

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*

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ABSTRACT

Xanthomonas campestris is a Gram-negative bacterium that causes bacterial blight in cruciferous plants. The quorum sensing (QS) system is a key regulatory mechanism for the pathogen's virulence. The RpfB-dependent QS system is a major signaling pathway in *X. campestris*. In this study, we investigated the effect of salicylic acid (SA) on the QS system of *X. campestris*. We found that SA treatment significantly reduced the production of the QS signal 3-oxo-C₁₂-HSL and the expression of the RpfB-dependent QS genes *rpfB* and *rpfF*. This effect was associated with an increase in the culture and cytoplasmic pH of the bacteria. The pH increase was caused by the production of ammonia (NH₃) from the degradation of urea. The pH increase and the production of NH₃ were dependent on the RpfB-dependent QS system. In vitro, the addition of NH₃ to the culture medium also reduced the production of 3-oxo-C₁₂-HSL and the expression of *rpfB* and *rpfF*. These results suggest that SA activates the RpfB-dependent QS signal turnover via altering the culture and cytoplasmic pH in *X. campestris*.

IMPORTANCE

The quorum sensing (QS) system is a key regulatory mechanism for the virulence of *Xanthomonas campestris*. The RpfB-dependent QS system is a major signaling pathway in *X. campestris*. In this study, we investigated the effect of salicylic acid (SA) on the QS system of *X. campestris*. We found that SA treatment significantly reduced the production of the QS signal 3-oxo-C₁₂-HSL and the expression of the RpfB-dependent QS genes *rpfB* and *rpfF*. This effect was associated with an increase in the culture and cytoplasmic pH of the bacteria. The pH increase was caused by the production of ammonia (NH₃) from the degradation of urea. The pH increase and the production of NH₃ were dependent on the RpfB-dependent QS system. In vitro, the addition of NH₃ to the culture medium also reduced the production of 3-oxo-C₁₂-HSL and the expression of *rpfB* and *rpfF*. These results suggest that SA activates the RpfB-dependent QS signal turnover via altering the culture and cytoplasmic pH in *X. campestris*.

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KEYWORDS

Xanthomonas campestris

Plant diseases caused by *Xanthomonas campestris* (1–3). *X. campestris* (4). *X. campestris* (5). *X. campestris* *vir* *Agrobacterium tumefaciens* (6–8). *repABC* *A. tumefaciens*, *fil* *N-* *Pectobacterium carotovorum* *Pseudomonas syringae* *syringae* (10–11). *hrpA* *in vitro* *Erwinia amylovora* (12). *Xanthomonas oryzae* (12). (13). *Xanthomonas campestris* *campestris* (14). *Xanthomonas campestris* *campestris* (15). *Xanthomonas campestris* *campestris* (14, 16, 17). *Xanthomonas campestris* *campestris* (18–20). *cis-11-H* *Burkholderia cenocepacia* (*cis-2-*) *Xanthomonas* (21). (22–24). (25). (25, 26). *Xanthomonas campestris* *campestris*, *Xanthomonas* *Xanthomonas campestris* *campestris*, β - (27–2). *Xanthomonas* *Arabidopsis*, *Nicotiana benthamiana*, (30–32). *Xanthomonas campestris* *campestris*, *Xanthomonas campestris* *campestris*, *fil*

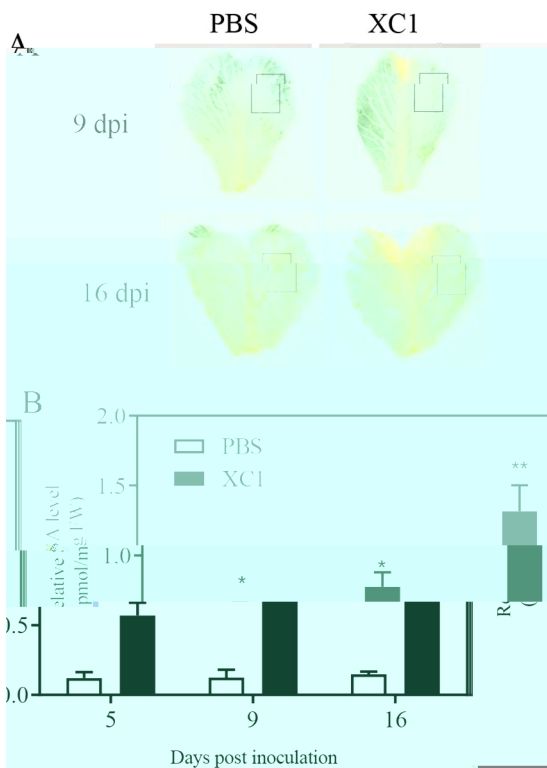


FIG 1 *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage. Heatmaps (A) and bar chart (B) showing relative SA levels in cabbage leaves at 5, 9, 16, and 21 dpi. (A) Heatmaps showing SA levels in cabbage leaves at 9 and 16 dpi for PBS and XCI treatments. (B) Bar chart showing relative SA levels (pmol/mg FW) in cabbage leaves at 5, 9, 16, and 21 dpi for PBS and XCI treatments. Error bars represent standard deviation. (*, $P \leq 0.05$; **, $P \leq 0.01$).

flavonoid biosynthesis pathway (Kobayashi et al., 2010). The results of this study show that *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage leaves. This is the first time that SA biosynthesis in cabbage leaves has been shown to be promoted by *Xanthomonas campestris* pv. *campestris*.

RESULTS

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

The results of this study show that *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage leaves. This is the first time that SA biosynthesis in cabbage leaves has been shown to be promoted by *Xanthomonas campestris* pv. *campestris*. The results of this study show that *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage leaves. This is the first time that SA biosynthesis in cabbage leaves has been shown to be promoted by *Xanthomonas campestris* pv. *campestris*. The results of this study show that *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage leaves. This is the first time that SA biosynthesis in cabbage leaves has been shown to be promoted by *Xanthomonas campestris* pv. *campestris*.

Exogenous addition of SA induces DSF and BDSF turnover. *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage leaves. This is the first time that SA biosynthesis in cabbage leaves has been shown to be promoted by *Xanthomonas campestris* pv. *campestris*. The results of this study show that *Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage leaves. This is the first time that SA biosynthesis in cabbage leaves has been shown to be promoted by *Xanthomonas campestris* pv. *campestris*.

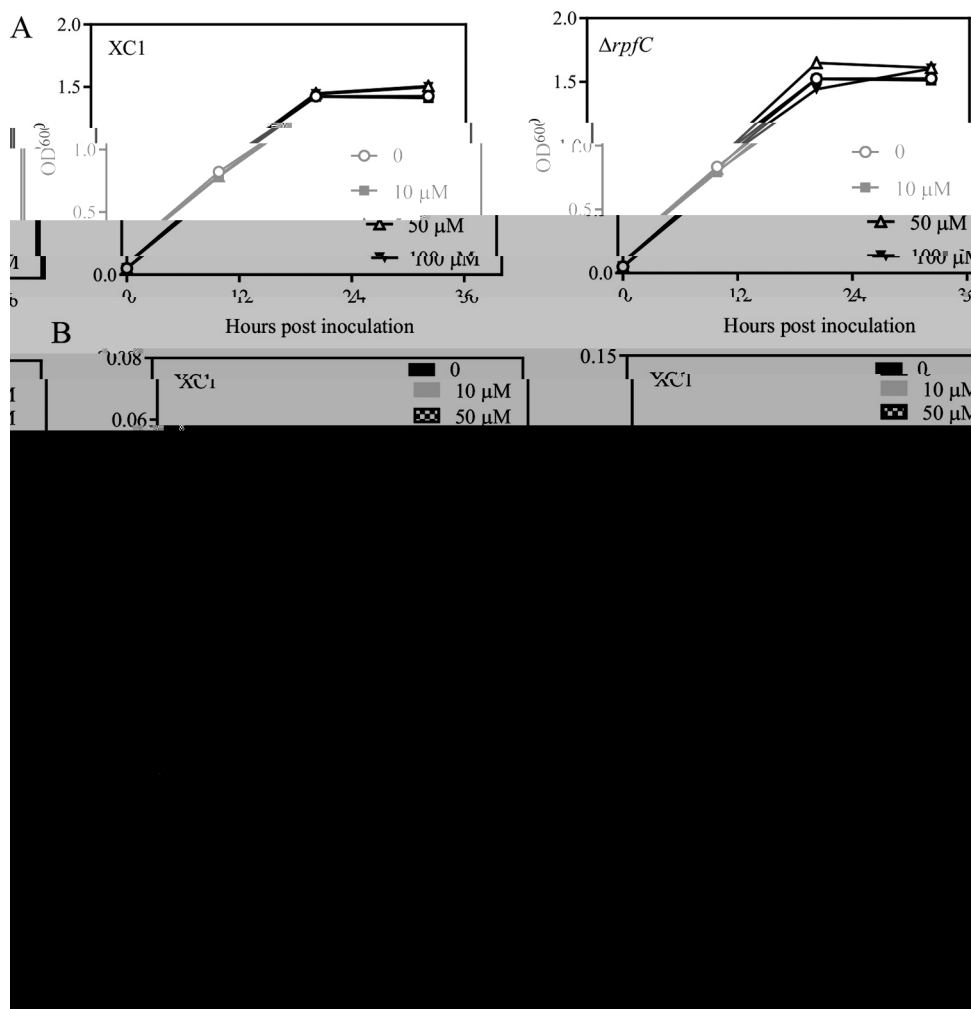


FIG 2 Growth curves and XCC1 production of *Xanthomonas campestris* strains XC1 and $\Delta rpfC$. (A) Growth curves of XC1 and $\Delta rpfC$ strains in the presence of 10, 50 and 100 μM of XCC1. (B) XCC1 production in the presence of 10, 50 and 100 μM of XCC1. Error bars represent standard deviation. Statistical significance was determined by two-tailed t-test (*, $P \leq 0.05$; **, $P \leq 0.01$).

Xanthomonas campestris ssp. *campestris* strains XC1 and $\Delta rpfC$ were grown in the presence of 10, 50, and 100 μM XCC1. The growth curves showed that the presence of XCC1 did not significantly affect the growth of either strain (Fig. 2A). The XCC1 production of XC1 and $\Delta rpfC$ strains was measured in the presence of 10, 50, and 100 μM XCC1. The results showed that the XCC1 production of XC1 and $\Delta rpfC$ strains was not significantly affected by the presence of XCC1 (Fig. 2B). The XCC1 production of XC1 and $\Delta rpfC$ strains was measured in the presence of 10, 50, and 100 μM XCC1. The results showed that the XCC1 production of XC1 and $\Delta rpfC$ strains was not significantly affected by the presence of XCC1 (Fig. 2B).

Xanthomonas campestris ssp. *campestris* strains XC1 and $\Delta rpfC$ were grown in the presence of 10, 50, and 100 μM XCC1. The growth curves showed that the presence of XCC1 did not significantly affect the growth of either strain (Fig. 2A). The XCC1 production of XC1 and $\Delta rpfC$ strains was measured in the presence of 10, 50, and 100 μM XCC1. The results showed that the XCC1 production of XC1 and $\Delta rpfC$ strains was not significantly affected by the presence of XCC1 (Fig. 2B).

(0.015 μl) ... (μl. 2). ... 12 ...
 ... 1 ... (...).
 ... ΔrpfC.
 10 μl ... ΔrpfC
 (μl. 2). ... 50 ... 100 μl ...
 ... ΔrpfC ... 12 ...
 ... 24 ... 36 ... (μl. 2).
 ... ΔrpfC ... 50 ... 100 μl ... 36
 0.17 μl ... 0.05 μl ... 12.1% ... 3.5%
 ... (1.40 μl), ΔrpfC ... (μl. 2).

Endogenous production of SA induces DSF and BDSF turnover.

Xanthomonas campestris ... *campestris*
 ... 3- ... 4- ... (35,
 36). ... *Xanthomonas cam-*
pestris ... *campestris*, ... ΔrpfC pchAB,
 ... pchAB ... ΔrpfC ... (μl. 3). pchAB,
 ... *Pseudomonas aeruginosa* (37).
 ... ΔrpfC ... ΔrpfC pchAB ...
 ΔrpfC pchAB ... 55.1 μl ... 12 ... 5.5 μl ... 24 ... 124.6 μl ... 36
 ... ΔrpfC ... (μl. 3 ...).
 ... ΔrpfC pchAB ... 24 ... 36
 ... ΔrpfC ... (μl. 3 ...).
 ... ΔrpfC pchAB ... 36 ... 0.05 μl ... 1.4%
 ... 3.5 μl ... ΔrpfC ... (μl. 3). ... ΔrpfC pchAB ...
 36 ... 0.04 μl ... 1.1% ... 2.2 μl ... ΔrpfC ...
 (μl. 3).

SA-induced DSF and BDSF turnover is dependent on rpfB.

Xanthomonas campestris ... *campestris* (28).
 rpfB, ...
 ΔrpfB, ΔrpfB ... rpfB ... att 7 ...
 (ΔrpfB rpfB), ... rpfB ... rpfC ... (... ΔrpfBC
), ... ΔrpfBC ... rpfB ... att 7 ...
 (... ΔrpfBC rpfB). ... ΔrpfB ... ΔrpfB rpfB
 ... 100 μl ...
 100 μl ... ΔrpfB ...
 ... ΔrpfB rpfB ... 0.002 ... 0.02 μl ... 36
 ... 22.2% ... 25%
 (0.00 ... 0.08 μl, ...) (μl. 4 ...).
 ... ΔrpfBC ... ΔrpfBC rpfB ...
 ... 100 μl ...
 100 μl ... ΔrpfBC (μl. 4 ...).
 ... 100 μl ... ΔrpfBC rpfB
 (μl. 4 ...). ... ΔrpfBC rpfB ...
 0.23 ... 0.15 μl ... 36 ...
 ... (2.2 ... 2.1 μl, ...) (μl. 4 ...).

SA does not affect the expression of rpfB and rpfF.

Xanthomonas campestris ... *campestris*
 ...
 ... (10 ... 100 μl) ... rpfB ... rpfF ...
 ... 1 rpfB-gusA ... 1 rpfF-gusA

rpfB, *rpfF*, *rpfB*, *rpfF*, *rpfB-gusA*, *rpfF-gusA* (μ . 3).
 1 *rpfB-gusA*, *rpfF-gusA* (μ . 3).
 1 *rpfB-gusA*, *rpfF-gusA* (μ . 3).
 1 *rpfB-gusA*, *rpfF-gusA* (μ . 3).
 (2). *rpfB-gusA*, *rpfF-gusA* (μ . 3).
 1 Δ *rpfC* (μ . 3), *rpfB-gusA*, *rpfF-gusA* (μ . 3).

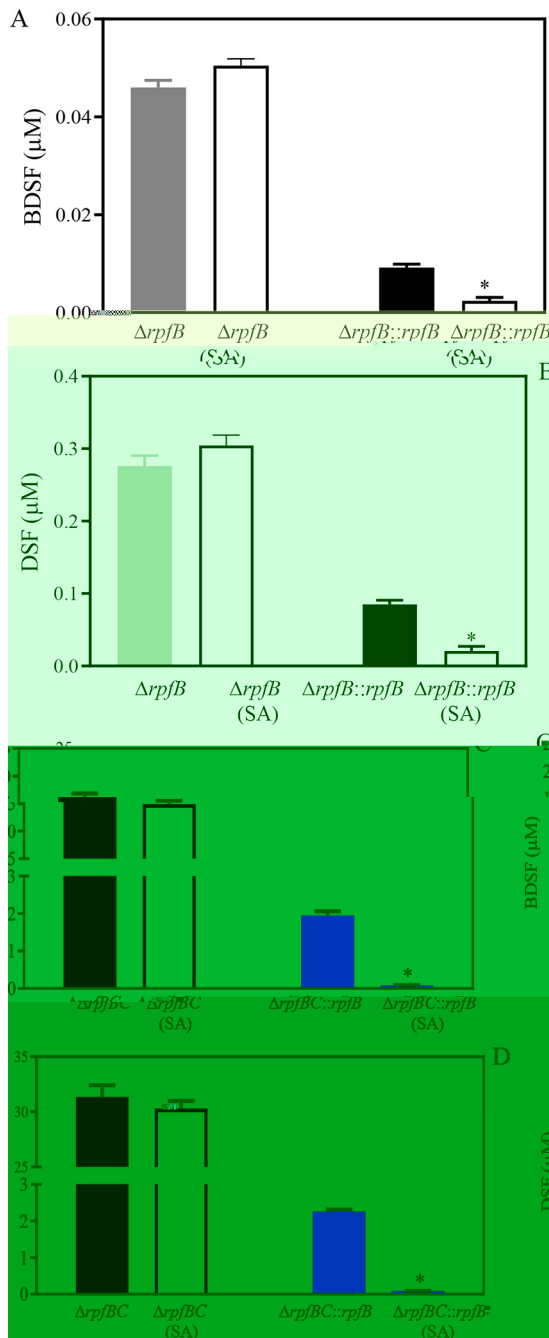


FIG 4 *rpfB* and *rpfBC* deletion mutants of *Xanthomonas campestris* produce significantly less BDSF and DSF than their wild-type counterparts. $\Delta rpfB$ and $\Delta rpfBC$ mutants were grown in 100 μ l of 24 h-old *rpfB* and *rpfC* complemented cultures. $\Delta rpfB::rpfB$ and $\Delta rpfBC::rpfB$ complemented cultures were grown in 50 μ l of 24 h-old *rpfB* and *rpfC* complemented cultures. Error bars represent standard deviation. Asterisks indicate significant differences between wild-type and mutant strains (*, $P \leq 0.05$).

24 h-old cultures (4.4 \pm 36 μ M, $n = 5$). $\Delta rpfC$ mutant produced significantly less DSF than wild-type (1 μ M, $n = 5$). *Xanthomonas campestris* *campestris* complemented cultures produced significantly less BDSF and DSF than wild-type (0.5 μ M, $n = 5$). *Xanthomonas campestris* *campestris* complemented cultures produced significantly less DSF than wild-type (0.5 μ M, $n = 5$).

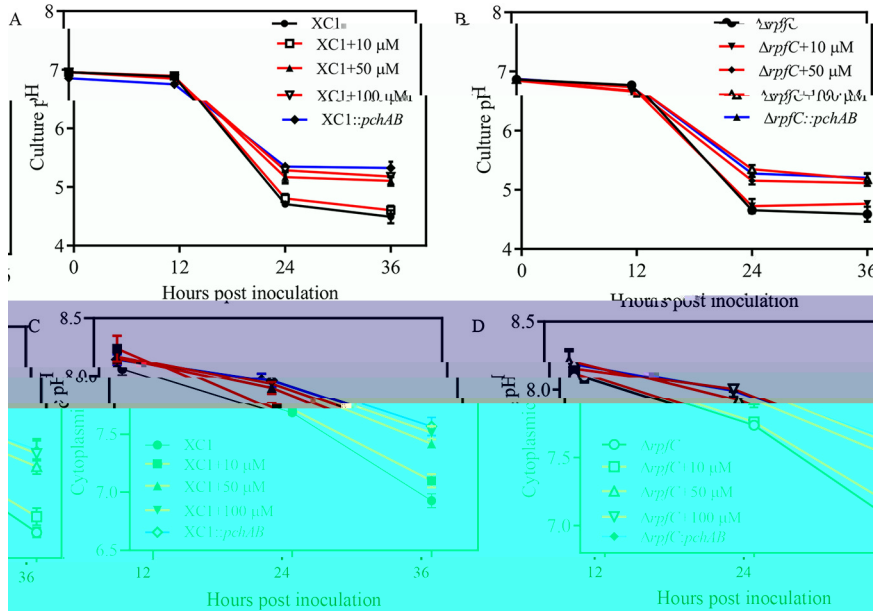


FIG 5 Culture pH of *Xanthomonas campestris* pv. *campestris* strains $\Delta rpfC$, $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, and $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA $\Delta rpfC::pchAB$. Culture pH was monitored in YXS medium at 12, 24, and 36 hpi. The pH of the culture medium was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter. The cytoplasmic pH was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter.

(3.8), (4.1), (4.2), (4.3), (4.4), (4.5), (4.6), (4.7), (4.8), (4.9), (5.0), (5.1), (5.2), (5.3), (5.4), (5.5), (5.6), (5.7), (5.8), (5.9), (6.0), (6.1), (6.2), (6.3), (6.4), (6.5), (6.6), (6.7), (6.8), (6.9), (7.0), (7.1), (7.2), (7.3), (7.4), (7.5), (7.6), (7.7), (7.8), (7.9), (8.0), (8.1), (8.2), (8.3), (8.4), (8.5), (8.6), (8.7), (8.8), (8.9), (9.0), (9.1), (9.2), (9.3), (9.4), (9.5), (9.6), (9.7), (9.8), (9.9), (10.0).

Exogenous addition of SA or endogenous production of SA significantly prevents *Xanthomonas campestris* pv. *campestris* culture pH and cytoplasmic pH decrease.

$\Delta rpfC$, $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, and $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA $\Delta rpfC::pchAB$. Culture pH was monitored in YXS medium at 12, 24, and 36 hpi. The pH of the culture medium was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter. The cytoplasmic pH was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter.

$\Delta rpfC$, $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, and $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA $\Delta rpfC::pchAB$. Culture pH was monitored in YXS medium at 12, 24, and 36 hpi. The pH of the culture medium was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter. The cytoplasmic pH was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter.

$\Delta rpfC$, $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA, $\Delta rpfC::pchAB$, and $\Delta rpfC$ $\Delta rpfC$ + 10 μ M, 50 μ M, 100 μ M SA $\Delta rpfC::pchAB$. Culture pH was monitored in YXS medium at 12, 24, and 36 hpi. The pH of the culture medium was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter. The cytoplasmic pH was measured in a 100 μ l aliquot using a pH meter. Error bars represent standard deviation. The pH of the cytoplasm was measured in a 100 μ l aliquot using a pH meter.

Increasing the pH of XYS medium triggers BDSF and DSF turnover in an RpfB-dependent manner.

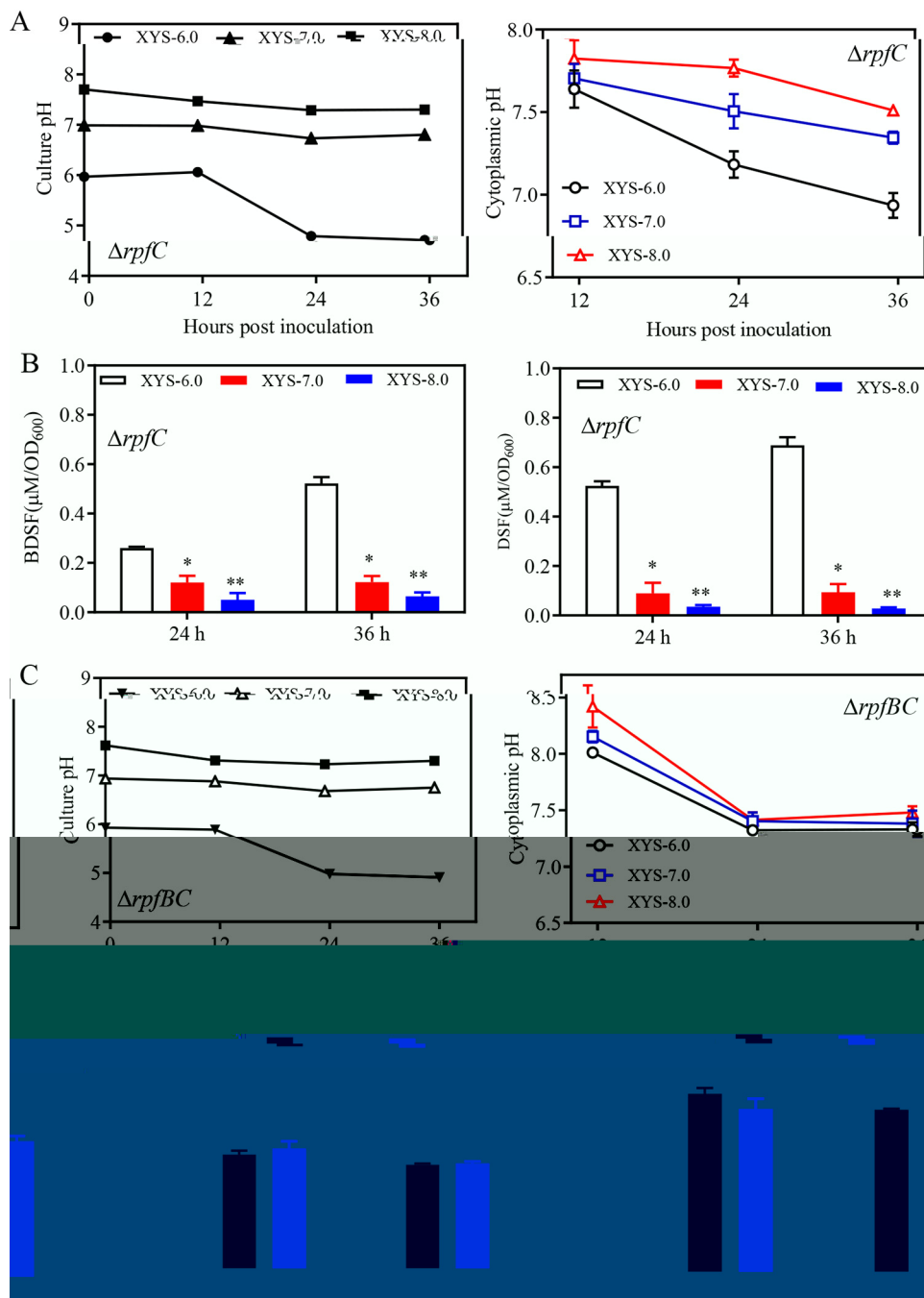


FIG 6 Growth and pH changes in *Xanthomonas campestris* strains. (A) Culture pH (left) and cytoplasmic pH (right) of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfC$ at 0, 12, 24, and 36 h. (B) BDSF (left) and DSF (right) production of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfC$ at 24 and 36 h. (C) Culture pH (left) and cytoplasmic pH (right) of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfBC$ at 0, 12, 24, and 36 h. The bar chart at the bottom shows BDSF production of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfBC$ at 24 and 36 h. Error bars represent standard deviation. (*, $P \leq 0.05$; **, $P \leq 0.01$).

Table 1. Culture pH (left) and cytoplasmic pH (right) of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfC$ at 0, 12, 24, and 36 h. Table 2. BDSF (left) and DSF (right) production of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfC$ at 24 and 36 h. Table 3. Culture pH (left) and cytoplasmic pH (right) of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfBC$ at 0, 12, 24, and 36 h. The bar chart at the bottom shows BDSF production of *X. campestris* strains XY5-6.0, XY5-7.0, and XY5-8.0 in the presence of $\Delta rpfBC$ at 24 and 36 h. Error bars represent standard deviation. (*, $P \leq 0.05$; **, $P \leq 0.01$).

$\Delta rpfC$... 6.0 ± 0.0 ($n=6$).
 $rpfB$... $\Delta rpfBC$... $\Delta rpfBC$...
 -7.0 ± 0.0 , ...
 5.12 ± 0.12 ... $(4.) \pm 24$... 36 ...
 -6.0 ($n=6$). ... $\Delta rpfBC$...
 5.1 ± 0.3 ... $(7.4) \pm 24$...
 36 ... ($n=6$). ...
 $\Delta rpfBC$... ($n=6$).

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

(2%). ... *in vitro* ...
 -2.8 ... (27, 2%).
 1 ...
 $(250 \mu l, 50 \mu l, 300 \mu l, 1 \mu l)$...
 7.4 ... 2 $250 \mu l$...
 $25 \mu l, 100 \mu l, 2, 100 \mu l, 100 \mu l$... $(n=4)$ $2, 4, 7.2$ ($n=7$).
 $(n=1)$...
 1 ... 71.2% ...
 2 ... ($n=4$) ($n=7$).

in vitro ...
 $150 \mu l, 10 \mu l, 2, 2 \mu l$...
 0.1% ... $-100, 5 \mu l, 0.5 \mu l, 100 \mu l, 15 \mu l$... ($n=7.2$) (27, 2%).
 $(n=7)$.
 $150 \mu l, 100 \mu l, 2, 4$...
 $60 \mu l$...
 $(n=7)$.

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

$6.0, 7.0, 8.0$...
 $(n=6, 7, 8)$.
 $250 \mu l, 37, 15, 30, 60 \mu l$...
 $6, 7, 8$... ($n=8$).
 $10 \mu l$...
 $(n=6), (n=7), (n=8)$ ($n=8$).
 (K_m) ... $12.3 \mu l$...
 $(n=6), 6.5 \mu l$... $(n=7), 3.6 \mu l$... ($n=8$) ($n=8$).
 $6, 7, 8$... $100 \mu l$...
 $37, 30 \mu l$... ($n=6$), ...
in vitro ...

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

campestris ...
 (40) .
 1 ($1+$...) ... $(10 \mu l$...
 $100 \mu l)$...
 $(n=7)$.
 1 ...
 $1+$... 1 ... $\Delta rpfC$, ...

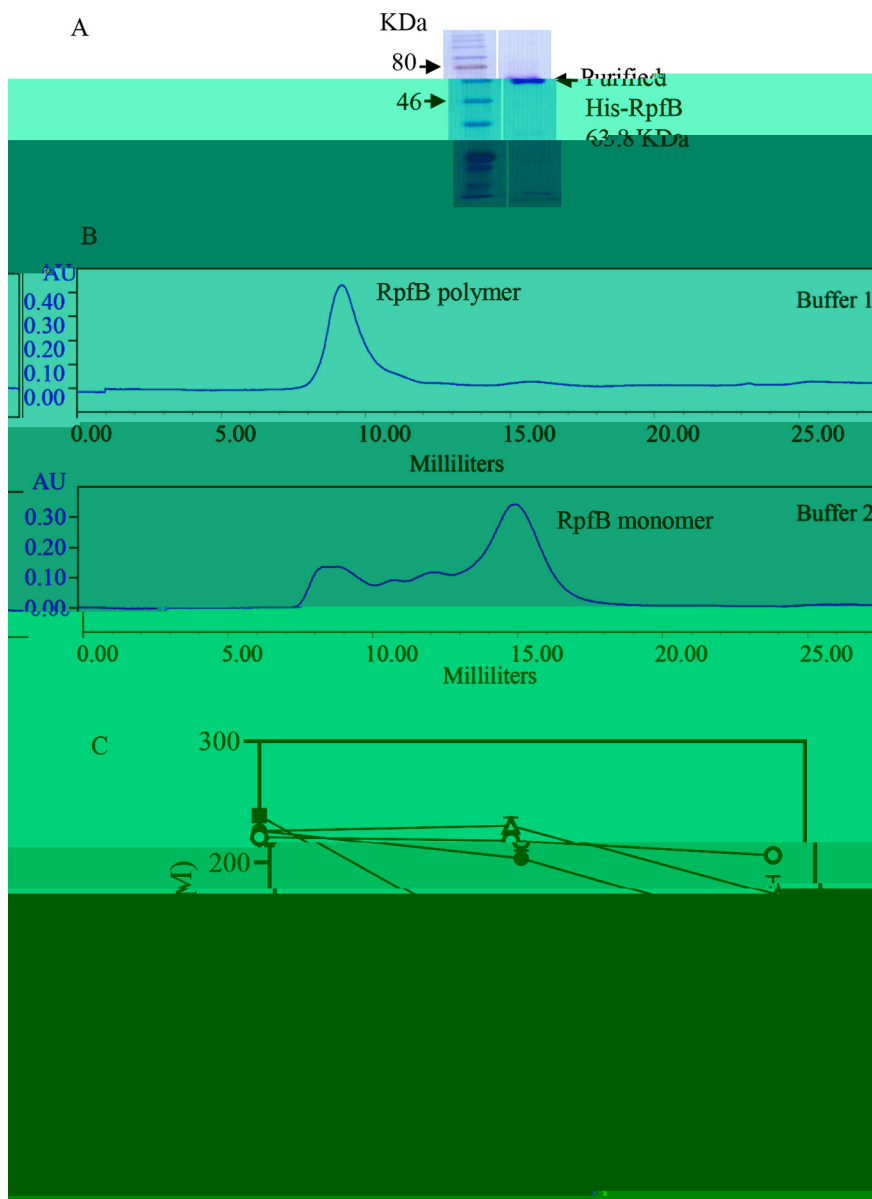


FIG 7 *In vitro* characterization of the purified His-RpfB. (A) Purified His-RpfB was analyzed by SDS-PAGE and Western blotting. The molecular weight markers (kDa) are indicated on the left. The purified His-RpfB is indicated by the arrow on the right. (B) Size exclusion chromatography (SEC) analysis of purified His-RpfB in Buffer 1 (top) and Buffer 2 (bottom). The x-axis represents the elution volume (Milliliters) and the y-axis represents the absorbance units (AU). The peaks are labeled RpfB polymer (top) and RpfB monomer (bottom). (C) Surface plasmon resonance (SPR) analysis of purified His-RpfB. The sensorgram shows the binding of RpfB to the sensor chip (filled circles) and the fit (open circles). The binding curve is labeled with A and B. The y-axis represents the response units (RU) and the x-axis represents the time (minutes).

The *in vitro* characterization of the purified His-RpfB was performed using SDS-PAGE and Western blotting. The molecular weight markers (kDa) are indicated on the left. The purified His-RpfB is indicated by the arrow on the right. The size exclusion chromatography (SEC) analysis of purified His-RpfB in Buffer 1 (top) and Buffer 2 (bottom) is shown. The x-axis represents the elution volume (Milliliters) and the y-axis represents the absorbance units (AU). The peaks are labeled RpfB polymer (top) and RpfB monomer (bottom). The surface plasmon resonance (SPR) analysis of purified His-RpfB is shown. The sensorgram shows the binding of RpfB to the sensor chip (filled circles) and the fit (open circles). The binding curve is labeled with A and B. The y-axis represents the response units (RU) and the x-axis represents the time (minutes).

DISCUSSION

The *in vitro* characterization of the purified His-RpfB was performed using SDS-PAGE and Western blotting. The molecular weight markers (kDa) are indicated on the left. The purified His-RpfB is indicated by the arrow on the right. The size exclusion chromatography (SEC) analysis of purified His-RpfB in Buffer 1 (top) and Buffer 2 (bottom) is shown. The x-axis represents the elution volume (Milliliters) and the y-axis represents the absorbance units (AU). The peaks are labeled RpfB polymer (top) and RpfB monomer (bottom). The surface plasmon resonance (SPR) analysis of purified His-RpfB is shown. The sensorgram shows the binding of RpfB to the sensor chip (filled circles) and the fit (open circles). The binding curve is labeled with A and B. The y-axis represents the response units (RU) and the x-axis represents the time (minutes).

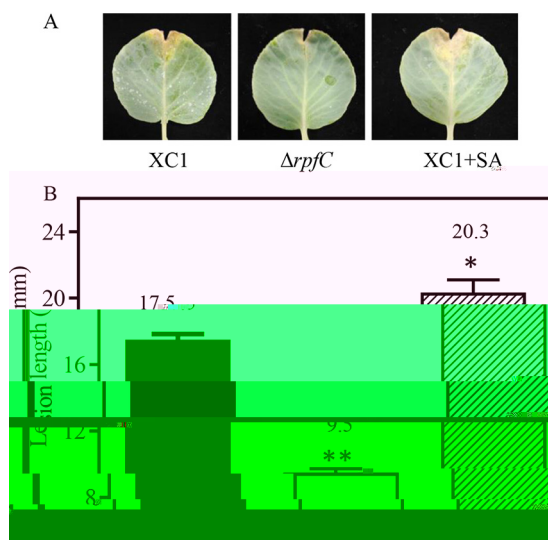


FIG 9 Phenotypic and growth characteristics of *Xanthomonas campestris* strains. (A) Representative leaf spots of *X. campestris* strains XC1, $\Delta rpfC$, and XC1+SA. (B) Lesion length (mm) of *X. campestris* strains XC1, $\Delta rpfC$, and XC1+SA. Error bars represent standard deviation. Significance markers: * ($P \leq 0.05$), ** ($P \leq 0.01$).

in planta, *X. campestris* strains XC1, $\Delta rpfC$, and XC1+SA were able to infect Arabidopsis leaves. The lesion length of *X. campestris* strains XC1, $\Delta rpfC$, and XC1+SA was significantly different ($P \leq 0.05$). The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.01$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.05$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.01$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.05$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.01$) from the other two strains.

Escherichia coli (45). *Xanthomonas campestris* strain XC1+SA was able to infect Arabidopsis leaves. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.05$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.01$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.05$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.01$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.05$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.01$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.05$) from the other two strains. The lesion length of *X. campestris* strain XC1+SA was significantly different ($P \leq 0.01$) from the other two strains.

campestris . *campestris* .

E. coli, .

(12).

(45).

(4).

(4%).

Xanthomonas campestris . *campestris* .

(52).

(7).

(4).

(1).

(1).

(1).

(1).

Xanthomonas campestris . *campestris* .

Xanthomonas campestris . *campestris* .

Xanthomonas .

Stenotrophomonas maltophilia .

Burkholderia .

Lysobacter, *Leptospirillum*, *Frateuria*, *Luteibacter*, *Rhodanobacter*, *Methylobacillus flagellates*, .

Thiobacillus denitrificans .

fi .

fi .

fi .

fi .

fi .

fi .

fi .

fi .

fi .

fi .

MATERIALS AND METHODS

Bacterial strains and culture conditions.

Xanthomonas campestris . *campestris* .

campestris .

campestris .

campestris .

campestris .

campestris .

campestris .

... 7 = 1. ... *Xanthomonas campestris* ... (53). ...

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

... (33). ... (33). ... 0.51 ... 4.0 ... 0.1% ... (54). ... 50 μ ... (34). ... 10 μ ... 0.1% ... (60/40) ... 0.4% ...

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

... *Xanthomonas campestris* ... (55). ... 20 ... 4.0 ... 20 ... 0.1 ... 5 μ ... 4.6 ... 150 ... 0.1% ... (50/20) ... 0.4% ...

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

... (21) ... 2 ... (2). ... 0.1 ... β- ... 16 ... 2 ... 25 ... 100 ... 100 ... 2⁶ ... 100 ... (2⁴)₂ ... 10 ... (7.2). ... 25 ... 100 ... 100 ... 2⁶ ... 100 ... (2⁴)₂ ... 25 ... 100 ... 100 ... 2⁶ ... 100 ... (2⁴)₂ ... 250 ... 7.2. ... 200 ... 25 ... 100 ... 100 ... 2⁶ ... 100 ... (2⁴)₂ ... 7.2).

In vitro

... (2). ... 100 ... 2 ... 4 ... 2 ... 10 ... 2 ... 2 ... 0.1% (/) ... -100, 5 ... 0.5 ... 0.3 ... 10 μ ... 500 μ ... 2 ... 15, 30, 45, 60 ... 4.0 ... 11 ... 2-

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

... gusA- ... rpfF ... rpfB ... (24). ... β- ... (34). ... 12 ... 36 ... 2 ... (11) ... 5,000 ... 17 ... 10 ... (1x, 7.4). ... 20 μ ... 0.1% (/) ... 40 μ

... 5,000 I 51 4 100 μ ... 250 μ ... (111 41 ... β - ... 501 I ,511 ... 111 ... 4.0). ... 37 151 ... (200 μ) ... 4.00 μ 0.21 ... 6- ... 365 I 455 I ...

Western blotting. ... 5% (V) ... 15,000 ... (24). ... (2011 ... 0.151 ... 0.1% / ... 20). ... 16,500 ... ()- ... 21001) ... 610 ...

Measurement of culture and cytoplasmic pH. ... 31 ... *Xanthomonas campestris* ... *campestris* ... 5,000 I 51 4 ... *Xanthomonas campestris* ... *campestris* ... *Xanthomonas campestris* ... (34). ... 5332 6) ... 674264) ... 11 -2 ... *Xanthomonas campestris* ... *campestris* ... 1.

... 444 510 I ... 547 ... 610 I ... 12 ... *Xanthomonas campestris* ... *campestris* ... 600 0.6 ... 6.0, 7.0, 7.5, ... 61 ... 250 μ ... *m*- ... 201 ...

Virulence assay of *Xanthomonas campestris* pv. *campestris* strains in cabbage. ... *Xanthomonas campestris* ... *campestris* ... ()-1) ... (34). ... Δ *prfC* ... 12 ... 600 0.1 ... 1 (1+ ... 100 μ ... 12 ... 5,000 I 51 4 ... 600 0.1 ... 600 0.1 ... 30 ... 4.0% ... 10,000 ... 2 ... 15 ...

Statistical analyses. ... () ... 5.0). ... *F* ... *P* ... <0.05.

SUPPLEMENTAL MATERIAL

- FIG S1, ... 1.21 ...
- FIG S2, ... 0.41 ...
- FIG S3, ... 1.61 ...
- FIG S4, ... 1.11 ...
- FIG S5, ... 0.61 ...
- FIG S6, ... 0.31 ...
- FIG S7, ... 1.41 ...
- TABLE S1, ... 0.021 ...

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... fi ... 31. 72231 ... 32172355 ...

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