Xanthomonas campestris Promotes Diffusible Signal Factor Biosynthesis and Pathogenicity by Utilizing Glucose and Sucrose from Host Plants

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The plant pathogen *Xanthomonas campestris* pv. *campestris* produces diffusible signal factor (DSF) quorum sensing (QS) signals to regulate its biological functions and virulence. Our previous study showed that *X. campestris* pv. *campestris* utilizes host plant metabolites to enhance the biosynthesis of DSF family signals. However, it is unclear how *X. campestris* pv. *campestris* benefits from the metabolic products of the host plant. In this study, we observed that the host plant metabolites not only boosted the production of the DSF family signals but also modulated the expression levels of DSF-regulated genes in *X. campestris* pv. *campestris*. Infection with *X. campestris* pv. *campestris* induced changes in the expression of many sugar transporter genes in *Arabidopsis thaliana*. Exogenous addition of sucrose or glucose, which are the major products of photosynthesis in plants, enhanced DSF signal production and *X. campestris* pv. *campestris* pathogenicity in the *Arabidopsis* model. In addition, several sucrose hydrolase-encoding genes in *X. campestris* pv. *campestris* and sucrose invertase-encoding genes in the host plant were notably upregulated during the infection process. These enzymes hydrolyzed sucrose to glucose and fructose, and *in trans* expression of one of these enzymes, CINV1 of *A. thaliana* or XC_0805 of *X. campestris* pv. *campestris*, enhanced DSF signal biosynthesis in *X. campestris* pv. *campestris* in the presence of sucrose. Taken together, our findings demonstrate that *X. campestris* pv. *campestris* applies multiple strategies to utilize host plant sugars to enhance QS and pathogenicity.

Quorum sensing (QS) is widely employed by microorganisms to coordinate group behavior in a cell density-dependent

manner (Deng et al. 2011; He and Zhang 2008; Wang et al. 2004). QS is a cell-to-cell communication process consisting of the production, release, and perception of small diffusible signal molecules. A range of QS signals have been identified in various bacterial species. Among these signals, the diffusible signal factor (DSF) family of signaling molecules represents an important type of QS signal that controls diverse biological functions, such as biofilm formation, motility, virulence, and antibiotic resistance in gram-negative bacterial pathogens (Deng et al. 2011; He and Zhang 2008; He et al. 2009; Ryan and Dow 2011). DSF was originally identified in *Xanthomonas campestris* pv. *campestris* (Barber et al. 1997), which is the causal agent of black rot of crucifers (Vicente and Holub 2013; Williams 1980). *X. campestris* pv. *campestris* is a vascular pathogen that typically enters the xylem of plants through structures at the leaf margin called hydathodes (Vicente and Holub 2013; Williams 1980). Genetic and biochemical analyses showed that the biosynthesis of DSF signals from carbohydrates is dependent on the DSF synthase RpfF and the membrane-associated DSF sensor RpfC in *X. campestris* pv. *campestris* (He et al. 2006a; Zhou et al. 2015a). Mutation of RpfF abolishes DSF family signal production and results in reduced virulence factor production (Barber et al. 1997; Deng et al. 2015, 2016; He et al. 2010).

Photosynthesis is a process that occurs in the mesophyll cells of green plant leaves, in which carbon dioxide is fixed to synthesize carbohydrates (Slewiniski 2011), which are then broken down into sugars and imported into the sieve element-companion cell (SE/CC) complex of the collection phloem for energy metabolism (Braun 2012). The SWEET (sugars will eventually be exported transporters) proteins are a recently identified class of sugar transporters that function as uniporters to facilitate the diffusion of sugars across cell membranes while also mediating sucrose efflux from phloem parenchyma into the phloem apoplasm (Chen 2014; Chen et al. 2010, 2012). SWEETs mediate the bidirectional, pH-independent transport of sugars across the plasma membrane or tonoplast and contribute to a wide range of physiological processes that involve sugar efflux (Chardon et al. 2013; Guo et al. 2014; Lin et al. 2014). As reported previously, SWEET-mediated sugar transport is not only essential for carbohydrate distribution but also required for pathogen resistance (Chen 2014; Chen et al. 2010, 2012, 2015). The overexpression of *AtSWEET4* in *Arabidopsis thaliana* increases plant size and causes plants to accumulate glucose and fructose, which

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enhances the growth of *Pseudomonas syringae* pv. *phaseolicola* NPS3121 (Liu et al. 2016). In contrast, the mutation of *AtSWEET4* in *A. thaliana* leads to plants that, compared with wild-type plants, are smaller in size, have reduced glucose and fructose content, and have enhanced resistance to *Botrytis cinerea* infection (Chong et al. 2014; Liu et al. 2016).

The primary goal of pathogens is to access nutrients from their hosts to promote their reproduction and virulence. *Xanthomonas* spp. can live in the intercellular space (apoplast) of plants, where it acquires carbohydrates for energy and carbon (Tang et al. 2005). Our previous study showed that glucose

from the host plant is an active component that specifically stimulates DSF family signal production in *X. campestris* pv. *campestris* (Deng et al. 2015). In this study, we showed that *X. campestris* pv. *campestris* targets not only the SWEET proteins, which facilitate the diffusion of sugars across the cell membranes and mediate sucrose efflux from phloem parenchyma into the phloem apoplast, but, also, sucrose hydrolases to hydrolyze sucrose to glucose for the DSF family signal synthesis. This type of host-pathogen interaction would almost certainly increase the survival and pathogenicity of pathogens. Our findings provide new insight into the crosstalk between

Fig. 1. Differential gene expression profiles between the *Xanthomonas campestris* pv. *campestris* wild-type strain Xc1 in the absence and presence of cabbage juice and Xc1 and the *rpfF* mutant in the presence of cabbage juice, as measured by RNA-Seq ($\log_2$ fold change ≥ 1). **A**, Gene ontology (GO) term enrichment analysis of differentially expressed genes between Xc1_C and Xc1. **B**, Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of the selected genes confirmed the RNA-Seq results for Xc1_C versus Xc1. **C**, GO term enrichment analysis of differentially expressed genes between Xc1_C and *rpfF*_C. **D**, qRT-PCR analysis of the selected genes confirmed the RNA-Seq results of Xc1_C versus *rpfF*_C. **E**, Venn diagrams showing the overlap of genes from different backgrounds. Divergently regulated genes are not depicted in these Venn diagrams. Abbreviations of Xc1_C and *rpfF*_C indicate that the Xc1 and *rpfF* mutant were cultured in nutrient yeast glycerol medium supplemented with cabbage juice.

host plants and pathogens that may be used for the development of new methods to control disease.

RESULTS

The exogenous addition of cabbage juice affects the expression levels of a wide range of genes in *X. campestris* pv. *campestris*.

To study whether the metabolic products of the host plant influence the gene expression profile of pathogens, we analyzed and compared the transcriptome profiles of the *X. campestris* pv. *campestris* wild-type Xc1 strain cultured with or without Chinese cabbage juice, using RNA-Seq. Differential gene expression analysis showed that 550 genes exhibited altered expression (with a $\log_2$ -fold change ≥ 1.0) in the wild-type strain Xc1 with the addition of Chinese cabbage juice (Fig. 1A; Supplementary Table S1). These differentially expressed genes are associated with a range of biological functions, including motility and cell attachment, stress tolerance, virulence regulation, transcriptional regulation, membrane components, transport, multidrug resistance, metabolism, detoxification, and signal transduction (Fig. 1A). According to the previous study, some of these target genes are modulated by the DSF QS system in *X. campestris* pv. *campestris* (He et al. 2006b). Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of the selected genes confirmed the RNA-Seq results (Fig. 1B).

Cabbage juice affects the expression levels of many DSF-regulated genes.

To comprehensively assess the influence of the metabolic products of the host plant on the DSF QS system in *X. campestris* pv. *campestris*, we analyzed the transcriptomes of the wild-type strain Xc1 and the $\Delta rpfF$ deletion mutant cultured in the presence of cabbage juice, using RNA-Seq. Differential gene expression analysis showed that 339 genes exhibited altered expression (with a $\log_2$ fold change ≥ 1.0) in the $\Delta rpfF$ mutant compared with their expression in the wild-type Xc1 strain, which we confirmed by qRT-PCR analysis (Fig. 1C and D). These differentially expressed genes are also associated with a range of biological functions (Fig. 1C). We compared the profiles of the differentially expressed genes in the DSF-deficient mutant and the wild-type Xc1 strain after the addition of Chinese cabbage juice and found substantial overlap in these genes between the strains (Fig. 1E). From these observations, we concluded that Chinese cabbage juice may have an important effect on the DSF QS system in *X. campestris* pv. *campestris*.

Cabbage juice significantly increases the production of DSF family signals.

Our previous study showed that the addition of Chinese cabbage ethanol extract enhances DSF family signal biosynthesis in *X. campestris* pv. *campestris* (Deng et al. 2015). Based on our finding that Chinese cabbage juice can modulate DSF-regulated genes, we next investigated the effect of Chinese cabbage juice on the production profile of DSF family signals. Given that *X. campestris* pv. *campestris* produces at least five DSF family signals (Deng et al. 2015, 2016), we measured the production of the two major signals, DSF and BDSF (*cis*-2-dodecenoic acid), by *X. campestris* pv. *campestris* in the presence or absence of Chinese cabbage juice. The results showed that exogenous addition of the juice to the *rpfC* deletion mutant, which overproduces DSF family signals, slightly increased the bacterial growth rate (Fig. 2A) but notably increased the production of both DSF and BDSF by 260.97 and 1303.9%, as revealed by liquid chromatography-mass

spectrometry (LC-MASS) analysis at 12 h postinoculation, respectively (Fig. 2B and C; Supplementary Fig. S1).

Cabbage juice contains large amounts of sucrose and glucose that stimulate DSF family signal synthesis.

It was previously shown that carbohydrates, especially glucose, can be used by *X. campestris* pv. *campestris* to synthesize DSF family signals (Deng et al. 2015; Zhou et al. 2015b). Since the exogenous addition of Chinese cabbage juice significantly increased the production of BDSF and DSF by *X. campestris*

Fig. 2. Influence of the exogenous addition of Chinese cabbage juice on signal production of the diffusible signal factor (DSF) family in *Xanthomonas campestris* pv. *campestris*. **A**, The growth curve of the *rpfC* mutant in the absence (●) and presence (■) of cabbage juice. **B**, Time course analysis of the production of DSF and **C**, BDSF in the *rpfC* mutant in the absence (●) and presence (■) of cabbage juice. Data are the mean $\pm$ standard deviation of three independent experiments. One asterisk (*), $P < 0.1$; two (**), $P < 0.05$; three (***), $P < 0.01$; four (****), $P < 0.001$ (unpaired *t* test).

pv. *campestris* (Fig. 2), we conducted LC-MASS to analyze the carbohydrate components in the cabbage juice. The results showed that there are plenty of sucrose and glucose carbohydrates in the juice, the concentrations of which were 3.42 mM and 34.86 mM, respectively (Fig. 3A to D; Supplementary Fig. S2). Given that glucose can be easily used by *X. campestris* pv. *campestris* to synthesize the DSF family signals (Deng et al. 2015), we investigated the effect of the exogenous addition of sucrose and glucose on the DSF family signal synthesis on a large time scale (from 0 to 24 h). Consistent with the previous findings, we observed that the exogenous addition of 15 mM glucose slightly increased the growth rate of the $\Delta rpfC$ mutant (Fig. 3E) and significantly enhanced the production of DSF and BDSF, by 128.85 and 170.98%, at 4 h postinoculation, respectively (Fig. 3F and G; Supplementary Fig. S3). After culturing for 24 h, the addition of glucose increased the production of DSF and BDSF by 615.1 and 702.9%, respectively (Fig. 3F, 3G). Interestingly, in agreement with our previous findings, the exogenous addition of 15 mM sucrose showed no detectable effect on the DSF family signal synthesis in *X. campestris* pv. *campestris* at the early stage (Fig. 3F and G). However, at 24 h postinoculation, the addition of sucrose increased the production of DSF and BDSF by 167.51 and 181.52%, respectively

(Fig. 3F and G). Since glucose is first converted to acetyl-CoA, which is then used as the carbon source for the biosynthesis of DSF family signals, and sucrose would be first catalyzed to glucose and fructose, it is not hard to understand why the addition of glucose promotes DSF family signal production at an earlier stage than sucrose.

X. campestris pv. *campestris* induces *AtSWEET* expression in *A. thaliana*.

The sugar efflux systems of host plants have been reported to be hijacked by pathogens to access nutrients (Chen 2014). We tested whether the 17 *Arabidopsis* SWEET family members are the targets of *X. campestris* pv. *campestris* during the infection process. qRT-PCR analysis showed that Xc1 infection caused an increase in the expression levels of several transporter genes in *A. thaliana* (Supplementary Fig. S4), which could potentially alter the efflux of sugars at the site of infection to enhance pathogenicity.

The exogenous addition of sugars enhances

X. campestris pv. *campestris* virulence toward *A. thaliana*.

As an effective plant model, *A. thaliana* has been widely used to understand the molecular mechanisms of host-plant

Fig. 3. Influence of the exogenous addition of sugars on signal production by the diffusible signal factor (DSF) family in *Xanthomonas campestris* pv. *campestris*. **A to D**, Ultra-performance liquid chromatography-mass spectrometry analysis of the standards glucose (A) and sucrose (B) and the glucose (C) and sucrose (D) concentrations in *Arabidopsis thaliana*. **E**, Growth curve of the $rpfC$ mutant in the absence (●) and presence of sucrose (▲) and glucose (■). **F**, Time course analyses of the production of DSF and **G**, BDSF in the $rpfC$ mutant in the absence (●) and presence of sucrose (▲) and glucose (■). Data are the mean $\pm$ standard deviation of three independent experiments. One asterisk (*), $P < 0.1$; two (**), $P < 0.05$; three (***), $P < 0.01$; four (****), $P < 0.001$ (unpaired t test).

interactions. Our results showed that *A. thaliana* juice contains substantial amounts of glucose and sucrose (Supplementary Fig. S5). Because the addition of sugars to *X. campestris* pv. *campestris* significantly increased the production of DSF family signals, which play an important role in regulation of virulence, we next tested the effect of the addition of glucose and sucrose on the ability of the *X. campestris* pv. *campestris* wild-type strain Xc1 to infect *A. thaliana*. Xc1 was inoculated into *A. thaliana* Col-0 leaves, using syringe infiltration to manually deliver the pathogen into the apoplasts of leaves through the stomata, which are natural leaf openings (Katagiri et al. 2002). The addition of sugars promoted the virulence of the bacterial pathogen (Fig. 4), as the inoculated leaves formed yellow spots, which were enlarged in the presence of 10% added sugars (Fig. 4C to E). Measurements of the *X. campestris* pv. *campestris* colony-forming units (CFUs) in the inoculated leaves provided additional support for these results. The exogenous addition of 10% glucose or sucrose increased the number of Xc1 CFUs from $2.88 \times 10^6 \text{ mL}^{-1}$ to $5.91 \times 10^6 \text{ mL}^{-1}$ and $7.26 \times 10^6 \text{ mL}^{-1}$ at 3 days postinoculation, respectively (Fig. 4F).

The expression of some sucrose hydrolase-encoding genes is up-regulated in both the plant and pathogen during the infection process.

Glucose is the carbon source that is directly used for the biosynthesis of DSF family signals. Sucrose is the predominant

carbohydrate present in the intercellular spaces of photosynthetically active tissues (Chen et al. 2010, 2012). Since there are several potential sucrose hydrolases in both *A. thaliana* and *X. campestris* pv. *campestris*, we next investigated whether Xc1 infection affects the expression levels of the genes encoding these enzymes. qRT-PCR analysis revealed that infection by Xc1 induced the expression of several sucrose hydrolase genes in *A. thaliana* (Fig. 5A). In addition, three sucrose hydrolase genes in Xc1 exhibited induced expression after the exogenous addition of cabbage juice (Fig. 5B).

To further confirm their enzymatic activity, the candidate plant and *X. campestris* pv. *campestris* enzymes CINV1 and XC_0805, which have Glyco_hydro_100 and Alpha-amylase domains, respectively (Fig. 5C), were purified, using affinity chromatography (Fig. 5D). The GST-XC_0805 and GST-CINV1 fusion proteins contain 879 and 793 amino acids and have calculated molecular weights of 97.44 and 90.98 kDa, respectively (Fig. 5D). In vitro enzyme activity analysis showed that CINV1 and XC_0805 catalyzed the formation of glucose and fructose after the addition of sucrose (Fig. 5E and F; Supplementary Fig. S6).

In trans expression of CINV1 and XC_0805 in the *rpfC* mutant promotes DSF production in the presence of sucrose.

To further explore the effect of the sucrose hydrolases on DSF synthesis in *X. campestris* pv. *campestris*, CINV1 and

Fig. 4. Influence of the exogenous addition of sugars on the virulence of *Xanthomonas campestris* pv. *campestris* in *Arabidopsis thaliana*. **A**, *A. thaliana* without *X. campestris* pv. *campestris* inoculation. **B to E**, *A. thaliana* inoculated with medium (B) Xc1 (C) Xc1 supplemented with 10% glucose (D), and Xc1 supplemented with 10% sucrose (E). **F**, The number of Xc1 cells in each *A. thaliana* leaf at 3 days postinoculation. Data are the mean $\pm$ standard deviation of three independent experiments. One asterisk (*), $P < 0.1$; two (**), $P < 0.05$; three (***), $P < 0.01$; four (****), $P < 0.001$ (unpaired *t* test).

XC_0805 were expressed in trans in the Xc1 $\Delta rpfC$ mutant. The transconjugants overexpressing CINV1 or XC_0805 were cultured in nutrient yeast glycerol (NYG) medium in the absence and presence of sucrose. In the absence of sucrose, no obvious difference in DSF production was observed among the

$\Delta rpfC$, $\Delta rpfC$ (CINV1), and $\Delta rpfC$ (XC_0805) strains. In contrast, the overexpression of CINV1 and XC_0805 in the $\Delta rpfC$ mutant in the presence of sucrose increased DSF production by 30.95 and 34.27% at 4 h postinoculation, respectively (Fig. 6). These results suggest that the overexpression of sucrose

Fig. 5. Analysis of the enzyme activity of sucrose hydrolases and sucrose invertases. **A**, Comparison of the relative fold changes of sucrose invertase-encoding genes from *Arabidopsis thaliana* with or without the inoculation of Xc1. **B**, Comparison of relative fold changes of sucrose hydrolase-encoding genes from *Xanthomonas campestris* pv. *campestris* in the absence and presence of cabbage juice. **C**, Domain structure analysis of XC_0805 and CINV1. **D**, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of XC_0805 (lanes a and b) and CINV1 (lanes c and d) proteins. **E**, Analysis of the products of XC_0805 and **F**, CINV1 to hydrolyze sucrose. Data are the mean $\pm$ standard deviation of three independent experiments. One asterisk (*), $P < 0.1$; two (**), $P < 0.05$; three (***), $P < 0.01$; four (****), $P < 0.001$ (unpaired t test).

hydrolases significantly increased the production of glucose for DSF biosynthesis in the presence of sucrose.

DISCUSSION

Our previous study revealed that *X. campestris* pv. *campestris* utilizes glucose from a Chinese cabbage ethanol extract as the carbon source for DSF signal biosynthesis (Deng et al. 2015). The results of our current study further indicated the complex interaction between *X. campestris* pv. *campestris* and the host plant. The crosstalk between the host and *X. campestris* pv. *campestris* is a two-way process. An analysis of transcriptome profiles suggested that the host plant juice affected the expression of many genes in Xc1 (Fig. 1A). On the other hand, Xc1 targeted the SWEET systems in *A. thaliana* to gain access to nutrients and carbon sources to enhance DSF biosynthesis and pathogenicity (Figs. 2 and 4). Since some pathogens are known to hijack the host sugar efflux systems to access nutrients (Chen 2014, 2010), our results provide additional evidence in support of this type of interaction. However, the greater significance of this study is that we describe the first example of the possible interaction between the DSF QS system in a pathogen and the SWEET sugar transporter system in a host plant. Since both the DSF and SWEET systems are widely distributed (Chen 2014; Chen et al. 2010; Deng et al. 2011), our findings offer the first insight into this interesting crosstalk. In addition, whether there are some potential interactions between the SWEET systems and other QS systems, such as the AHL and AI-2 family signals, merits further investigation.

A total of 17 SWEET family members have been identified in *A. thaliana* (Chen et al. 2010, 2012). Previous studies suggested that the expression of *AtSWEET4* and *VvSWEET4* was induced in response to *Botrytis cinerea* infection (Chen 2014; Chen et al. 2010, 2012). Host sugar efflux systems are typically targeted by pathogens, and the modulation of SWEET mRNA levels by pathogens probably alters sugar efflux at the site of infection and has an impact on pathogen pathogenicity and plant immunity (Chen 2014; Chen et al. 2010, 2012). Our results consistently confirmed that Xc1 infection significantly induced *AtSWEET2*, *AtSWEET4*, *AtSWEET8*, *AtSWEET10*, and *AtSWEET15* mRNA levels in *A. thaliana* leaves. While exogenous addition of glucose or sucrose enhances *X. campestris* pv. *campestris* virulence toward *A. thaliana* (Fig. 4). It was recently reported that *X. oryzae* pv. *oryzae* utilizes a type III secretion system to secrete transcription activator-like (TAL) effectors to directly target the expression of specific *SWEET* genes in rice (Bogdanove 2014). However, amino acid alignment analysis indicated that there are no orthologs of these specific TAL effectors in *X. campestris* pv. *campestris*, suggesting that *X. campestris* pv. *campestris* may use different effectors or have a different mechanism to induce *SWEET* gene expression in *A. thaliana* (Denancé et al. 2018).

Our results indicated that both glucose and sucrose could be efficiently utilized by *X. campestris* pv. *campestris* to synthesize DSF family signals (Fig. 3). However, a notable delay in DSF production was observed when *X. campestris* pv. *campestris* cells were supplemented with sucrose compared with glucose. The primary reason for this result could be that, while glucose is first converted to acetyl-CoA for DSF family signal biosynthesis, sucrose needs to be catalyzed to glucose and fructose. This result raises the question of what the roles are for sucrose hydrolases in DSF family signal synthesis. Our results support an important role of both plant and pathogen sucrose hydrolases in the infection process. These enzymes efficiently hydrolyzed sucrose to glucose and fructose (Fig. 5E and F), and intriguingly, the expression of some plant and pathogen sucrose hydrolases were induced during the infection process. *In trans*

expression of sucrose hydrolases in Xc1 significantly increased the production of the DSF signal (Fig. 6). In general, these results suggest a complex mechanism by which *X. campestris* pv. *campestris* targets and utilizes sucrose hydrolases to provide adequate glucose for energy metabolism and DSF biosynthesis during the infection process (Fig. 7).

In conclusion, in this study we determined, for the first time, that *X. campestris* pv. *campestris* targets sugar transporters and sucrose hydrolases to utilize sugars of the host plant as nutrients and substrates for DSF signal biosynthesis to promote its pathogenicity (Fig. 7).

MATERIALS AND METHODS

Biological materials and growth conditions.

The strains and plants used in this work are listed in Supplementary Table S3, and the *X. campestris* pv. *campestris* strains used were described previously (He et al. 2006a and b). The bacteria were maintained at 30°C in Luria Bertani (LB) or NYG medium supplemented with the following antibiotics,

Fig. 6. Influence of *in trans* expression of *CINVI* and *XC_0805* on diffusible signal factor (DSF) production in the *rpfC* mutant. **A**, The cell densities and **B**, DSF signal production of the Xc1 strain or the Xc1 overexpressing *XC_0805* or *CINVI* that were cultured for 4 h in the absence and presence of sucrose. Data are the mean ± standard deviation of three independent experiments.

when necessary: rifampicin, 25 $\mu\text{g ml}^{-1}$; kanamycin, 50 $\mu\text{g ml}^{-1}$; and gentamicin, 50 $\mu\text{g ml}^{-1}$. Sugars were added to the media at the indicated final concentrations. All *A. thaliana* plants used in this study were in the Columbia-0 ecotype background. *A. thaliana* seeds were soaked in sterile water in the dark at 4°C for 3 days, after which they were washed with 70% ethanol for 1 min, were sterilized in 5% sodium hypochlorite for 5 min, and finally, were washed with sterilized water four or five times. The seeds were sown on half-strength Murashige and Skoog solid agar medium (half-strength Murashige and Skoog basal salt mixture, 0.8% agar for solid media, pH = 5.8 to 5.9) containing 3% sucrose. After germination, the plants were grown in small pots filled with a mixture of Professional Growing Mix soil and vermiculite (3:1 ratio) or solid agar media in a climate-controlled room at 23°C (day) and 21°C (night). Light was set at 30,000 Lux illumination under a 10-h-day and 14-h-night regime. Chinese cabbage was minced using an electric juicer, and supernatants were collected, using sterile 0.45- μm Acrodisc syringe filters, and were stored at -20°C.

RNA-Seq analysis.

RNA was isolated from the *X. campestris* pv. *campestris* strains using an RNeasy protect bacteria mini kit (Qiagen). RNA purity was assessed using a NanoPhotometer spectrophotometer (Implen), and the concentration of RNA was measured using a Qubit RNA assay kit with a Qubit 2.0 Fluorometer (Life Technologies). RNA integrity was assessed using an RNA Nano 6000 assay kit for the Bioanalyzer 2100

system (Agilent Technologies). Sequencing libraries were generated using a NEBNext Ultra Directional RNA library prep kit for Illumina (NEB). After the products were purified using an AMPure XP system, the library quality was assessed using an Agilent Bioanalyzer 2100 system. The clustering of the index-coded samples was performed on a cBot cluster generation system using TruSeq PE cluster kit v3-cBot-HS (Illumina). After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform to generate paired-end reads. Reference genome and gene model annotation files were downloaded directly from the genome website. Building of the reference genome index and alignment of the clean reads to the reference genome were both performed using Bowtie2-2.2.3. HTSeq v0.6.1 was used to count the number of reads that mapped to each gene, and the reads per kilobase million of each gene was calculated based on the length of a given gene and the number of reads that mapped to it (Mortazavi et al. 2008).

Differential expression analysis.

Differential expression analysis was performed using the DESeq R package (1.18.0). DESeq provides statistical routines to determine differential gene expression using a model based on a negative binomial distribution. The differentially expressed genes were identified as described by Audic and Claverie (1997). The original *P* values were corrected for multiple testing by following the false discovery rate procedure, and genes with adjusted *P* values below 0.05 were considered significant.

Fig. 7. Schematic representation of the cross-talk between *Arabidopsis thaliana* and *Xanthomonas campestris* pv. *campestris*. Infection with *X. campestris* pv. *campestris* causes the activation of sugar transporter and sucrose invertase gene expression in *A. thaliana*, which boosts the hydrolysis of sucrose to glucose and the transport of sugars across the cell membrane. *X. campestris* pv. *campestris* utilizes sugars, especially glucose, as the substrates for diffusible signal factor (DSF) family signal biosynthesis, which promotes pathogenicity.

Real-time qPCR.

RT-PCR was performed using a cDNA synthesis kit (Tiangen), according to the manufacturer's instructions. Specific RT-PCR primers were used to amplify 100- to 300-bp internal fragments of different genes. Real-time qPCR was performed using PowerUp SYBR green master mix (Invitrogen), according to the manufacturer's instructions on a 7300plus PCR system (Thermo Scientific). The 16S ribosomal RNA house-keeping gene was used to normalize prokaryotic gene expression values, and actin (*ACT8*) was used to normalize eukaryotic gene expression values. The relative expression levels of the target genes were calculated using the quantitation-comparative cycle threshold ($\Delta\Delta CT$) method.

Analysis of DSF family signals.

The DSF-overproducing *rpfC* strain, an *rpfC* deletion mutant derived from the wild-type strain Xc1, was grown in NYG medium overnight. The cultures were centrifuged, and the bacterial cells were resuspended in fresh NYG medium to a high initial cell density (optical density at 600 nm [OD_{600}] = of 2.0). Next, the bacterial cells were grown at 30°C, with slow shaking at 150 rpm, in the absence or presence of cabbage juice (1:1, vol/vol) or sugars at different concentrations as indicated. The cultures were centrifuged, and the supernatants were extracted twice with ethyl acetate (1:1, vol/vol). After the ethyl acetate was evaporated, the residues were dissolved in 0.5 ml of methanol and were subjected to ultraperformance liquid chromatography in tandem with mass spectrometry (UPLC/MS) profiling. An Acquity UPLC BEH C18 column (2.1 × 50 mm) (Waters) was employed for chromatography. Solution A was composed of 0.01% formic acid in water, and solution B was composed of 0.01% formic acid in MeOH. A linear gradient elution of solution B from 10 to 100% was applied for 10 min, and the column was re-equilibrated with 10% B for an additional 3 min. The entire eluate from the column was introduced into the quadrupole time-of-flight mass (Q-TOF) spectrometer. Ion detection was conducted in electrospray ionization mode, using a source capillary voltage of 2.0 kV, a source temperature of 120°C, a desolvation temperature of 350°C, a cone gas flow of 50 liters per hour (N₂), and a desolvation gas flow of 600 liters per hour (N₂).

Analysis of sugars.

To analyze the sugar components in plants, the plant juice was filtered, was mixed with methanol (1:1, vol/vol), and was loaded onto a Waters UPLC-MS system (Waters).

An Acquity UPLC Amide column (2.1 × 100 mm, Waters) was employed for chromatography. Solution A was composed of 0.1% NH₄OH in water, and solution B was composed of 0.01% formic acid in acetonitrile. An isocratic elution of 75% B was performed for 10 min. The entire column eluate was introduced into a Q-TOF mass spectrometer. Ion detection was conducted under the same conditions as described above.

Infection analysis.

Overnight cultures of *X. campestris* pv. *campestris* were diluted in sterile water to an OD_{600} of 0.1, in the absence or presence of different concentrations of sugars, and were injected into the leaves of adult soil-grown *A. thaliana*, using a syringe. Leaves were collected, milled, and diluted at 3 days postinoculation and were then plated on LB plates supplemented with rifampicin to determine the bacterial CFUs for each leaf. The experiment was repeated three times with four replicates for each sample.

Protein expression and purification.

The coding regions of the *XC_0805* gene (GenBank accession number NC_007086.1) from *X. campestris* pv. *campestris*

and the *CINV1* gene (GenBank accession number AT1G35580) from *A. thaliana* cDNA were amplified, with the primers listed in Supplementary Table S4, and were fused to the expression vector pGEX-6P-1. The fusion gene constructs were transformed into *Escherichia coli* BL21 (DE3). The transformed cells were grown in LB medium at 37°C to an OD_{600} of 0.6. One millimolar IPTG (isopropyl β -D-thiogalactoside) was added to the cell culture, and the growth temperature was adjusted to 18°C for 12 h. The affinity purification of GST-XC_0805 and GST-CINV1 fusion proteins were performed following the method described previously (Tao et al. 2010). The fusion protein was eluted and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Enzyme activity analysis.

Sucrose hydrolysis reactions were performed in a mixture containing phosphate buffered saline (pH 7.5) and TME buffer (60 mM Tris-HCl, 10 mM MgCl₂, and 1 mM EDTA, pH 7.5), as previously described (Tao et al. 2010). Sucrose was dissolved in water and was added to the mixture at a final concentration of 9 mM, while the final concentrations of XC_0805 and CINV1 were 5 μ M. The reaction mixture was incubated at 30°C and was stopped by placing sample tubes in boiling water for 5 min. The levels of sugars were measured via UPLC-MS spectrometry.

In trans expression of XC_0805 and CINV1 in the rpfC mutant.

The coding regions of *XC_0805* and *CINV1* were amplified by PCR and were cloned into the multiple cloning site of the expression plasmid pBBR1-MCS5. The resulting constructs were conjugated into the Xc1 *rpfC* deletion mutant using tri-parental mating, with pRK2013 as the mobilizing plasmid.

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