



# The Plant Defense Signal Salicylic Acid Activates the RpfB-Dependent Quorum Sensing Signal Turnover via Altering the Culture and Cytoplasmic pH in the Phytopathogen *Xanthomonas campestris*

Kai Song,<sup>a</sup> Bo Chen,<sup>a</sup> Ying Cui,<sup>a</sup> Lian Zhou,<sup>b</sup> Kok-Gan Chan,<sup>c,d</sup> Hong-Yan Zhang,<sup>e</sup> Ya-Wen He<sup>a</sup>

<sup>a</sup>State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic and Developmental Sciences, SJTU-NLBP Joint R&D Center on Biopesticides and Biofertilizers, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University (SJTU), Shanghai, China

<sup>b</sup>Zhiyuan Innovative Research Center, Shanghai Jiao Tong University, Shanghai, China

<sup>c</sup>Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

<sup>d</sup>Faculty of Applied Sciences, UCSI University, Kuala Lumpur, Malaysia

<sup>e</sup>Shanghai Nong Le Biological Products Company Limited (NLBP), Shanghai, China

## ABSTRACT

Salicylic acid (SA) is a plant defense signal that activates the RpfB-dependent quorum sensing (QS) signal turnover in the phytopathogen *Xanthomonas campestris*. In this study, we investigated the mechanism of SA-induced QS signal turnover in *X. campestris*. We found that SA treatment significantly reduced the production of the QS signal 3-oxo-C<sub>12</sub>-homoserine lactone (3-oxo-C<sub>12</sub>-HSL) in *X. campestris* cultures. This reduction was associated with a decrease in the expression of the *rpfB* gene, which encodes the RpfB protein responsible for the synthesis of 3-oxo-C<sub>12</sub>-HSL. The reduction in 3-oxo-C<sub>12</sub>-HSL production was not due to a change in the growth of *X. campestris* cultures, as indicated by the similar optical density (OD<sub>600</sub>) values. Instead, we observed a significant decrease in the cytoplasmic pH of *X. campestris* cultures treated with SA. This decrease in pH was associated with an increase in the production of the QS signal 3-oxo-C<sub>12</sub>-HSL. The increase in 3-oxo-C<sub>12</sub>-HSL production was not due to a change in the expression of the *rpfB* gene, as indicated by the similar *rpfB* mRNA levels. Instead, we observed a significant increase in the activity of the RpfB protein, which is responsible for the synthesis of 3-oxo-C<sub>12</sub>-HSL. The increase in RpfB activity was associated with a decrease in the cytoplasmic pH of *X. campestris* cultures. In vitro, we found that the activity of RpfB protein is significantly increased at a lower pH. These results suggest that SA-induced QS signal turnover in *X. campestris* is mediated by altering the culture and cytoplasmic pH, which in turn affects the activity of the RpfB protein.

## IMPORTANCE

Salicylic acid (SA) is a plant defense signal that activates the RpfB-dependent quorum sensing (QS) signal turnover in the phytopathogen *Xanthomonas campestris*. In this study, we investigated the mechanism of SA-induced QS signal turnover in *X. campestris*. We found that SA treatment significantly reduced the production of the QS signal 3-oxo-C<sub>12</sub>-homoserine lactone (3-oxo-C<sub>12</sub>-HSL) in *X. campestris* cultures. This reduction was associated with a decrease in the expression of the *rpfB* gene, which encodes the RpfB protein responsible for the synthesis of 3-oxo-C<sub>12</sub>-HSL. The reduction in 3-oxo-C<sub>12</sub>-HSL production was not due to a change in the growth of *X. campestris* cultures, as indicated by the similar optical density (OD<sub>600</sub>) values. Instead, we observed a significant decrease in the cytoplasmic pH of *X. campestris* cultures treated with SA. This decrease in pH was associated with an increase in the production of the QS signal 3-oxo-C<sub>12</sub>-HSL. The increase in 3-oxo-C<sub>12</sub>-HSL production was not due to a change in the expression of the *rpfB* gene, as indicated by the similar *rpfB* mRNA levels. Instead, we observed a significant increase in the activity of the RpfB protein, which is responsible for the synthesis of 3-oxo-C<sub>12</sub>-HSL. The increase in RpfB activity was associated with a decrease in the cytoplasmic pH of *X. campestris* cultures. In vitro, we found that the activity of RpfB protein is significantly increased at a lower pH. These results suggest that SA-induced QS signal turnover in *X. campestris* is mediated by altering the culture and cytoplasmic pH, which in turn affects the activity of the RpfB protein.

**Editor** Anne K. Vidaver, University of Nebraska-Lincoln

**Copyright** © 2022 Song et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Ya-Wen He, yawenhe@sjtu.edu.cn.

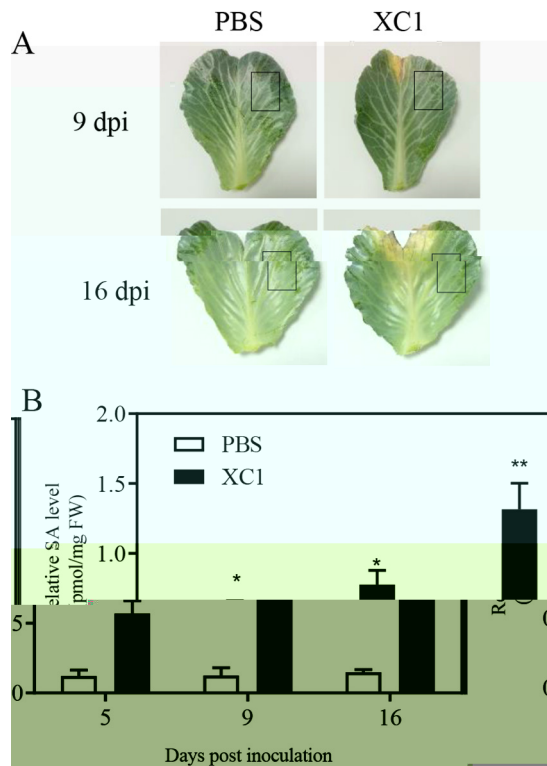
The authors declare no conflict of interest.

**Received** 8 December 2021

**Accepted** 15 February 2022

**Published** 7 March 2022





**FIG 1** *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage. Cabbage leaves were inoculated with *Xanthomonas campestris* pv. *campestris* (1) for 5, 9, and 16 days. The relative SA level in cabbage leaves was determined by HPLC-MS/MS. The relative SA level was normalized to the SA level in the control (PBS) at the same time point. The relative SA level was expressed as the ratio of the SA level in the infected leaves to the SA level in the control leaves. The relative SA level was significantly higher in the infected leaves than in the control leaves at 9 and 16 dpi ( $P < 0.05$  and  $P < 0.01$ , respectively). Error bars represent the standard deviation. The relative SA level was significantly higher in the infected leaves than in the control leaves at 9 and 16 dpi ( $P < 0.05$  and  $P < 0.01$ , respectively). Error bars represent the standard deviation. The relative SA level was significantly higher in the infected leaves than in the control leaves at 9 and 16 dpi ( $P < 0.05$  and  $P < 0.01$ , respectively). Error bars represent the standard deviation.

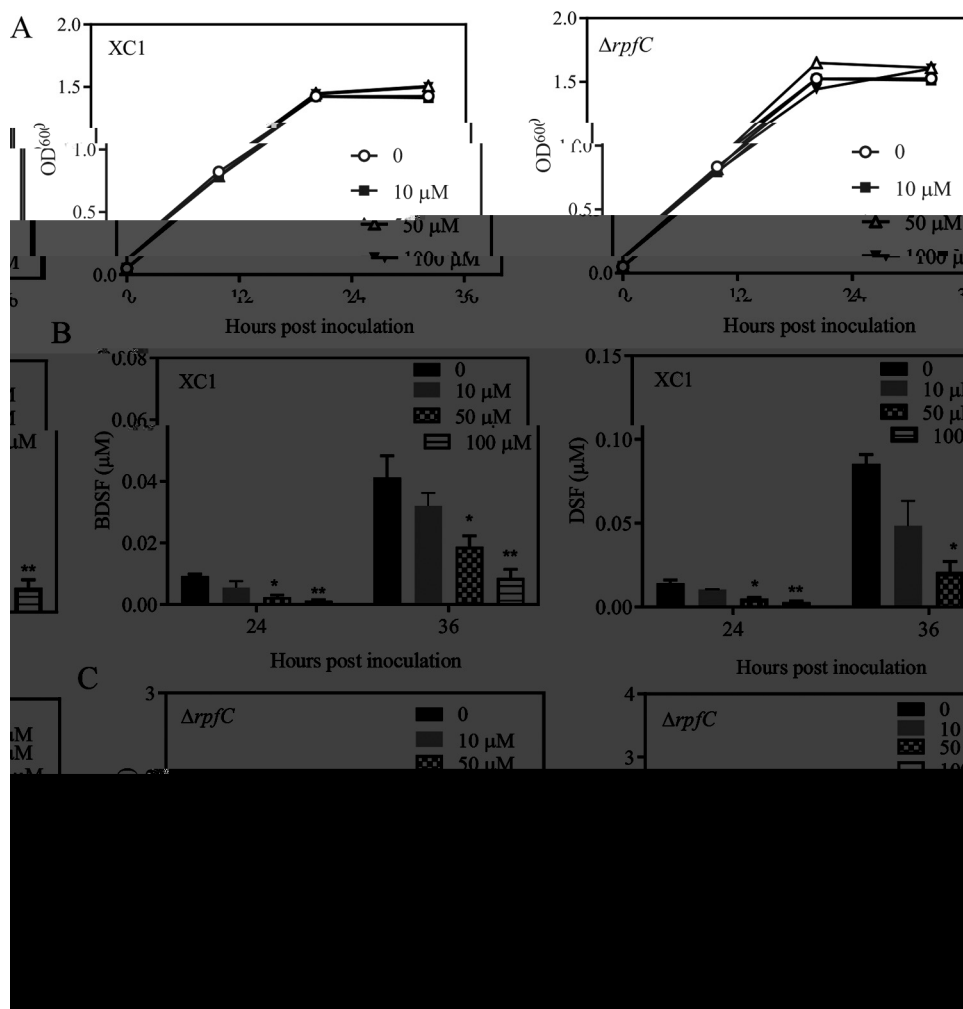
flavonoid biosynthesis pathway. The relative SA level in cabbage leaves was determined by HPLC-MS/MS. The relative SA level was normalized to the SA level in the control (PBS) at the same time point. The relative SA level was expressed as the ratio of the SA level in the infected leaves to the SA level in the control leaves. The relative SA level was significantly higher in the infected leaves than in the control leaves at 9 and 16 dpi ( $P < 0.05$  and  $P < 0.01$ , respectively). Error bars represent the standard deviation. The relative SA level was significantly higher in the infected leaves than in the control leaves at 9 and 16 dpi ( $P < 0.05$  and  $P < 0.01$ , respectively). Error bars represent the standard deviation.

## RESULTS

### *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage.

The relative SA level in cabbage leaves was determined by HPLC-MS/MS. The relative SA level was normalized to the SA level in the control (PBS) at the same time point. The relative SA level was expressed as the ratio of the SA level in the infected leaves to the SA level in the control leaves. The relative SA level was significantly higher in the infected leaves than in the control leaves at 9 and 16 dpi ( $P < 0.05$  and  $P < 0.01$ , respectively). Error bars represent the standard deviation. The relative SA level was significantly higher in the infected leaves than in the control leaves at 9 and 16 dpi ( $P < 0.05$  and  $P < 0.01$ , respectively). Error bars represent the standard deviation.

**Exogenous addition of SA induces DSF and BDSF turnover.** *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage. *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage. *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage. *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage. *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage.

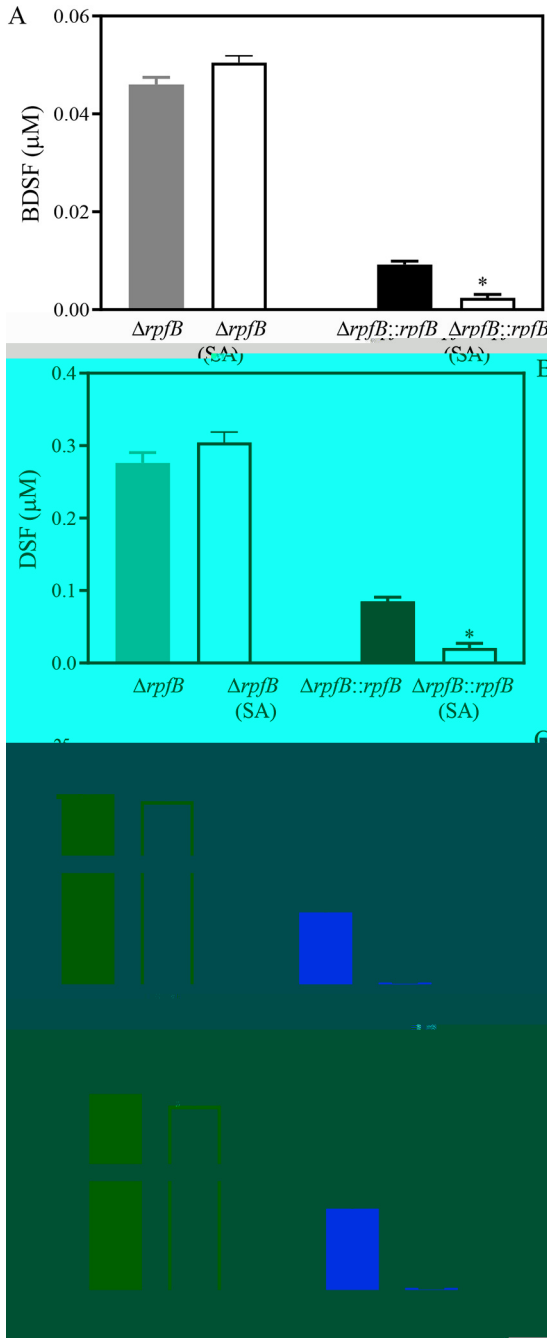


**FIG 2** Growth and virulence of *Xanthomonas campestris* strains. (A) Growth curves of *X. campestris* strains XC1 (○) and  $\Delta rpfC$  (□) at 0, 10, 50, and 100  $\mu\text{M}$  concentrations. (B) BDSF and DSF production by XC1 at 24 and 36 hours post-inoculation. (C) BDSF and DSF production by  $\Delta rpfC$  at 24 and 36 hours post-inoculation. Error bars represent standard deviation. Statistical significance is indicated by asterisks (\*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ).

*Xanthomonas campestris*, *X. campestris* (3- ) 4- (33, 34), fi, *Xanthomonas campestris*, *X. campestris* in vitro. *Xanthomonas campestris*, *X. campestris* (1- ). fi 100  $\mu\text{M}$ ,  $\Delta rpfC$  /  $\Delta rpfB$  (1- 2), *Xanthomonas campestris*, *X. campestris* fi 10  $\mu\text{M}$ , 50  $\mu\text{M}$ , 100  $\mu\text{M}$ , 10 100  $\mu\text{M}$  ff 1. (1- 2). fi 10  $\mu\text{M}$  fi 1. ff (1- 2). fi 50 100  $\mu\text{M}$  fi 1. fi 24 36 (1- 2). (1- 2). fi 36 0.033, 0.020, 0.014  $\mu\text{M}$  fi 10, 50  $\mu\text{M}$ , 100  $\mu\text{M}$  3. %, 23.5%, 16.4%, fi





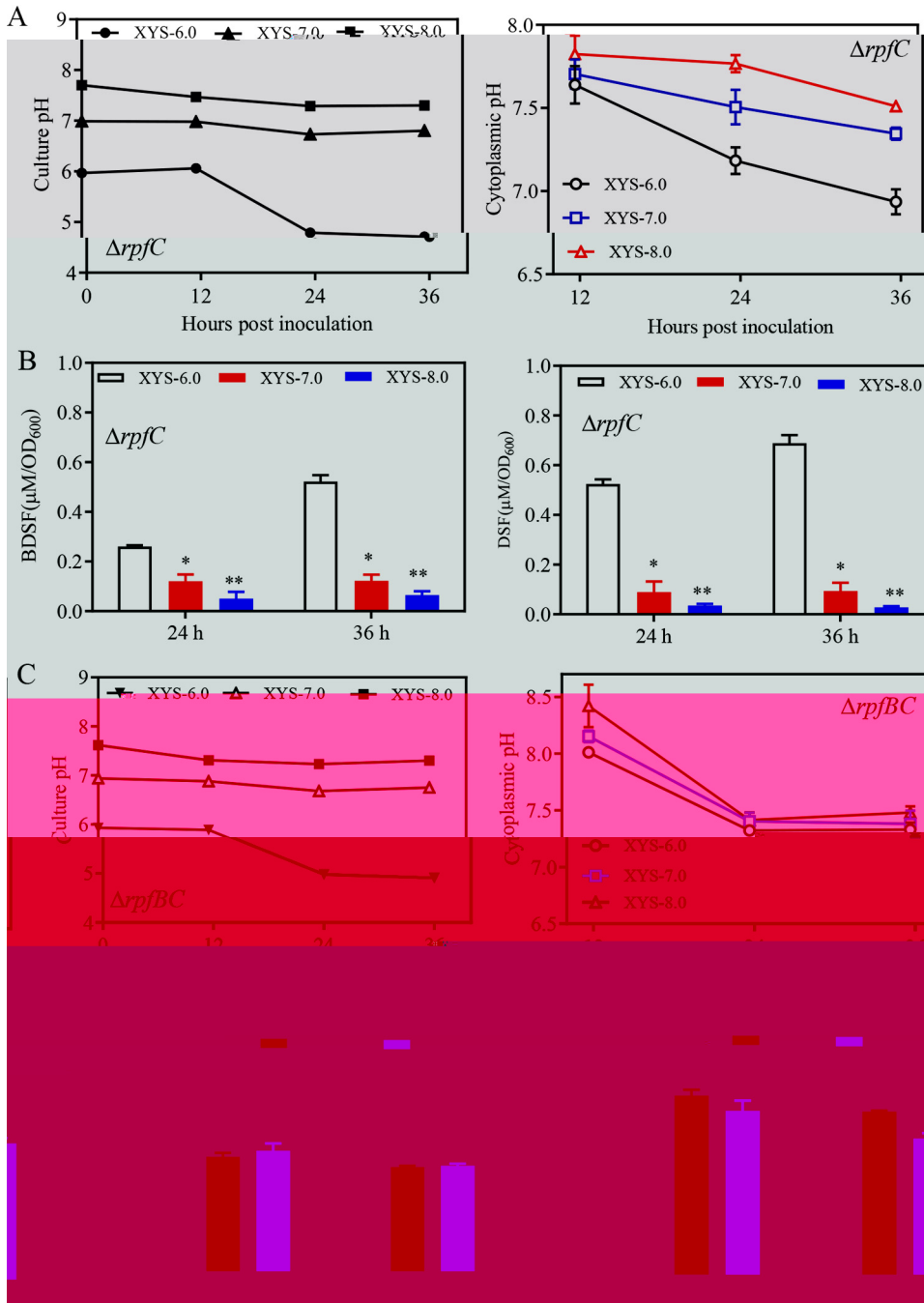


**FIG 4** *rpfB* deletion affects quorum sensing molecule production. (A) *rpfB* deletion ( $\Delta rpfB$ ) and complementation ( $\Delta rpfB::rpfB$ ) of *rpfB* in *Xanthomonas campestris* strain 8004.  $\Delta rpfB$  and  $\Delta rpfB::rpfB$  ( $\Delta rpfB$ ) were grown in 100 μM BDSF for 24 h. (B) *rpfB* deletion ( $\Delta rpfB$ ) and complementation ( $\Delta rpfB::rpfB$ ) of *rpfB* in *Xanthomonas campestris* strain 8004.  $\Delta rpfB$  and  $\Delta rpfB::rpfB$  ( $\Delta rpfB$ ) were grown in 100 μM DSF for 24 h. (C) Growth curves of *rpfB* deletion ( $\Delta rpfB$ ) and complementation ( $\Delta rpfB::rpfB$ ) of *rpfB* in *Xanthomonas campestris* strain 8004.  $\Delta rpfB$  and  $\Delta rpfB::rpfB$  ( $\Delta rpfB$ ) were grown in 100 μM BDSF for 24 h.  $\Delta rpfB$  and  $\Delta rpfB::rpfB$  ( $\Delta rpfB$ ) were grown in 50 μM DSF for 24 h. Error bars represent standard deviation. (\*,  $P \leq 0.05$ ).

24 h,  $P = 4.4 \times 10^{-36}$  ( $n = 5$ ). *rpfB* complementation ( $\Delta rpfB::rpfB$ ) significantly reduced the production of BDSF ( $P = 1 \times 10^{-5}$ ) and DSF ( $P = 1 \times 10^{-5}$ ) in *Xanthomonas campestris* strain 8004. *rpfB* complementation ( $\Delta rpfB::rpfB$ ) significantly reduced the growth of *Xanthomonas campestris* strain 8004 in 100 μM BDSF ( $P = 1 \times 10^{-2}$ ) and 50 μM DSF ( $P = 1 \times 10^{-2}$ ) for 24 h.







**FIG 6** Growth and acid production of *Xanthomonas campestris* strains. (A) Culture pH and cytoplasmic pH of *X. campestris* strains (XY5-6.0, XY5-7.0, XY5-8.0) and their  $\Delta rpfC$  mutants over 36 hours. (B) BDSF and DSF production by *X. campestris* strains and their  $\Delta rpfC$  mutants at 24 and 36 hours. (C) Culture pH and cytoplasmic pH of *X. campestris* strains (XY5-6.0, XY5-7.0, XY5-8.0) and their  $\Delta rpfBC$  mutants over 36 hours. Error bars represent standard deviation. (\*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ).

XY5-6.0 (6.0), XY5-7.0 (7.0), XY5-8.0 (8.0),  $\Delta rpfC$  XY5-6.0 (6.06),  $\Delta rpfC$  XY5-7.0 (7.0),  $\Delta rpfC$  XY5-8.0 (8.0). BDSF (μM/OD<sub>600</sub>) at 24 h: XY5-6.0 (0.28), XY5-7.0 (0.12), XY5-8.0 (0.08),  $\Delta rpfC$  XY5-6.0 (0.52),  $\Delta rpfC$  XY5-7.0 (0.12),  $\Delta rpfC$  XY5-8.0 (0.08). DSF (μM/OD<sub>600</sub>) at 24 h: XY5-6.0 (0.52), XY5-7.0 (0.12), XY5-8.0 (0.08),  $\Delta rpfC$  XY5-6.0 (0.70),  $\Delta rpfC$  XY5-7.0 (0.12),  $\Delta rpfC$  XY5-8.0 (0.08). Culture pH at 0 h: XY5-6.0 (6.0), XY5-7.0 (7.0), XY5-8.0 (7.8),  $\Delta rpfBC$  XY5-6.0 (6.0),  $\Delta rpfBC$  XY5-7.0 (7.0),  $\Delta rpfBC$  XY5-8.0 (7.8). Culture pH at 12 h: XY5-6.0 (6.0), XY5-7.0 (7.0), XY5-8.0 (7.4),  $\Delta rpfBC$  XY5-6.0 (6.0),  $\Delta rpfBC$  XY5-7.0 (7.0),  $\Delta rpfBC$  XY5-8.0 (7.4). Culture pH at 24 h: XY5-6.0 (5.0), XY5-7.0 (6.8), XY5-8.0 (7.3),  $\Delta rpfBC$  XY5-6.0 (5.0),  $\Delta rpfBC$  XY5-7.0 (6.8),  $\Delta rpfBC$  XY5-8.0 (7.3). Culture pH at 36 h: XY5-6.0 (4.9), XY5-7.0 (6.8), XY5-8.0 (7.3),  $\Delta rpfBC$  XY5-6.0 (4.9),  $\Delta rpfBC$  XY5-7.0 (6.8),  $\Delta rpfBC$  XY5-8.0 (7.3). Cytoplasmic pH at 12 h: XY5-6.0 (8.1), XY5-7.0 (8.1), XY5-8.0 (8.4),  $\Delta rpfBC$  XY5-6.0 (8.1),  $\Delta rpfBC$  XY5-7.0 (8.1),  $\Delta rpfBC$  XY5-8.0 (8.4). Cytoplasmic pH at 24 h: XY5-6.0 (7.4), XY5-7.0 (7.4), XY5-8.0 (7.4),  $\Delta rpfBC$  XY5-6.0 (7.4),  $\Delta rpfBC$  XY5-7.0 (7.4),  $\Delta rpfBC$  XY5-8.0 (7.4). Cytoplasmic pH at 36 h: XY5-6.0 (7.4), XY5-7.0 (7.4), XY5-8.0 (7.4),  $\Delta rpfBC$  XY5-6.0 (7.4),  $\Delta rpfBC$  XY5-7.0 (7.4),  $\Delta rpfBC$  XY5-8.0 (7.4).

$\Delta rpfC$  ... 6.0 ... 0.6 ...

$rpfB$  ...  $rpfC$  ...  $\Delta rpfBC$  ...  $\Delta rpfBC$  ...

5, 12 ... 24, 36 ...

-6.0 (r.t. 6) ...

0.1, 0.3, 12 ... (7.4) 24 ...

36 ... (r.t. 6) ...

$\Delta rpfBC$  ... (r.t. 6) ...

**Establishment of an *in vitro* RpfB-dependent DSF turnover system.**

(2) ... *in vitro* ...

(27, 2) ...

-2 ...

( ) ...

(250  $\mu$ M, 50  $\mu$ M, 300  $\mu$ M, 1  $\mu$ M ... 250  $\mu$ M ...

7.4) ... 250  $\mu$ M ...

25  $\mu$ M, 100  $\mu$ M, 100  $\mu$ M, 100  $\mu$ M ... 7.2 (r.t. 7) ...

(r.t. 7) ...

ff 1 ... (r.t. 7) ... 71.2% ...

ff 2 ... (r.t. 7) ...

*in vitro*

150  $\mu$ M, 10  $\mu$ M, 2, 2 ...

0.1% ... -100, 5 ... 0.5 ... 100  $\mu$ M ... 15  $\mu$ M ... (r.t. 7.2) (27, 2) ...

(r.t. 7) ...

150  $\mu$ M, 100  $\mu$ M ...

2, 4 ...

60 ... (r.t. 7) ...

***In vitro* RpfB-dependent DSF turnover activity increases with pH and is independent of SA.**

6.0, 7.0, ... 0 ...

(r.t. 6, 7) ...

250  $\mu$ M ... 37 ... 15, 30, 60 ...

6, 7, ... (r.t. 6) ...

10  $\mu$ M ... (r.t. 6), (r.t. 7), ... (r.t. 6) ...

( $K_m$ ) ... 12.3  $\mu$ M ...

(r.t. 6), 6.5  $\mu$ M ... (r.t. 7), 3.6  $\mu$ M ... (r.t. 6) ...

6, 7, ...

100  $\mu$ M ... (r.t. 6) ...

37 ... 30 ... (r.t. 6) ...

*in vitro* ...

**SA-treated XC1 exhibits increased virulence in cabbage. *Xanthomonas campestris* ...**

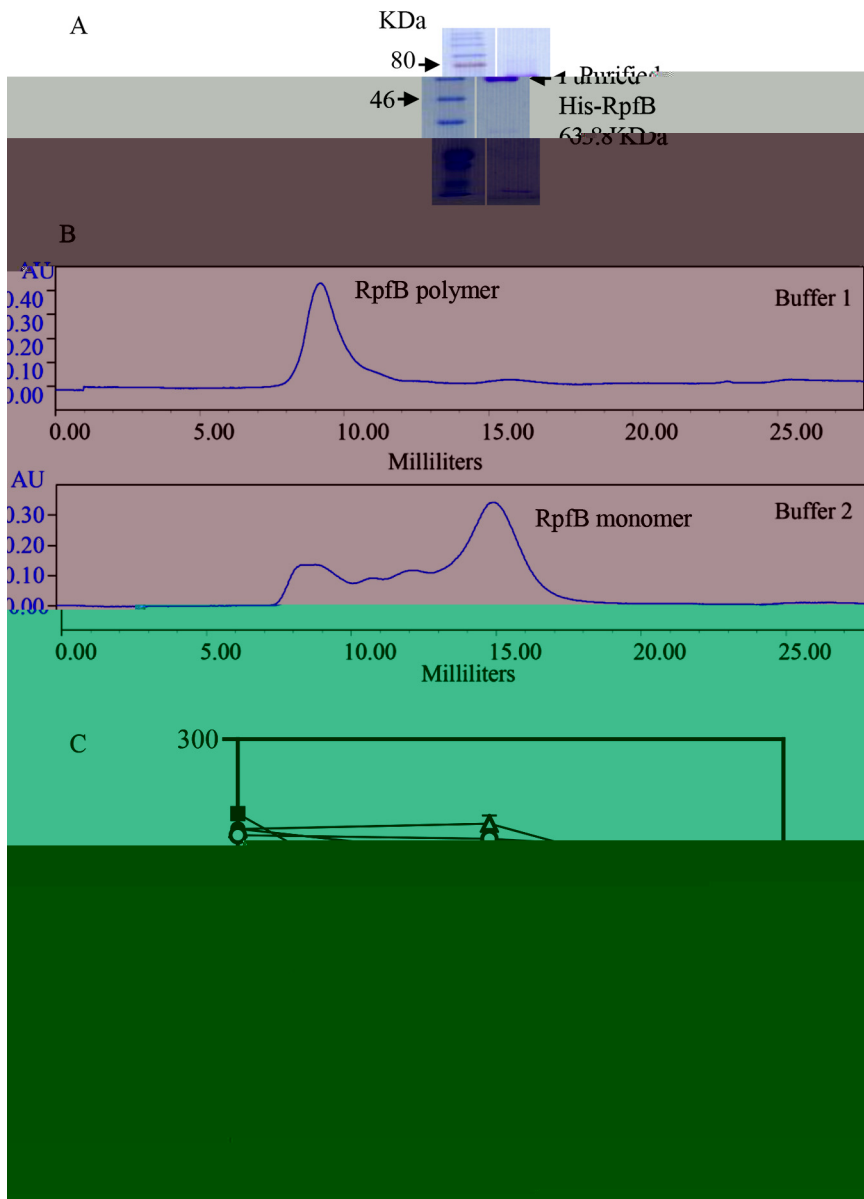
*campestris* ... (40) ...

1 (1+) ... (10  $\mu$ M ...

100  $\mu$ M) ... (r.t. 7) ...

1 ...

1+ ... 1 ...  $\Delta rpfC$  ...

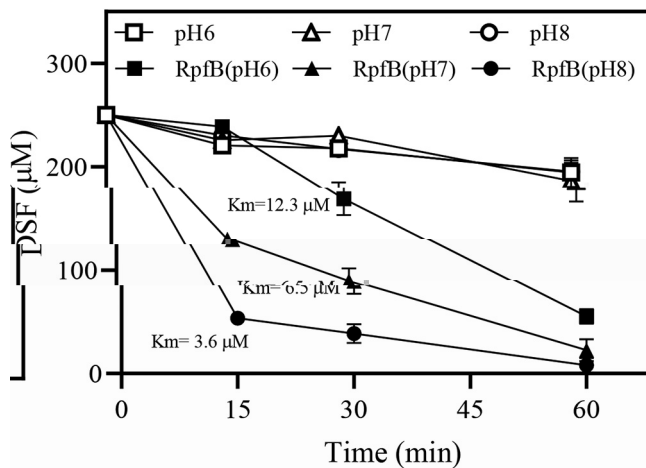


**FIG 7** *In vitro* self-assembly of purified His-RpfB. (A) Purification of His-RpfB from a His-tagged RpfB expression strain. The purified His-RpfB is shown in the lane labeled "Purified His-RpfB". Molecular weight markers are indicated in kDa. (B) Size exclusion chromatography (SEC) coupled with multi-angle laser light scattering (MALS) analysis of purified His-RpfB. The top panel shows SEC-MALS analysis of His-RpfB in Buffer 1 (100 mM NaCl, 50 mM Tris-HCl, pH 7.4), showing a peak at approximately 10.5 mL. The bottom panel shows SEC-MALS analysis of His-RpfB in Buffer 2 (100 mM NaCl, 25 mM Tris-HCl, pH 7.2), showing a peak at approximately 14.5 mL. (C) *In vitro* self-assembly of purified His-RpfB. His-RpfB is self-assembled in the presence of His-RpfB. The self-assembly is shown as a horizontal line connecting two points, with a 300 μm scale bar.

of purified His-RpfB in the presence of purified His-RpfB. The self-assembly of His-RpfB was quantified by measuring the absorbance at 280 nm of the supernatant and the pellet. The  $\Delta rpfC$  mutant, which lacks the RpfC protein, was used as a control. The self-assembly of His-RpfB in the  $\Delta rpfC$  mutant was significantly reduced compared to the wild-type strain. The self-assembly of His-RpfB in the wild-type strain was approximately 16% of the total His-RpfB, while in the  $\Delta rpfC$  mutant it was approximately 1%. The self-assembly of His-RpfB in the wild-type strain was significantly higher than in the  $\Delta rpfC$  mutant. The self-assembly of His-RpfB in the wild-type strain was approximately 16% of the total His-RpfB, while in the  $\Delta rpfC$  mutant it was approximately 1%. The self-assembly of His-RpfB in the wild-type strain was significantly higher than in the  $\Delta rpfC$  mutant. The self-assembly of His-RpfB in the wild-type strain was approximately 16% of the total His-RpfB, while in the  $\Delta rpfC$  mutant it was approximately 1%. The self-assembly of His-RpfB in the wild-type strain was significantly higher than in the  $\Delta rpfC$  mutant.

## DISCUSSION

The self-assembly of RpfB is a process that is regulated by the presence of RpfC. The self-assembly of RpfB is a process that is regulated by the presence of RpfC. The self-assembly of RpfB is a process that is regulated by the presence of RpfC. The self-assembly of RpfB is a process that is regulated by the presence of RpfC. The self-assembly of RpfB is a process that is regulated by the presence of RpfC. The self-assembly of RpfB is a process that is regulated by the presence of RpfC.



**FIG 8** *in vitro* degradation of DSF by RpfB at different pH levels. The graph shows the concentration of DSF (µM) over time (min) for three pH levels (6, 7, 8) with and without RpfB. The RpfB series show a faster decrease in DSF concentration over time. The  $K_m$  values for the RpfB series are:  $K_m=12.3 \mu\text{M}$  for pH6,  $K_m=6.5 \mu\text{M}$  for pH7, and  $K_m=3.6 \mu\text{M}$  for pH8.

(5, 41).  
(42, 43).  
(3, 5, 11).  
*Xanthomonas campestris*, ... *campestris*  
(17, 1, 20, 44).  
*Xanthomonas campestris*, ... *campestris*  
*Xanthomonas campestris*, ... *campestris*,  
*N-*  
*P. carotovorum*  
*Pectobacterium aroidearum*.  
*P. carotovorum* 1, ... 2.5- 10.0-  
f. 24 f. ...  
(11). *A. tumefaciens* 5, ... (6 µM  
f. 6 ) ... *attKLM*  
*N-3-*  
(3 ... ), *A. tumefaciens* (6).  
24 36 ... (1, 2 3).  
10- 100-µM ... *rpfF*  
*rpfB*,  
*Xanthomonas campestris*, ... *campestris* (21, 2 ) (1, 3).  
*Xanthomonas campestris*, ... *campestris*  
*P. carotovorum*, *P. aroidearum*, ... *A. tumefaciens* (6, 11),





*campestris* . . . *campestris*

*E. coli*,

ff-

(45).

(4).

(4).

*Xanthomonas campestris* . . . *campestris*

1 (52).

1 (7).

1

*Xanthomonas campestris* . . . *campestris*

*Xanthomonas campestris* . . . *campestris*.

*Xanthomonas*

*Stenotrophomonas mal-*

*tophilia* *Burkholderia*

*Lysobacter*, *Leptospirillum*, *Frateuria*, *Luteibacter*, *Rhodanobacter*, *Methylobacillus flagel-*

*lates*, *Thiobacillus denitrificans*,

fi f

## MATERIALS AND METHODS

### Bacterial strains and culture conditions.

*Xanthomonas campestris* . . . *campestris*  
2 (0.7 . . . 0.2 . . . )  
0.0625% . . . 7.0), (5 . . . 10 . . . )  
*E. coli* . . . 5 $\alpha$   
*E. coli* . . . 37  
25  $\mu$  . . . 50  $\mu$  . . . 20  $\mu$  . . .  
100  $\mu$  . . .  
f 600 ( . . . 600).

### Gene deletion and functional complementation analysis in *Xanthomonas campestris* pv. *campestris*.

(~500 . . . ) f  
*Xanthomonas campestris* . . . *campestris*  
50  $\mu$  . . . 5% ( . . . )

*Xanthomonas campestris* (53).  
 1.

**Extraction and quantitative analyses of SA in *Xanthomonas campestris* pv. *campestris* cultures and in plant leaf tissues.**

(33).  
 (33). fl, 0.5  
 4.0  
 0.1  
 0.05%  
 (25/75) 1  
 25 75%  
 16 (,000) f 2  
*Xanthomonas campestris*  
 600 f 0.1  
 (1,000) f (200) 5, 16  
 (54). 50 μ  
 (34). fl, 10 μ  
 (4.6 150, 5 μ)  
 0.1% f (60/40) /  
 40 fl f 0.4  
 ( ) ( )

**Extraction, purification, and quantitative analysis of BDSF and DSF using UPLC-TOF MS.**

*Xanthomonas campestris* pv. *campestris* (55).  
 4.0 20  
 30  
 0.1  
 4.6 150) ( )  
 0.1% f ( 0/20) /

**RpfB expression, purification, and *in vitro* DSF turnover activity assays. *E. coli***

(3)  
 2  
 0.1  
 1 f 16  
 100 ( )<sub>2</sub> 4r 10  
 25 ( )  
 25 ( )  
 100 ( )  
 100 ( )  
 100 ( )  
 100 ( )  
 200 f  
 100 ( )<sub>2</sub> 4 ( )  
 7.2)

*In vitro*  
 (2).  
 100 2 4 2 4r 10 2r 2  
 0.1% ( ) -100, 5, 0.5, 0.3  
 10 μ f  
 2 f 15, 30, 45, 60 f 500 μ  
 4.0 f 1 2-f

**Construction of *gusA*-dependent reporter strains to monitor *rpfF* and *rpfB* transcriptional activities and GUS assays.**

*gusA*-*rpfF* *rpfB*  
 (2). β-  
 (34). fl, 12  
 36 2 (1) f, 1,000 ( )  
 (17) f 10 (1×, 7.4).  
 1 f 20 μ f 0.1% ( ) 40 μ



... 250 μm (1 × 4- ... β- ... 37 f, 15 ... (200 μm) ... 00 μm f 0.2 ... 6- ... f 365 ... 455

**Western blotting.**

... 15,000 ... 5% ( / . ) ... f ( 2 ). ... ff ( 20 ... 0.15 ... 0.1% ... 20). ... 16,500 ... ( )- ... 21001) ... 610

**Measurement of culture and cytoplasmic pH.**

... *Xanthomonas campestris*, ... *campestris* ... 5,000 ... 4 ... *Xanthomonas campestris*, ... *campestris* ... *Xanthomonas campestris*, ... *campestris* ... ( 3 ). ... 5332 6) ... 67 264)

... *Xanthomonas campestris*, ... *campestris* ... 1.

... 4 ... 510 ... 610 ... 12

... *Xanthomonas campestris*, ... *campestris* ... 600 f 0.6 ... 6.0, 7.0, 7.5, ... 0. ... 6 ... f 250 μm ... m- ... f f 20

**Virulence assay of *Xanthomonas campestris* pv. *campestris* strains in cabbage.**

... *Xanthomonas campestris*, ... *campestris* ... ( ... f ... -1) ... (34). ... Δ*prfC* ... 12 ... ff ... fi ... 600 f 0.1. ... 1 ( ... + ... 100 μm ... 12 ... 5,000 ... f 5 ... 4 ... ff ... fi ... 600 f 0.1. ... 600 ... f 0.1. ... fi ... 30 ... f 0% ... f 10,000 ... 2 ... f 15

**Statistical analyses.**

... ( ... ) ... f ... ff ... F ... fi ... P ... f < 0.05.

**SUPPLEMENTAL MATERIAL**

- FIG S1, ... fi , 1.2
- FIG S2, ... fi , 0.4
- FIG S3, ... fi , 1.6
- FIG S4, ... fi , 1.1
- FIG S5, ... fi , 0.6
- FIG S6, ... fi , 0.3
- FIG S7, ... fi , 1.4
- TABLE S1, ... fi , 0.02

## ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (grant numbers 3172231 and 32172355).

## REFERENCES

1. Zhang, Y., et al. (2004). Identification of a novel *Agrobacterium* strain for plant transformation. *Plant Cell Reports*, 23(1), 42-50. [DOI: 10.1146/annurev-arplant-070703-140421](#).
2. Wang, L., et al. (2006). Genetic engineering of *Agrobacterium* for plant transformation. *Plant Cell Reports*, 25(1), 323-329. [DOI: 10.1038/nrn0526](#).
3. Zhang, Y., et al. (2011). Identification of a novel *Agrobacterium* strain for plant transformation. *Plant Cell Reports*, 30(1), 1427-1433. [DOI: 10.1016/j.plcr.2011.10.002](#).
4. Zhang, Y., et al. (2020). Identification of a novel *Agrobacterium* strain for plant transformation. *Plant Cell Reports*, 39(1), 54-56. [DOI: 10.1016/j.plcr.2020.01.004](#).
5. Zhang, Y., et al. (2021). Identification of a novel *Agrobacterium* strain for plant transformation. *Plant Cell Reports*, 40(1), 72-76. [DOI: 10.1146/annurev-arplant-070703-140421](#).
6. Zhang, Y., et al. (2007). Identification of a novel *Agrobacterium* strain for plant transformation. *Plant Cell Reports*, 26(1), 117-121. [DOI: 10.1073/pnp.070466104](#).
7. Zhang, Y., et al. (2014). *Agrobacterium* strain for plant transformation. *Plant Cell Reports*, 33(1), 730-735. [DOI: 10.33044/PLCR.2014.00730](#).

35. ... 2011. *Xanthomonas campestris* ff. ... 3- ...  
24. 4 – 57. // ... /10.10.4/ ... -02-11-0031.
36. ... 2015. f. ... 4- ...  
*Xanthomonas campestris*, ... f. f. ...  
51 456. // ... /10.103 / ... 1 456.
37. ... 201 ... f ...  
3- ...  
110 16–32. // ... /10.1111/ ...  
.14064.
3. ... 201. ... f *E. coli* ...  
3 6 // ... /10.103 / 415.  
-01. -40560-3.
3. ... 2000. ... f ...  
21. –220 // ... /10.1016/ 0006-34. 5(00)7646 - ...
40. ... 2020. *Xanthomonas* ...  
1 415–427. // ...  
/10.103 / 4157. -020-0361-
41. ... 2011. ...  
0156. // ... /10  
.11. / ...0156.
42. ... 200. ... f ...  
47 177–206. // ...  
/10.1146/ ... .050 0 .135202.
43. ... 2020. ... f ...  
361–10. // ... /10.5423/  
.12.201.02. 5.
44. ... 2017. ff. ... f ...  
122 2–11. // ... /10.1111/ ...13307.
45. ff. ... f ...  
2021. ... fi ... ff. f ... ff- ...  
*Escherichia coli* -12. ...  
7 0072421. // ... /10.112 / ...00724-21.
46. ... 2014. ...  
*Xanthomonas campestris* ... *campestris* ...  
106712, ... 43 6. // ... /  
10.1371/ ... .00 43 6.
47. ... 2006. ...  
11. 17. –1 7. // ... /10  
.1007/ 10265-005-0257- ...
4. ... 200. ... fi- ...  
( ... ) ... ff- ...  
176  
4. 7–504. // ... /10.1016/, ...200.01.002.
4. ... 2017. ... f ... f ...  
10 1371–13 6. // ... /10.1016/  
.2017.0.01 ...
50. ... 2011. ... f ...  
f *Salmonella*, f ...  
2 6 2217 –221 5. // ... /10.1074/ ... 111.24525 ...
51. ... 201. ...  
*Enterobacteriaceae*. ... 10 0000 -1. // ... /10  
.112 / ...0000 -1.
52. ... 2006. ... f ff. ...  
*Xanthomonas campestris*, ... *campestris* ... fi ... f ...  
5. 610–622. // ... /10.1111/.1365-2. 5 .2005.04. 61. ...
53. ... 200. ... 7. ...  
*Xanthomonas* ... 2.  
111–117. // ... /10.1111/.1574-6 6 .200 .01707. ...
54. ... 2010. ... f ...  
5. 6–2. // ... /10.103 /  
.2010.37.
55. ... 201 ... f ...  
f ... ( ... ) f ... f ... f ...  
1673. 7–105. // ... /10.1007/ 7 -1-4 3. -730 -51 ...