RESEARCH ARTICLE

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* . *campestris* *Xanthomonas campestris* . *campestris* *Xanthomonas campestris* . *campestris* *Xanthomonas campestris* . *campestris* *rpfB* *rpfB* *rpfF* *rpfB*- . *In vitro* 6 *in vitro*. *Xanthomonas campestris* . *campestris* *Xanthomonas campestris* . *campestris*

IMPORTANCE *Xanthomonas campestris* . *campestris*

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KEYWORDS , , *Xanthomonas campestris*

P (1–3). ( ) (4). 3 (5). fi fi *vir* *Agrobacterium tumefaciens* (6–9). *repABC* ( ) *A. tumefaciens*, ( ). , fi *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- , *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* fi

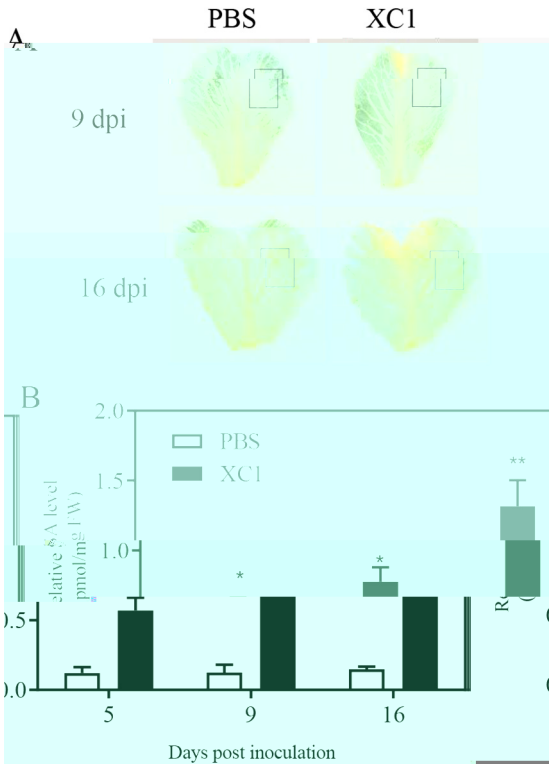

FIG 1 *Xanthomonas campestris* . *campestris* 1 *Brassica oleracea* . (1) 16 5, 16 1 (1×, 7.4), 5 (*, $P \leq 0.05$ **, $P \leq 0.01$).

Xanthomonas campestris . *campestris* *Xanthomonas campestris* . *campestris*.

RESULTS

***Xanthomonas campestris* pv. *campestris* infection promotes SA biosynthesis in cabbage.** 1 ( ) ( ) (1). ( ) (1). 0.5 / ( ) 5 , 0.74 / 1.31 / 16 . 4.6- .3- 5, 16 (1).

Exogenous addition of SA induces DSF and BDSF turnover. *Xanthomonas campestris* . *campestris* *Xanthomonas campestris* . *campestris* *Xanthomonas campestris* . *campestris* (21), *Xanthomonas campestris* . *campestris*

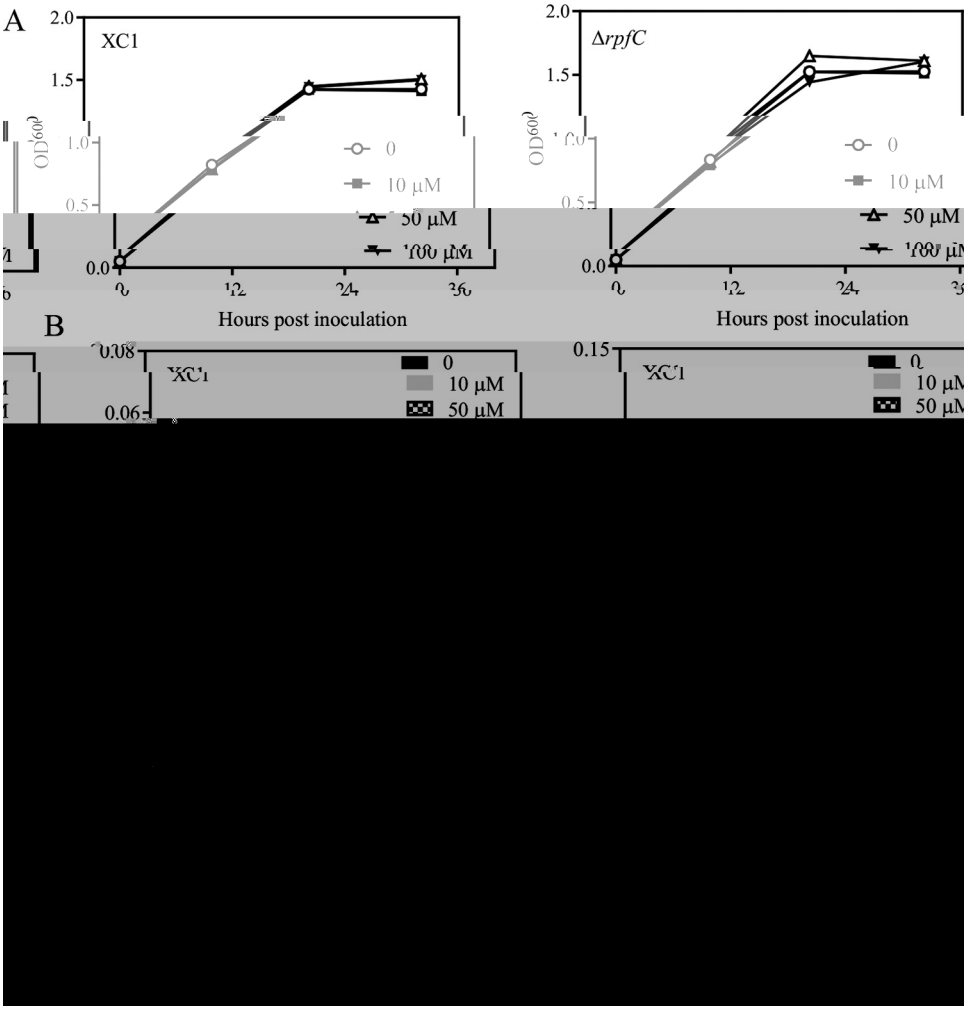

FIG 2 1 ΔrpfC. ( ) ΔrpfC 10 100 μl . ( ) 1 10 100 μl . ( ) ΔrpfC 10 100 μl . (*, $P \leq 0.05$ **, $P \leq 0.01$).

Xanthomonas campestris . *campestris* , 3- (3-) 4- (33, 34), *Xanthomonas campestris* . *campestris* *in vitro*. *Xanthomonas campestris* . *campestris* (. 1). 100 μl , 1, ΔrpfC / ΔrpfB (. 2), .

Xanthomonas campestris . *campestris* . 10 μl , 50 μl , 100 μl , 10 100 μl 1 (. 2). 10 μl 1 (. 2). , 50 100 μl 1 24 36 ( ) (. 2). 36 0.033, 0.020, 0.014 μl 10, 50 μl , 100 μl , 34.4%, 23.5%, 16.4%,

(0.05 μl) (. 2). 12
1 ( ).
Δ*rpfC*.
10 μl Δ*rpfC*
(. 2). 50 100 μl
Δ*rpfC* 12
24 36 (. 2).
Δ*rpfC* 50 100 μl 36
0.17 μl 0.05 μl , 12.1% 3.5%
(1.40 μl) Δ*rpfC* (. 2).

Endogenous production of SA induces DSF and BDSF turnover. *Xanthomonas campestris* . *campestris* 3- 4- (35, 36). *Xanthomonas campestris* . *campestris*, Δ*rpfC pchAB*, *pchAB* Δ*rpfC* (. 3). *pchAB* *Pseudomonas aeruginosa* (37). Δ*rpfC* Δ*rpfC pchAB* Δ*rpfC pchAB* 55.1 μl 12 , 5.5 μl 24 , 124.6 μl 36 , Δ*rpfC* (. 3). 12 Δ*rpfC pchAB* 24 36 Δ*rpfC* (. 3). Δ*rpfC pchAB* 36 0.05 μl , 1.4% 3.5 μl Δ*rpfC* (. 3). Δ*rpfC pchAB* 36 0.04 μl , 1. % 2.2 μl Δ*rpfC* (. 3). .

SA-induced DSF and BDSF turnover is dependent on rpfB. *rpfB* *Xanthomonas campestris* . *campestris* (2). *rpfB* , Δ*rpfB*, Δ*rpfB* *rpfB* *att* 7 (Δ*rpfB rpfB*), *rpfB* *rpfC* (Δ*rpfBC*), Δ*rpfBC* *rpfB* *att* 7 (Δ*rpfBC rpfB*). Δ*rpfB* Δ*rpfB rpfB* 100 μl . 100 μl Δ*rpfB* Δ*rpfB rpfB* (. 4). Δ*rpfB rpfB* 0.002 0.02 μl 36 , 22.2% 25% (0.00 0.0 μl ,) (. 4).
Δ*rpfBC* Δ*rpfBC rpfB* 100 μl . 100 μl Δ*rpfBC* (. 4). 100 μl Δ*rpfBC rpfB* (. 4). Δ*rpfBC rpfB* 0.23 0.15 μl 36 , (2.2 2.1 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*

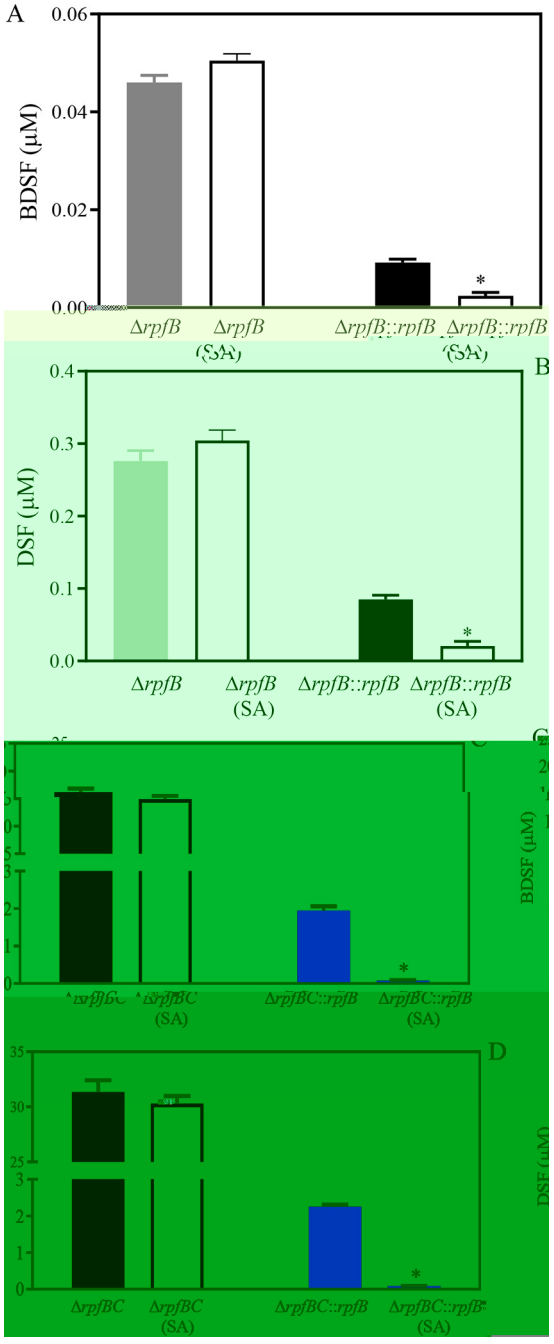

FIG 4 *rpfB* . ( ) ΔrpfB ΔrpfB *rpfB* (ΔrpfB *rpfB* 100 μl 24 . ( ) *rpfC* *rpfB* ΔrpfBC ΔrpfBC *rpfB* (ΔrpfBC *rpfB*) 0 50 μl 24 . (*, $P \leq 0.05$).

24 , 4.4 36 (. 5). ΔrpfC 1 (. 5). *Xanthomas campestris* . *campestris* . *Xanthomas campestris* . *campestris* 11 -2

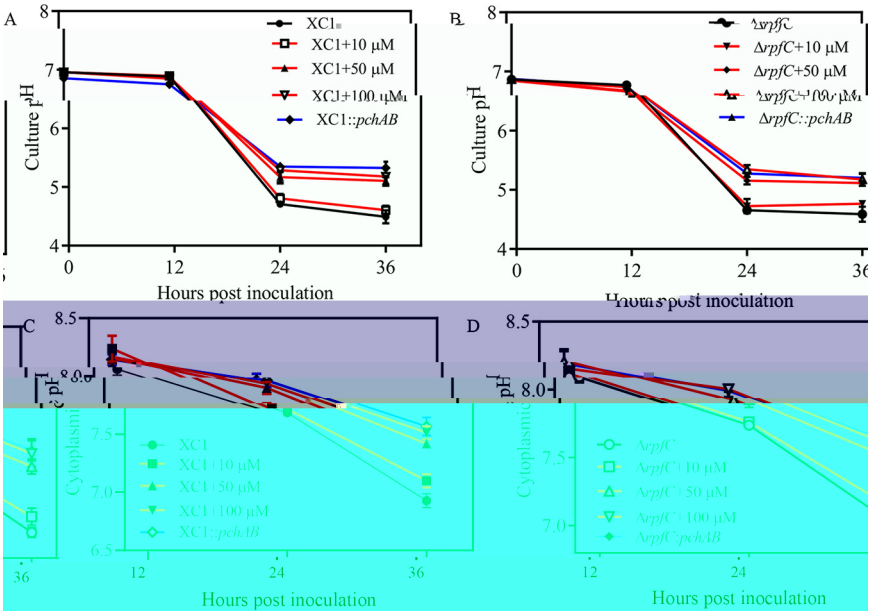

FIG 5 [illegible] 1 Δ*rpfC*. ( ) [illegible] 1, 1 [illegible] 10 100 μl , [illegible] 1 *pchAB*. ( ) [illegible] Δ*rpfC*, Δ*rpfC* [illegible] 10 100 μl , [illegible] Δ*rpfC pchAB*. ( ) [illegible] 1, 1 [illegible] 10 100 μl , [illegible] 1 *pchAB*. ( ) [illegible] Δ*rpfC*, Δ*rpfC* [illegible] 10 100 μl , [illegible] Δ*rpfC pchAB*. [illegible]

(3) (. 4). [illegible] (3), [illegible] (3). [illegible] 11 -2-[illegible] (/l) [illegible] (. 4). [illegible] 1 Δ*rpfC* [illegible] ~ .05 .07 12 ~6. 2 6. 36 (. 5).

Exogenous addition of SA or endogenous production of SA significantly prevents *Xanthomonas campestris* pv. *campestris* culture pH and cytoplasmic pH decrease. [illegible] 1 Δ*rpfC* [illegible] 10, 50, 100 μl 10 μl [illegible] 1 Δ*rpfC* (. 5). 50 100 μl [illegible] 12 , [illegible] 24 36 (. 5). [illegible] 36 [illegible] 24 (. 5).

[illegible] Δ*rpfC pchAB* 1 *pchAB* 12 [illegible] Δ*rpfC* 1 (. 5). [illegible] Δ*rpfC pchAB* 1 *pchAB* 24 36 [illegible] Δ*rpfC* 1 (. 5).

[illegible] 7.0 (, -7.0) [illegible] Δ*rpfC* -7.0 [illegible] 7.0 [illegible] (. 5). 10 μl 100 μl [illegible] Δ*rpfC* [illegible] Δ*rpfC* (. 5). [illegible]

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

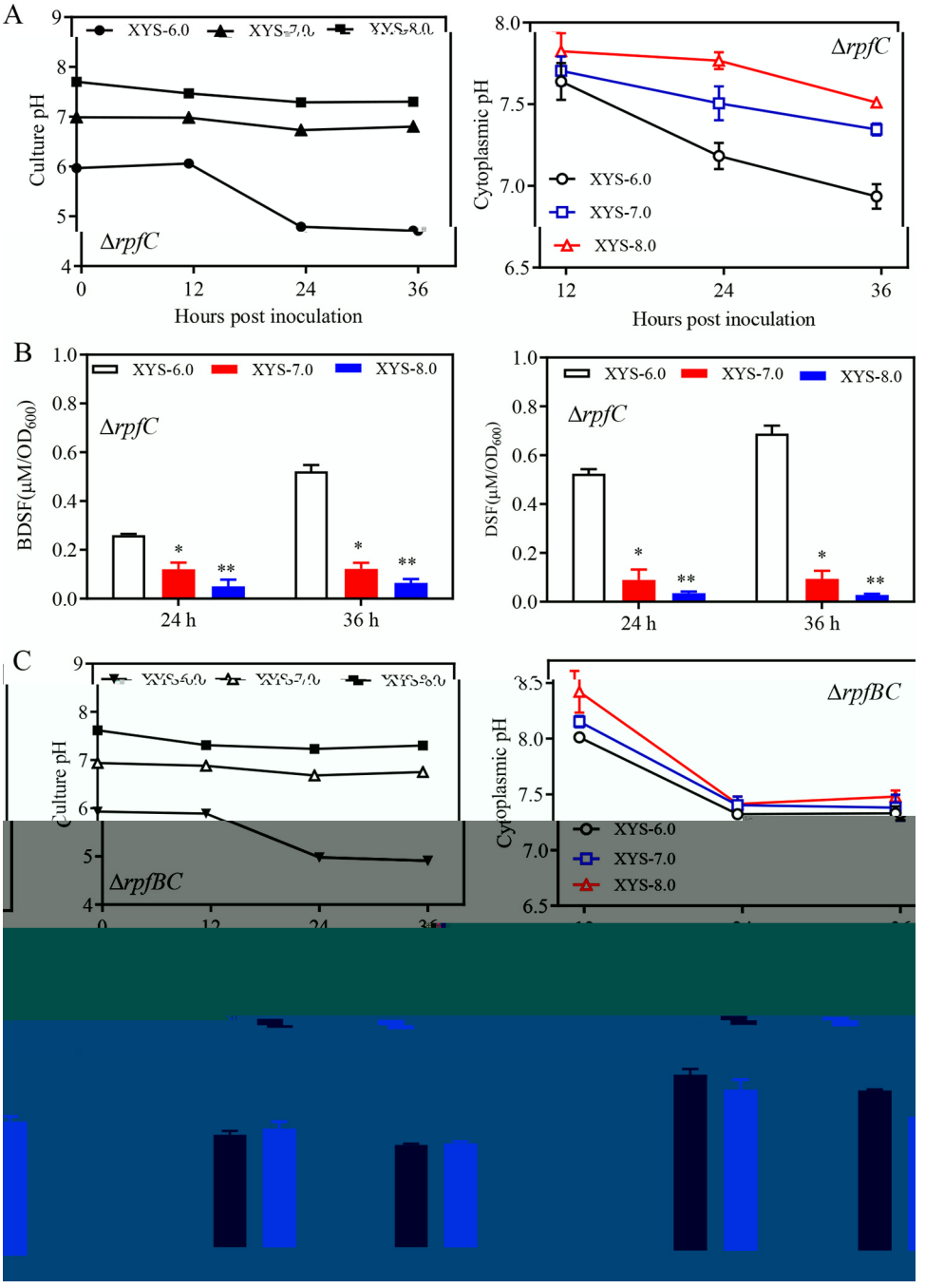

FIG 6 *Xanthomonas campestris* . *campestris* . ( ) ΔrpfC 6.0 -6.0, -7.0, -7.0. ( ) ΔrpfC 24 36 . ( ) *rpfC* *rpfB* ΔrpfBC -6.0, -7.0, -7.0. ( ) ΔrpfBC 24 36 (*, $P \leq 0.05$ **, $P \leq 0.01$).

-6.0 (6.0), -7.0 (7.0), -7.0 (7.0), . ΔrpfC ΔrpfC -7.0 -7.0 6.06 12 4.7 24 -6.0, 4.71 36 (. 6). ΔrpfC (. 6).

Δ*rpfC* 6.0 8.0 (6).

rpfB *rpfC* Δ*rpfBC* Δ*rpfBC* -7.0 -8.0, 5 12 (4) 24 36 -6.0 (6). Δ*rpfBC* 8.1 8.3 12 (7.4) 24 36 (6). Δ*rpfBC* (6).

Establishment of an *in vitro* RpfB-dependent DSF turnover system. (28). *in vitro* (27, 28). -28 ( ) 1 (250 , 50 , 300 , 1 , 7.4) 2 250 , 25 , 100 , 100 ( )2 7.2 (7). 1 (-), 71.2% 2 (+) (7).

in vitro 150 , 10 , 2 , 0.1% -100, 5 , 0.5 , 100 μ , 15 μ (7.2) (27, 28). (7). 150 100 2 4 60 (7).

***In vitro* RpfB-dependent DSF turnover activity increases with pH and is independent of SA.** 6.0, 7.0, 8.0 (6, 7, 8,). 250 , 37 15, 30, 60 , 6, 7, 8 (8). 10 μ , (6), (7), (8) (8). (K_m) 12.3 μ (6), 6.5 μ (7), 3.6 μ (8) (8).

6, 7, 8 100 μ . 37 30 , fi (6), *in vitro* .

SA-treated XC1 exhibits increased virulence in cabbage. *Xanthomonas campestris* . *campestris* (40). 1 (1+) (10 μ 100 μ) ( ) (7).

1 1+ , 1 Δ*rpfC*,

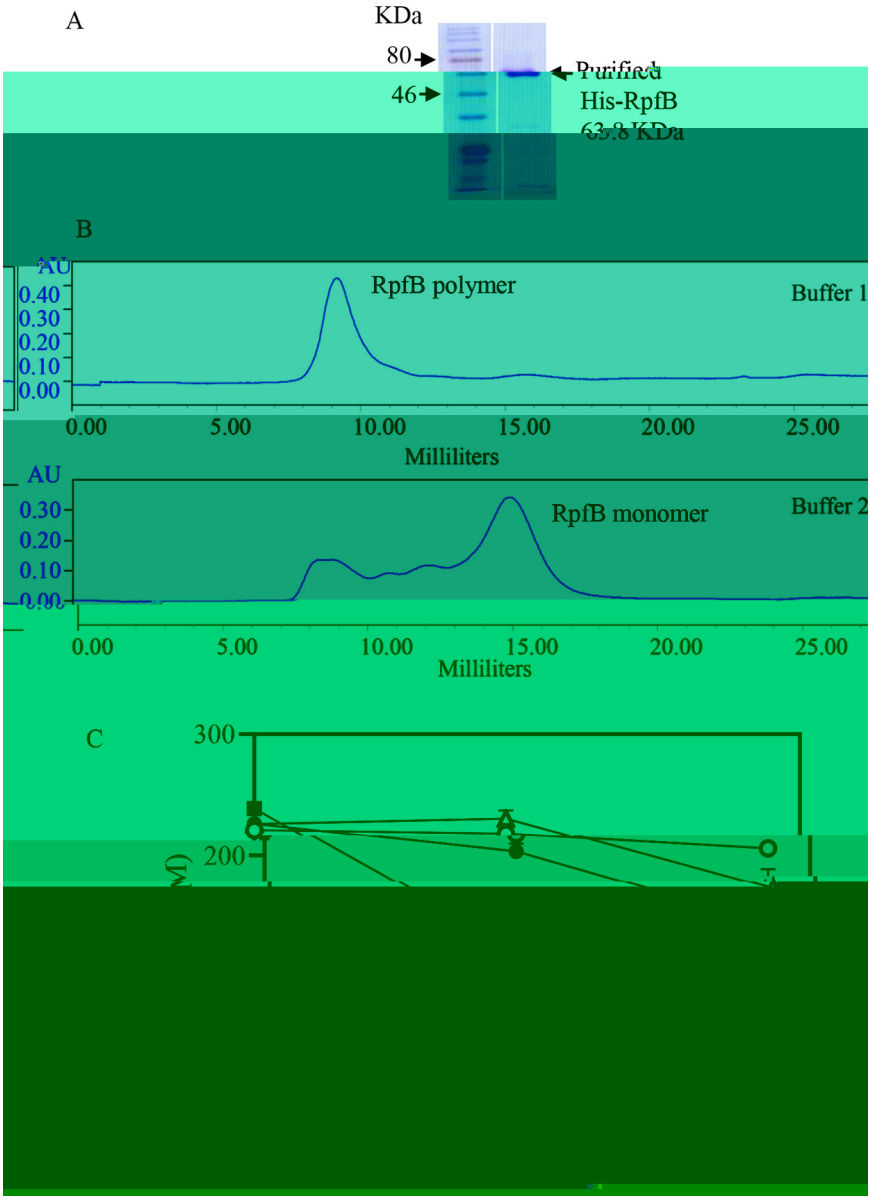

FIG 7 *In vitro* - . ( ) - fi -, . ( ) _ fi ( ) _ (-) 1 _ 2. - 1 250 , 50 , 300 1 (7.4), 2 250 , 25 , 100 , 100 100 ( )2 4 (7.2). ( ) *In vitro* - .

(-1) - 2 *ΔrpfC*, 1 ( ). 1+ 16% 1 ( ), *Xanthomonas campestris* . *campestris* - .

DISCUSSION

" "

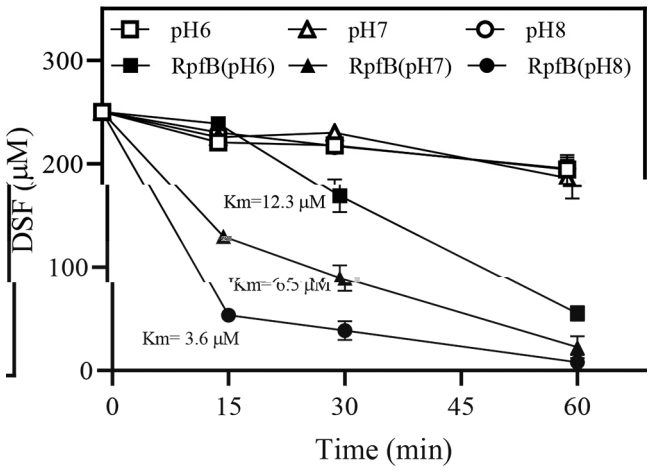

FIG 8 *in vitro* 6, 7, (6, 7,) 10 μ (6, 7,). (K_m)

(.,
) (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. *expI* *expR*
P. carotovorum 1 2.5- 10.0-
24
(11). *A. tumefaciens* 5 , (6 μ
6) *attKLM*
, *N*-3-
(3 -) *A. tumefaciens* (6).
24 36 (. 2 3).
10- 100-μ *rpfF*
rpfB,
Xanthomonas campestris . *campestris* (21, 2) (. 3).
Xanthomonas campestris . *campestris*
P. carotovorum, *P. aroidearum*, *A. tumefaciens* (6, 11),

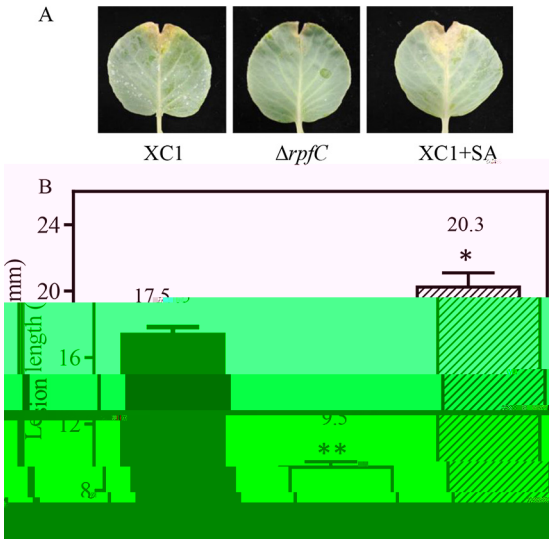

FIG 9 *Xanthomonas campestris* . *campestris* -1). ( ) 14 . ( ) 1, Δ*rpfC*, 1 (1+) 14 -1). 15 fi (*, $P \leq 0.05$ **, $P \leq 0.01$).

fi 24 36 (. 2 . 5) ( ). 1 Δ*rpfC* 24 36 (. 5). 1 Δ*rpfC* (. 5). *attKLM* 3 *A. tumefaciens* (6). *Escherichia coli* (45). *Xanthomonas campestris* . *campestris* , *β*- *Xanthomonas campestris* . *campestris* ( ). *rpfB* fi *Xanthomonas* (21, 2). *in vitro* ( ) *in planta* *Xanthomonas campestris* . *campestris* fi , *Xanthomonas campestris* . *campestris* (46). , 5 $^{-1}$, 0.0625% *Xanthomonas campestris* . *campestris* (21, 47). (48, 4). *Xanthomonas campestris* . *campestris* (.). , *Xanthomonas campestris* . *campestris* .

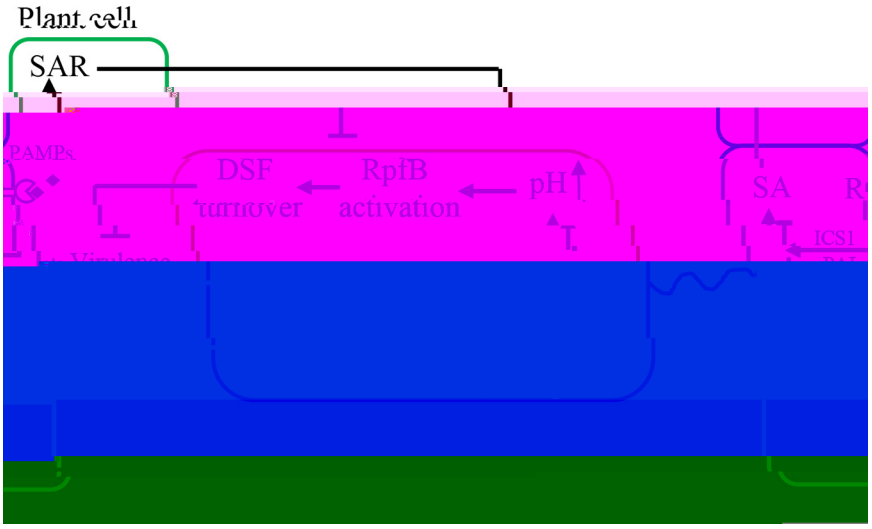

FIG 10 *Xanthomonas* *Xanthomonas campestris* . *campestris*- ( ) ( ) 1 (1)- ( ) *Xanthomonas campestris* . *campestris* *Xanthomonas campestris* . *campestris* ( ) *Xanthomonas campestris* . *campestris*, fi *Xanthomonas* .

fi 24 36 (. 5). fi (. 6). *in vitro* (. 7). 6 fi *in vitro* (.). (. 6), fi (. 10).

Xanthomonas campestris . *campestris* *Xanthomonas campestris* . *campestris* *Salmonella enterica* 25 50 (50). (51). *Xanthomonas campestris* . *campestris*. *Xanthomonas*

campestris . *campestris*

. *E. coli*,

(45).

(4).

(48).

Xanthomonas campestris . *campestris*

1 (52).

1 (. 7).

1

1

Xanthomonas campestris . *campestris*

Xanthomonas campestris . *campestris*.

Xanthomonas

Stenotrophomonas maltophilia *Burkholderia* ., *Lysobacter*, *Leptospirillum*, *Frateuria*, *Luteibacter*, *Rhodanobacter*, *Methylobacillus flagellates*, *Thiobacillus denitrificans*

MATERIALS AND METHODS

Bacterial strains and culture conditions. 1 . *Xanthomonas campestris* . *campestris*

1 28 (0.7 , 0.2 , 1 ( )2 4, 0.1 2 6 , 0.01 , 0.001 2 4 , 5 , 0.0625% 7.0), (5 , 3 , 20), ( ) (5 , 10 , 10), ( ) (5 , 3 , 10 , 1 , 7.0). , , (,). *E. coli* 5α . *E. coli* 37 . 25 μ , 50 μ , ( ), 20 μ , ( ), 100 μ , ( ). 600 (600).

Gene deletion and functional complementation analysis in *Xanthomonas campestris* pv. *campestris*. (23). fl , fl (~500) 1 *Xanthomonas campestris* . *campestris* 50 μ , 5% (/)

Xanthomonas campestris pv. *campestris* (53).

Extraction and quantitative analyses of SA in *Xanthomonas campestris* pv. *campestris* cultures and in plant leaf tissues. (33). (33). , 0.5 4.0 , 0.1 5 μl, 4.6 150). 0.05% (25 75 /) 1 $^{-1}$. ( ) 25 75% 16 (8,000) 2 *Xanthomonas campestris* *campestris* $_{600}$ 0.1 (1×, 7.4) 0.0% 16 (8,000). (200) 5, 16 (54). 50 μ ( ) (34). 10 μ (4.6 150 , 5 μl) 0.1% (60/40 /) 40 0.4 $^{-1}$. ( ) ( ) ( ) (55). , 20 4.0 20 30 0.1 5 μl, 4.6 150) ( ). 0.1% (80/20 /) 0.4 $^{-1}$.

Extraction, purification, and quantitative analysis of BDSF and DSF using UPLC-TOF MS. *Xanthomonas campestris* *campestris* (55). , 20 4.0 20 30 . 0.1 5 μl, 4.6 150) ( ). 0.1% (80/20 /) 0.4 $^{-1}$.

RpfB expression, purification, and *in vitro* DSF turnover activity assays. *E. coli* 21(3) (2). 0.1 -β- ( ) 1 16 . 1 2 25 , 100 , 100 , 6 , 100 ($_4)_2$, 10 (7.2). ( ). 25 , 100 , 100 , 6 , 100 ($_4)_2$, 25 (7.2). 25 , 100 , 100 , 6 , 100 ($_4)_2$, 250 , 7.2 . 200 ( ) 25 , 100 , 100 , 6 , 100 ($_4)_2$ (7.2).

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

Construction of *gusA*-dependent reporter strains to monitor *rpfF* and *rpfB* transcriptional activities and GUS assays. *gusA*- *rpfF* *rpfB* (2). β- ( ) (34). , 12 36 2 . (1) 8,000 ( ) 17) 10 , (1×, 7.4). 1 20 μ 0.1% (/) 40 μ

Western blotting. [illegible] (20 [illegible], 0.15 [illegible] 0.1% / 20). [illegible] 21001) [illegible] 610 [illegible].

Measurement of culture and cytoplasmic pH. [illegible] *Xanthomonas campestris* pv. *campestris* [illegible] *Xanthomonas campestris* pv. *campestris* [illegible] *Xanthomonas campestris* pv. *campestris* [illegible] *Xanthomonas campestris* pv. *campestris* [illegible] 1.

[illegible] 610 [illegible]). [illegible] *Xanthomonas campestris* pv. *campestris* [illegible] 0.6 [illegible] 6.0, 7.0, 7.5, [illegible] 250 μl [illegible] 20 [illegible].

Virulence assay of *Xanthomonas campestris* pv. *campestris* strains in cabbage. *Xanthomonas campestris* pv. *campestris* [illegible] Δ*rpfC* [illegible] 12 [illegible] 100 μl 12 [illegible] 30 [illegible] 10,000 [illegible] 15 [illegible].

Statistical analyses. [illegible] 5.0). [illegible] *F* [illegible] *P* [illegible] <0.05.

SUPPLEMENTAL MATERIAL

[illegible]

FIG S1, [illegible]

FIG S2, [illegible]

FIG S3, [illegible]

FIG S4, [illegible]

FIG S5, [illegible]

FIG S6, [illegible]

FIG S7, [illegible]

TABLE S1, [illegible]

ACKNOWLEDGMENTS

fi _ _ (. 31 72231 _ 32172355 .- . .).

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