RESEARCH ARTICLE

WILEY

The phytopathogen utilizes the divergently transcribed / locus for 4-hydroxybenzoic acid recognition and degradation to promote virulence

Bo Chen[1] Rui-Fang Li[2] Lian Zhou[3] Jia-Hui Qiu[1] Kai Song[1] Ji-Liang Tang[4] Ya-Wen He[1]

5 XºJM-ɟ´ª 0- «º .«ºX³«Jº -«Jɟ >X´XJ³N|

-ºXN|«-ɟ-zÉ ?|J«z|J 0J- A-«z

$-ɟXzX ?|J«z|J 0J- A-«z C« ÆX³´ ºÉ

Correspondence

5 N³-M Jɟ 5 XºJM-ɟ´ª 0- «º .«ºX³«Jº -«Jɟ

?N X«NX´ J«T # -ºXN|«-ɟ-zÉ ?|J«z|J 0J-

Funding information

Abstract

Xanthomonas campestris *campestris* *Xcc*

Xcc

β

Xcc

Xcc *pobA* *pobR*

pobA

Xcc *pobA*

ZÄɟÉ -ÆX³ɟJ°° «z ÐzÏð XɟXª X«º´ ÇX³X TX«º Z XT MXºÇXX« º|X T ÆX³zX«ºɟÉ º³J«´N³ MXT *pobA* *pobR*

Ç|N| X«N-ª °J´´X´ º|X ÐzÏð XɟXª X«º´ ´|J³XT MÉ º|X -ÆX³ɟJ°° «z °3-ª -ºX³´ÿ * «JɟɟÉ

pobA *pobR* *Xcc*

Δ*pobR*

Xcc

KEYWORDS

pobA *pobR* *Xanthomonas campestris*

1 INTRODUCTION

et al *et al* *Xcc*

Xanthomonas campestris *campestris* *Xcc* *et al*

et al

Xcc

et al

Xcc

p *et al*
p
et al ăŭŭЛ 0Ä«z
et al

Brassica

et al
Xcc

et al

et al

ortho *β* *meta*
et al
β

Azotobacter chroococcum *Pseudomonas* *Rhizobium*
leguminosarum *Acinetobacter calcoaceticus* *Cupriavidus necator*
et al *et al*

pobA
Xcc

β *et al*

FIGURE 2 *pobR/pobA*

pobR pobA

Xcc

pobA

pobR ℓ$-ɣ-Ä³ Z zÄ³X NJ« MX Æ XÇXT Jº

2.2 The pobA/pobR locus is essential for 4-HBA degradation in XC1

pobA *pobR*

pca *pobA*

pobR

pobA *pobR*

Δ*pobA* Δ*pobR*

pobA *pobR* Δ*pobRA*

pobA Δ*pobRA*

pobR Δ*pobRA*

Δ*pobA* Δ*pobR* Δ*pobRA*

pobA

pobR *pobA*

Δ*pobRA*

pobR

pobR *pobR*

2.3 Defining the overlapping promoters of pobA and pobR

Xcc

pobA *pobR*

N´X⅋É -³zJ« ÊXT⅋ A- º| ´ X«T⅋ ðِ¢> $(ÇJ´ X ª °⅋-ÉXT º- TX«º ZÉ º|X
ðِ¢X«T´X²ÄX«NX -Z XJN| º³J«´N³ °º Ä´ «z ª >6 °³X°J³XT Z³- ª NX⅋´

pobA º³J«´N³ °º ÇJ´ TX«º Z XT Jº «ÄN⅋X-º TX ÐzðЬ ³X⅋Jº ÆX º- º|X
« º Jº -« N-T-« A+ ∃* zÄ³X ǏT∂⅋ J«T J °ÄºJº ÆX ÐzǐðX⅋ª X«º J«T
ÐžĂĂ X⅋X ª X«º´X²ÄX«NX ∃A$+A$+¢6

E coli σ
∃* zÄ³X ǏT∂⅋ A|X ³-⅋X -Z º|X °³XT NºXT ÐzǐðX⅋ª X«º -Z ;

ðِ¢> $(J«J⅋É´´ TX«º Z XT J º³J«´N³ °º´ºJ³º´ ºX ∃+

et al

pobR

FIGURE 3 *pobA*

gusA

µ

gusA

Δ*pobR*

p á ÏĂõÎ ºÇ - J´ºX³ ´ ¦´ẅ *p* á ÏĂäÎ º| ³XX
p á ÏĂĂäϑ £$ -ǰ- Ä³ Z zÄ³X NJ« MX

Δ*pobR* *gusA* *pobA*

pobA

Δ*pobR* Δ*pobRA* Δ*pobRA pobR*

pobA

2.5 pobR is transcribed in the absence of 4-HBA, the presence of 4-HBA advances its expression

pobR *gusA*

gusA

gusA

gusA

gusA

FIGURE 4 *pobR*

gusA

µ

α

´ z«Jß «ºX«´ ºÉ J´ ª XJ´Ä³XT Ä´ «z . ª JzX0

p å ÿÄðϑ ℓ$ -ß - Ä³

μM

μM

μM

μ μ μ μ

± ±

±

±

pobR *Xcc*

Xanthomonas

°³-ºXNºXT ´ ºX ZÄ⁗É N-ÆX³´ º|X -ÆX³⁗J°° «z ĎzĭŏX⁗Xª X«º´ -Z M-º|

2.8 PobR specifically binds to a 25-bp site within the overlapping promoters

pobA *pobR*

TABLE 1

Mutation	Dimerization	4-HBA binding (Kd) (μM)	4-HBA degradation ratio (%) at 18 hpi
	±	±	
	±		±
	±	±	
	±		
	±	±	±
	±		
	±	±	±
			±

Note: *p* å ÄŸÄðª J³X «T NJºXT MÉ T ZZX³X«º ⁗XººX³ J -³ MŸ

Xcc

Xcc
pobA *pobR*
gusA *gusA*

2.9 Both pobR and pobA are transcribed during XC1 infection inside Chinese radish

pobR
pobA *Xcc*
Xcc in planta

2.10 pobR is required for full pathogenicity of XC1 in Chinese radish and cabbage

3 DISCUSSION

Xcc

Xcc

pobA *pobR*

pobA *pobR*

N- ª °⅋XºX⅋É -ÆX³⅋J°° «z Dzĭŏ X⅋X ª X«º´ « º|X ³ °³- ª -ºX³ ³Xz -«´Ÿ

FIGURE 8 *pobR* *pobA*

gusA

Xcc *gusA*

pobR *pobA*

Xcc

Xcc

3.1 Biochemical basis for 4-HBA recognition and PobR dimerization

Streptomyces

et al

Acinetobacter

et al

S coelicolor

et al

Acinetobacter

S coelicolor *Xcc*

Acinetobacter *S coelicolor*

pobA

S coelicolor *et al*

et al

Xcc

ǯ* zÄ³X ?ŮJ⧈T∂ÿ A|X TXNJªX³ N-«Z-³ªJº -« ´ Ä«N-ª ª-« J«T ´

Xcc

Streptomyces

et al

Xcc

FIGURE 9

4 EXPERIMENTAL PROCEDURES

4.1 Bacterial strains and culture conditions

4.2 Gene deletion and functional complementation analysis

4.3 Point mutagenesis of target gene in plasmid DNA and in Xcc chromosome

4.9 Electrophoretic mobility shift assay (EMSA) and DNase I footprinting sequencing assay

et al

º|X ´X²ÄX«NX Ƶ³- ª ǄǴĬĂŬ º- ĬƄ M° « º|X -ÆX³Ƅ°° «z ³Xz -« Ä°

pobA

ǯðą +$$ +A++$+ A + Ĭą̊ Jºº|X ðą X«TŸA|X °³-MX´ ÇX³X´ÄM

µ Dză Dză

×

×

ǯ*Ä¤ * Ƅª ŵ 0J°J«ð

et al

4.10 Isothermal titration calorimetry analysis

=

µ

µ

4.11 We l

4.14 Virulence assay in Chinese radish and cabbage

Xcc *Raphanus sativus*

4.15 Statistical analysis

$p =$

$p <$

ACKNOWLEDGMENT

CONFLICT OF INTEREST

AUTHOR CONTRIBUTIONS

ORCID

Ya-Wen He

REFERENCES

p

pobA

Pseudomonas putida *Microbiology* *147*

Nature Reviews Microbiology *2*

Nature Reviews Microbiology *14*

Xanthomonas

FEMS Microbiology Reviews *34*

Pseudomonas aeruginosa *Nature Protocols* *1*

p

Acinetobacter calcoaceticus *Journal of Bacteriology* *176*

?Ä‼ , Ÿ I Ÿ‼ F Ä‼ 2Ÿ‼ = ‼ HŸ , Ÿ‼ 2 Ä‼ +Ÿ*Ÿ‼ 2Ä‼ +ŸÄŸ J«T AJ«z‼ 0Ÿ2Ÿ ƷŤĂğ

Xanthomonas campestris *campestris*

Scientific Reports 6

?Ä«‼ ?Ÿ‼ $ | X«‼ #Ÿ‼ 0 «‼ I Ÿ0Ÿ‼ I | - Ä‼ 2Ÿ‼ *J«z‼ HŸ2Ÿ‼ A | JÇJ ‼ $Ÿ‼ *et al*

Pseudomonas aeruginosa *Molecular*

Microbiology 104

AJ«‼ 0Ÿ F Ÿ‼ #XT«J³X¦‼ ;Ÿ‼ 2 Ä‼ 0Ÿ1Ÿ‼ ?N | «X TX³‼ #Ÿ‼ ?ÆJº - ´‼ Ÿ J«T , J | ĵM³ - N¦‼

Arabidopsis thali-