

# The phytopathogen *Xanthomonas campestris* utilizes the divergently transcribed *pobA* / *pobR* locus for 4-hydroxybenzoic acid recognition and degradation to promote virulence

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## Funding information

### Abstract

*Xanthomonas campestris* *campestris* Xcc

Xcc

$\beta$

Xcc

Xcc

*pobA* *pobR*

*pobA*

Xcc

*pobA*

N¥, CL^ >...s-n~ } L L} L~"%L~\$HL~"\$LHAL"ELL~"pL^HCL^nl~" ¥"~>~%B%ALH

*pobA* *pobR*

£ pSp L-B, } ..>%L%oPL~ } L L} L~"%L~>LHAY~"pL~, CL^ >...s-n~... } , "L.%f s-> ¥~

*pobA* *pobR* Xcc

$\Delta$ *pobR*

Xcc

### KEYWORDS

*pobA* *pobR* *Xanthomonas campestris*

## 1 INTRODUCTION

*Xanthomonas campestris* *campestris*

Xcc et al

et al Xcc

et al

Xcc

et al

Xcc

p  
et al  
p  
et al  
et al  
et al  
et al

Brassica

Xcc  
et al  
et al  
et al  
ortho  
 $\beta$   
 $\beta$   
meta  
et al

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

pobA

Xcc  
 $\beta$   
et al

FIGURE 2 *pobR/pobA*

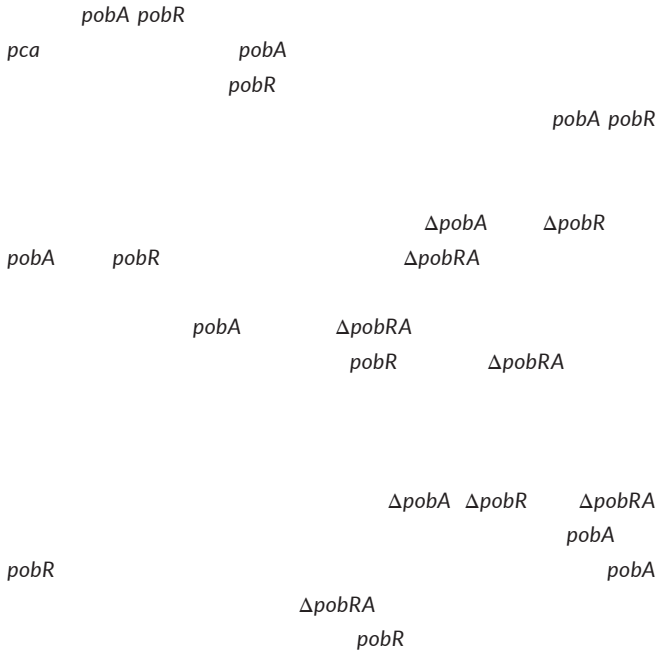
*pobR pobA*

Xcc

*pobR* , , Ÿ`TnŸ`L`B>~`AL`c`L`LH>~`  
*pobA*

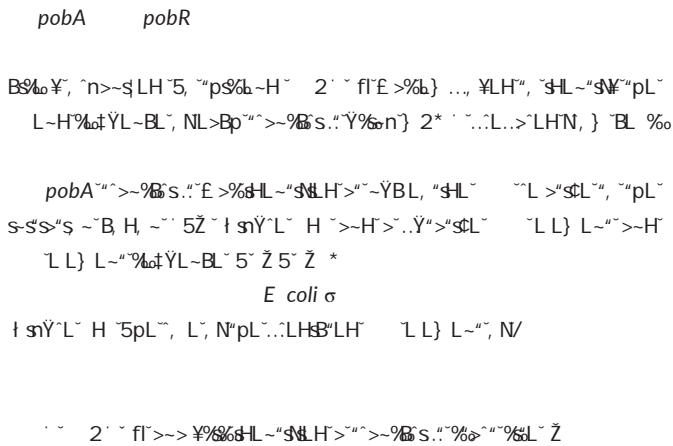
## 2.2 The *pobA/pobR* locus is essential for 4-HBA degradation in XC1

*pobR*    *pobR*



## 2.3 Defining the overlapping promoters of *pobA* and *pobR*

Xcc



*et al*  
*pobR*

FIGURE 3 *pobA*



$\Delta pobR$     *gusA*    *pobA*

$\Delta pobR$   $\Delta pobRA$      $\Delta pobRA$  *pobR*    *pobA*

*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*

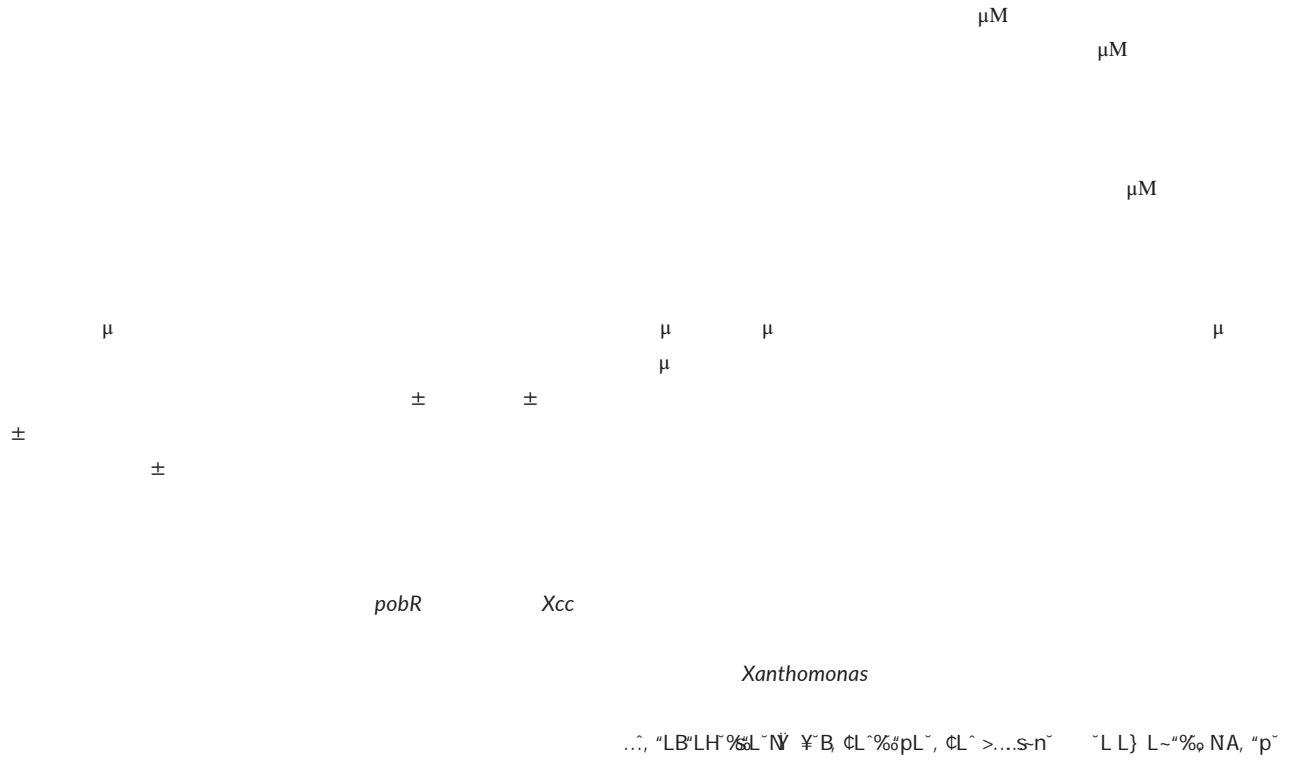
$\mu$

$\alpha$

$\frac{d\mu}{dt} = \alpha - \mu$

$\mu = \alpha$





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

*pobA*      *pobR*

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

TABLE 1

Note:  $p_{\text{pobR}}$  is the promoter of the *pobR* gene. The 4-HBA binding site is located within the overlapping promoter region of the *pobR* gene.



*Xcc*

*Xcc*

*pobA*

*pobR*

*gusA*

*gusA*

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

*pobA*

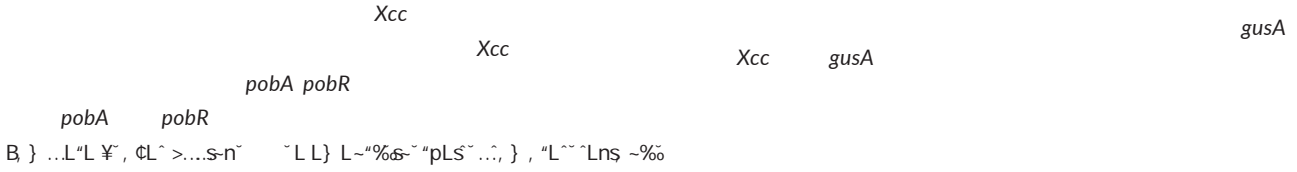
*Xcc*  
*Xcc in planta*

*pobR*

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

3 DISCUSSION

FIGURE 8 *pobR pobA*



3.1 Biochemical basis for 4-HBA recognition and *PobR* dimerization

*Streptomyces*  
*et al*

*Acinetobacter*

*et al*  
*S. coelicolor*

*et al*

*S. coelicolor*      *Acinetobacter*  
Xcc

*Acinetobacter*      *S. coelicolor*  
*pobA*

*S. coelicolor*      *et al*

*et al*

Xcc  
... f nY^L^3 > H ^5pL^HLB>} L^B -N ^} >^s ~\$%Y-B, } } , ~>-H^S%o

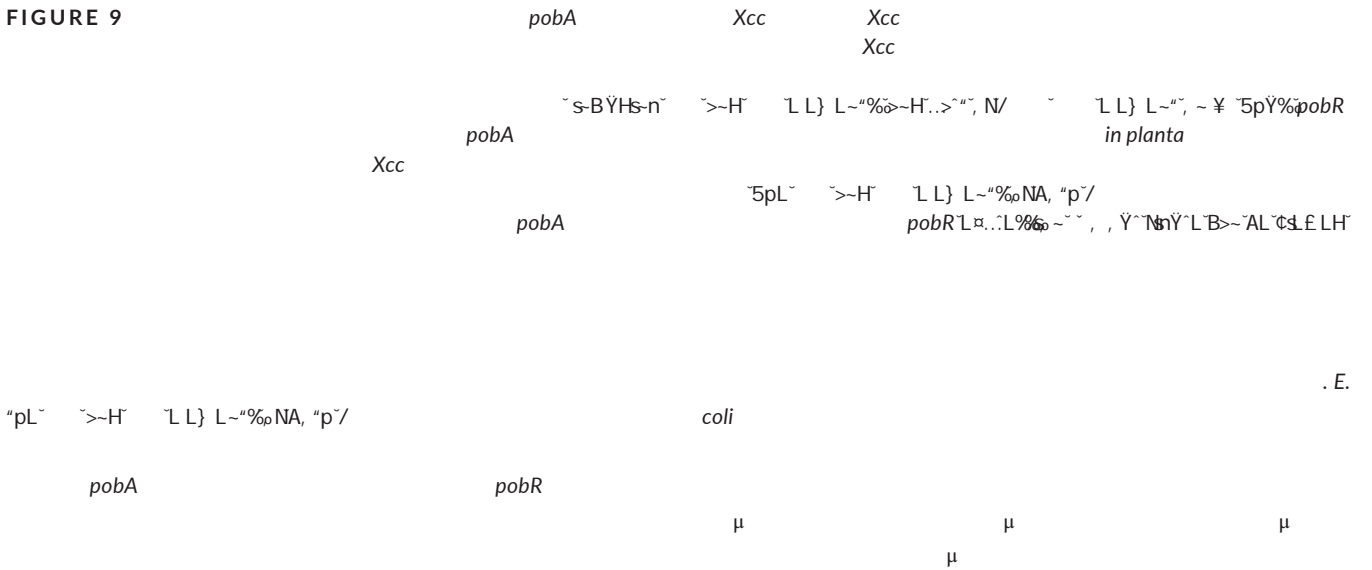
Xcc

*Streptomyces*

*et al*

Xcc

FIGURE 9



#### 4 EXPERIMENTAL PROCEDURES

##### 4.1 Bacterial strains and culture conditions

##### 4.2 Gene deletion and functional complementation analysis

*Xcc*

*Xcc*

*et al*

### 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*

"pL%...fYL~BL~N, } ~ ~", ~ ~ "A...s~"pL~, cL^ >...s-n~"Lns ~~Y...  
*pobA*

~ Ž ~ ~ ~ Ž 5Ž Ž ~ Ž ~ 5 ~ ~ Ž ~ ~ >"pL~ ~L~H 5pL... AL%& L~L~%A

μ

×

×

f Yv s} ~\$>..>~

*et al*

### 4.10 Isothermal titration calorimetry analysis

=

μ

μ

### 4.11 We

I

#### 4.14 Virulence assay in Chinese radish and cabbage

Xcc *Raphanus sativus*  
 ) >-n%PL-p, -n ~, ~B>AA>nL~ \$s-nN~n ~É >%L%LH'AY~"pL~ L>N

x

#### 4.15 Statistical analysis

Lx.L^\$ L~">~H>~%#%É >%o.L^N ^} LH^Y%o.n~\$) /~%oNÉ >^L~CL^%o~"

$$p =$$


$$p <$$

#### ACKNOWLEDGMENT

#### CONFLICT OF INTEREST

#### AUTHOR CONTRIBUTIONS

#### ORCID

Ya-Wen He 

#### REFERENCES

*Pseudomonas putida* *pobA* *Microbiology* 147  
 & B>~>-H'n, A>~LnY >"s ~, N  
*Nature Reviews Microbiology* 2

*Xanthomonas*  
*FEMS Microbiology Reviews* 34

*Pseudomonas aeruginosa* *Nature*  
*Protocols* 1

*Acinetobacter calcoaceticus* *Journal of Bacteriology* 176



3Y~z = : Y~&~1s~<z~&Y~Z~l~&Y~Z~5~>-H~5~>-n~\$&~

*Xanthomonas campestris*      *campestris*

*Scientific Reports* 6

3Y~3~pL~`~\$~`=\$~p, Y~&~l~>-n~&~5p>E>S~`~et al

*Pseudomonas aeruginosa*      *Molecular*

*Microbiology* 104

5>~\$:~LH->~Ly~/~&Y~\$%~3Bp~L3HL~`~3C>~, %ö~>-Hž >p A~, By~

*Arabidopsis thali-*