Fra1) (Figure 1A) and performed the enzymatic assays with Fra1. Data showed that Fra1 had little DGC activity (Figure 3), which indicates that the PAS domain of XvgA is a necessary element for the DGC activity. We also constructed XvgA E98G mutant with a site-directed mutagenesis. The kinetics of enzymatic reaction showed that XvgA E98G had little DGC activity (Figure 3). These results illuminate that Glutamic98 in XvgA is an active site to regulate the DGC activity. Consequently, the PAS domain is indispensable, and E98 is an active site for XvgA DGC activity.

Deletion of xvgA results in body defect and colony-growth delay of Xcc via interacting with light

Naturally, response to changes of environmental (intracellular or intercellular) condition requires a variety of receptors that can modulate gene expression and or enzyme activity, which might affect bacterial growth. Thus, we firstly constructed the in-frame deletion mutant of xvgA (DLTxvgA) and its complement strain (pLCxvgA). The growth of Xcc strains cultured in liquid MMXC medium was observed with Transmission Electron Microscope (TEM). As Figure 4 shows, the body diameter

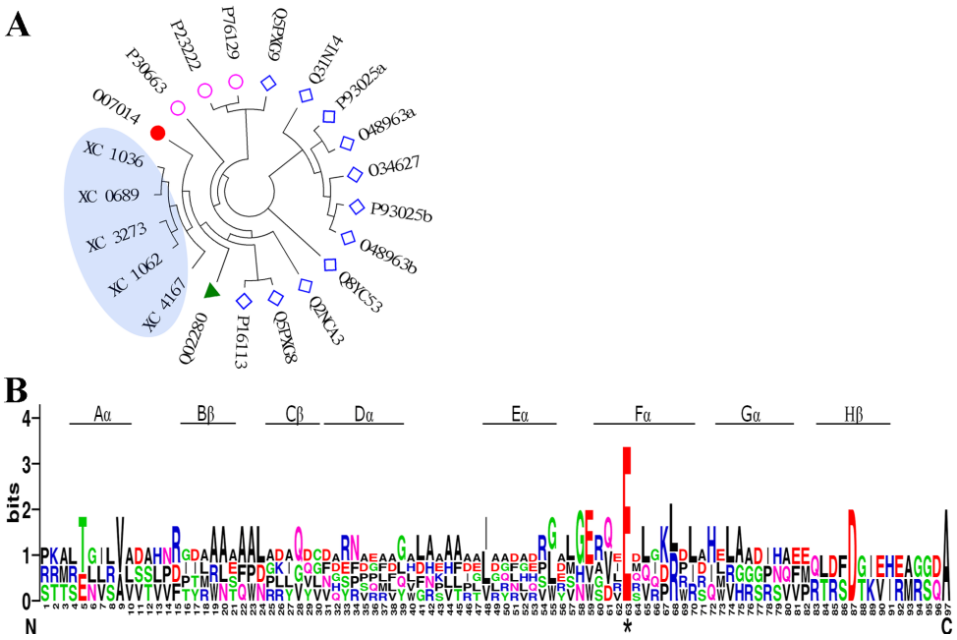

Figure 2. Bioinformatics analysis on PAS domain. A. The clustering tree of PAS domains on the basis of SST; B. Sequence features of the cluster of XvgA. In the clustering tree, the group of five PAS domains (circled with blue color) was closely linked to O07014, a putative red light sensor in *Bacillus subtilis* (Avila-Perez et al., 2010). XC_1036 indicates the PAS domain of XvgA. The sequence logo was created in WebLogo software, and the asterisk indicated the conservative site.

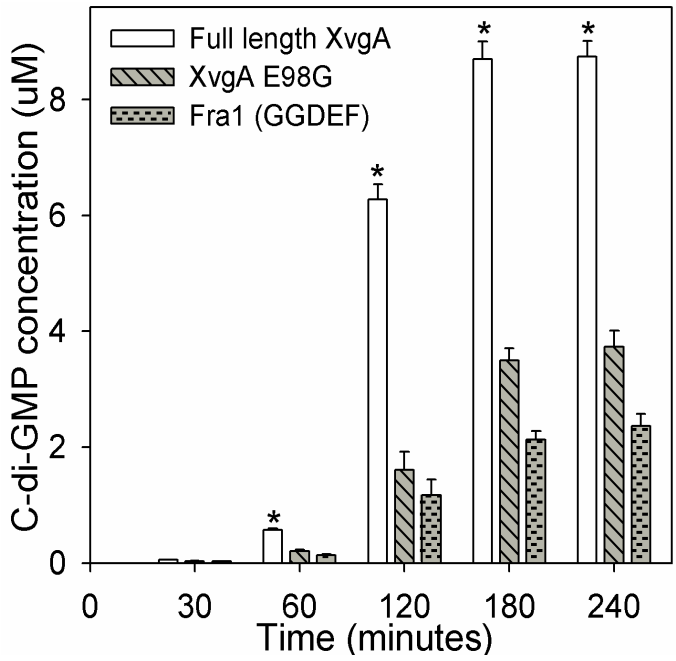

Figure 3. PAS domain is indispensible for DGC activity of XvgA. DGC activity of full length XvgA, XvgA E98G and Fra1 were measured. The DGC activity of full length XvgA was significantly higher than that of Fra1 and site-mutant protein of E98G, whose site is located on the PAS domain, therefore, DGC activity of XvgA is dependent on the PAS motif, and E98 is an active site for its function.

of wild type Xcc 8004 (Figure 4A) is significantly bigger than that of DLTxvgA (Figure 4B), the corresponding data was shown in Figure 4C. The Xcc strains were also cultured in liquid MMXC medium under red (4.30 mW/cm^2), far-red (3.36 mW/cm^2), white light (12000 lux) and dark conditions. In each condition, Xcc cell viability was estimated at the fourth and fifth day by plating on NYG agar plates and clony formation units (CFU) were numbered after two days growth at 28°C. As Figure 4D shows, bacterial population of wild type Xcc 8004 was significantly higher than that of DLTxvgA in dark condition, which indicates that deletion of xvgA results in Xcc growth defect. The mutation of xvgA reduced Xcc growth rate in the light conditions, therefore, we suggest that XvgA might regulate bacterial growth in a c-di-GMP dependent and light regulating fashions.

XvgA plays essential roles in Xcc virulence

The virulence assays were deployed on DLTxvgA and pLCxvgA, also an over expression complementary strain (pHMxvgA), which was constructed by a lac-promoter vector pHM1. The virulence of these strains was detected by measuring the lesion length after introducing bacteria into the vascular system of cabbage by leaf clipping. The mutant of xvgA gave a significant reduction in virulence (Figures 5A and B). The lesion length displayed an

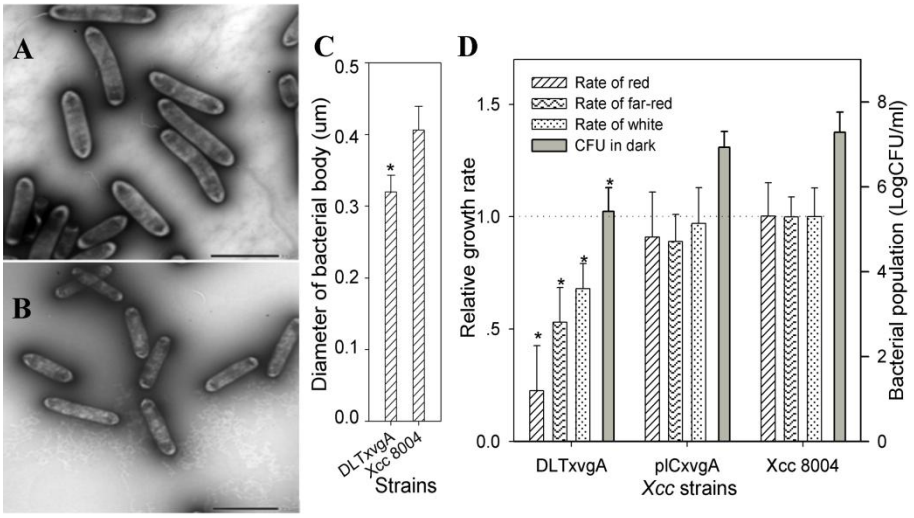

Figure 4. Roles of XvgA in the growth of *Xcc* strains. A. Wild type cultured on MMXC at 5th day was observed with TEM (scale bar: 2 µm). B. DLT*xvgA* cultured on MMXC at 5th day was observed with TEM (scale bar: 2 µm); C. Diameters of *Xcc* 8004 and DLT*xvgA*; D. Growth of bacterial population of *Xcc* strains. The bacterial population was an average value of strain cultrured at 4th and 5th days. Bacteria grown overnight in NYG medium were washed and re-suspended in 1 mM $MgCl_2$ to an OD of 600 nm of 0.1±0.01, and cultured in MMXC medium. CFU was numbered on NYG plates at the 4th and 5th days. Error bar indicates standard deviations (* P<0.05).

increasing trend with the increase of amount of XvgA protein (DLTxvgA < pLCxvgA < pHMxvgA) (Figure 5B), which demonstrates that XvgA is a positive regulator of Xcc virulence. Moreover, the bacterial population of DLTxvgA was significantly lower than that of Xcc 8004 in hosts (Figure 5C), which indicated that XvgA, a one component system in c-di-GMP signaling can promote Xcc growth in host leaves.

DISCUSSION

Bacteria adapt their physiologies in response to environmental stimuli through TCS, OCS and other signal transduction systems. Although extracellular signals are transmitted into the cell predominantly by TCS (Ulrich et al., 2005), OCS proteins, which are composed of an input domain directly fused to an output domain, provide a much simpler signal transduction design. In addition, OCS are evolutionarily older, display a great diversity of domains such as the bacterial PAS-HTH_8 and Cache-GGDEF domains (Ulrich et al., 2005), and appear to provide support for the notion that simpler systems are often more effective. In this study, we have identified an OCS (XvgA) involved in light (red or far-red) signaling (Figure 5), but no direct evidence was obtained in photochemistry. Therefore, XvgA was possibly involved in light response as a downstream component in the light signaling. In addition, we found that XvgA regulated Xcc motility via interacting oxygen signaling response. In the tests, motilities of Xcc strains were measured in both oxygen concentrations (1 vs. 20%); the details of results were shown in Figure 6. DLTxvgA was significantly decreased in motility compared to the complementary (pLCxvgA) and wild type strains in both oxygen concentrations, the corresponding diameters were shown in Figure 6C with slash and grey bars. In addition, the relative motility rate of the strains (Figure 6D) indicated that the motility of DLTxvgA was significantly increased, and with no significant difference between complementary and wild type strains. Hence, XvgA plays an important role in motility and regulates Xcc adaptation to oxygen stimulus. These mean that the PAS domain of XvgA is possibly involved in light and oxygen signaling simultaneously; nevertheless, these still need to be validated.

The PAS domain of XvgA is a signaling module and regulates the function of the output domain, GGDEF. When PAS domain is arose by signals, it can quickly convert them to a c-di-GMP signal that influences many bacterial behaviors including growth, motility and virulence (Figures 4, 5 and 6). The impaired growth and motility of xvgA mutants may be the cause of this strain's attenuated virulence because these defects will reduce Xcc fitness in plant hosts.

RpfG, a known c-di-GMP hydrolyse (PdeA), was previously found to positively regulate xvgA expression (Ryan et al., 2007), and Like xvgA, the rpfG mutant gave a significant reduction in Xcc virulence (Andrade et al., 2006). These findings indicate that RpfG might regulate

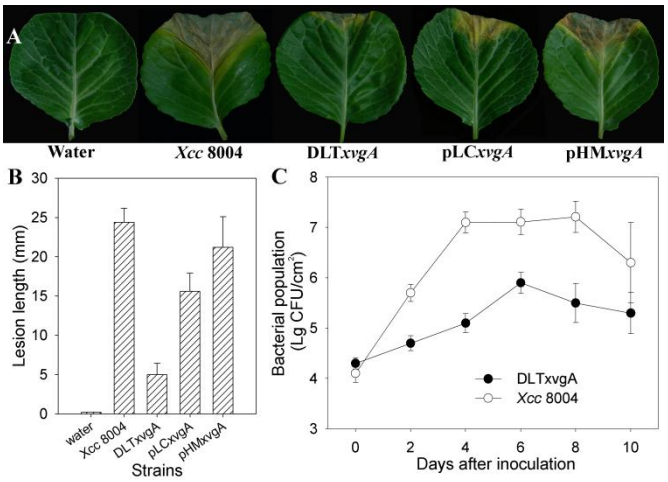

Figure 5. The effects of XvgA on *Xcc* virulence. A. The photos of lesion production; B. Lesion length in 15 leaf-lesion repeats; C. Bacterial population of DLT*xvgA* and *Xcc* 8004 in host leaves.

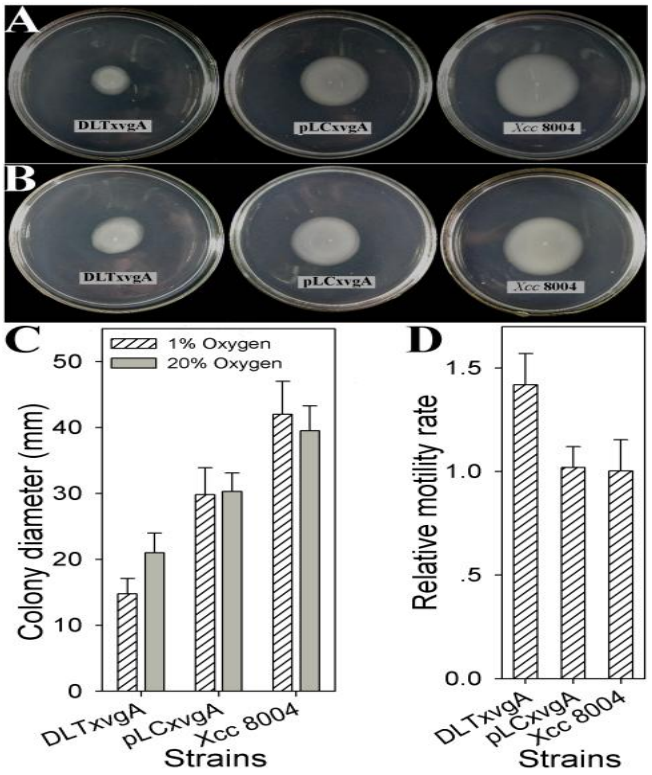

Figure 6. XvgA affects *Xcc* motility by sensing oxygen. A. Photos of the motility ofDLT*xvgA*, plC*xvgA* and wild type *Xcc* 8004 in 1% oxygen; B. Photos of the motility of DLT*xvgA*, plC*xvgA* and wild type *Xcc* 8004 in 20% oxygen; C. The corresponding diameters of the colony to the photos of the three strains; D. The relative motility rate of stains. The motility tests were conducted in 1% and 20% oxygen. The colony size in 1% (T0) versus 20% (T1) oxygen were measured and used in motility analysis. The motility rate was calculated as the ratio of T1 to T0. The relative motility rate was the ratio of the motility rate of mutant to that of the wild type. Error bar indicates standard deviations (*P<0.05).

virulence through XvgA. However, XvgA is a DGC (Figure 1) whose activity is opposite to that of RpfG. When rpfG was mutated, cellular c-di-GMP levels might increase, which would repress xvgA transcription. Conversely, if XvgA activity is reduced, cellular c-di-GMP will possibly decrease, which might in turn activate xvgA expression. These data demonstrate that XvgA might be involved in c-di-GMP homeostasis in the cell, where imbalances of c-di-GMP will result in impaired motility, growth, virulence, etc. Another interpretation of these observations is that c-di-GMP signaling pathways are temporally and or spatially separated (Kolter and Greenberg, 2006). If so, the cellular locations of c-di-GMP regulated by RpfG and XvgA may be different, and thus, differentially regulate gene expression or protein function.

In addition to signaling regulation, XvgA was involved in signal responses. When bacteria are grown in light or oxygen conditions, which involves in c-di-GMP signaling through the PAS domain of XvgA, in this way, c-di-GMP functions as a second messenger regulating the synthesis or activities of many growth and virulence related factors (Chang et al., 2001; Ryan et al., 2007; Hengge, 2009). Xcc motility was significantly inhibited by deletion of xvgA, and higher oxygen concentration also inhibited Xcc motility (compared to xvgA mutant), therefore, we deduced that higher oxygen concentration might inhibit XvgA activity. Two modes of regulation (light and oxygen) on the one component system, allow XvgA to quickly and effectively convert signals to c-di-GMP signals, and cause high adaptation of bacterial cells to environmental changes.

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