

# Sequence, Transcriptional Analysis and Chromosomal Location of the *Xanthomonas campestris* pv. *campestris* *uvrB* Gene

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## Abstract

The *uvrB* gene of *Xanthomonas campestris* pv. *campestris*, a Gram-negative plant pathogenic bacterium inhabiting soil and infected plants, was cloned and sequenced. This gene has the capacity to encode a polypeptide of 673 amino acid residues with a calculated molecular mass of 75.9 kDa. Its deduced amino acid sequence shows a high degree of similarity and possesses domain conservation to those of bacterial UvrB. The *uvrB* mutant, isolated by gene replacement, is extremely sensitive to ultraviolet irradiation. Like the situation in the *X. campestris* pv. *campestris* *recA* gene, no SOS box is present upstream of the *uvrB* gene. Northern blotting and transcriptional fusion assay with *lacZ* indicated that *X. campestris* pv. *campestris* *uvrB* is expressed constitutively at high levels and cannot be further induced by UV irradiation. These results suggest a regulatory mechanism different from that for the expression of *Escherichia coli* *uvrB*. Using a gene-tagging strategy in conjunction with pulsed-field gel electrophoresis, the *uvrB* gene was located near 1 o'clock on the *X. campestris* pv. *campestris* 17 chromosome (4.8 Mb) map, which is far apart from the *lexA-recA-recX* cluster near 5 o'clock.

## Introduction

The Gram-negative, yellow-pigmented *Xanthomonas campestris* pv. *campestris* is the causal agent of black rot in cruciferous plants (Williams, 1980). This bacterium is also known to produce an exopolysaccharide, xanthan gum, which finds a variety of applications in oil drilling, agriculture, cosmetics, and the food industry (Sandford and Baird, 1983).  $\phi$ Lf is a filamentous phage specifically infecting *X. campestris* pv. *campestris* (Tseng *et al.*, 1990). During the course of studying phage-host interactions, we have cloned from  $\phi$ Lf-sensitive *X. campestris* pv. *campestris* P20H a 7-kb DNA fragment required for  $\phi$ Lf infection. The upstream region of this fragment carries a *pil* gene cluster presumably involved in pilus biogenesis

(Lee and Tseng, 1999), whereas the downstream region contains a sequence similar to the N-terminal portion of bacterial *uvrB* genes. Since *X. campestris* pv. *campestris* persists in soil, infected plants, plant debris or asymptomatic plants near the acutely infected plants, it may encounter a variety of DNA-damaging conditions, including UV and various agrochemicals; therefore, it would be interesting to study the *uvrB* gene of this organism.

In *Escherichia coli*, *uvrA* and *uvrB* genes are part of the SOS system, a global DNA repair system consisting of more than 20 genes, which is induced by a variety of treatments causing DNA damage or interruption of DNA replication (Little and Mount, 1982; Walker, 1984). Induction of this system, known as the SOS response, results in increased DNA repair capacity and synthesis of RecA protein (Radman, 1975). UvrA, UvrB and UvrC proteins form an enzyme complex, (A)BC excinuclease, which can catalyze the initial reaction of the excision repair system (Friedberg *et al.*, 1995). Expression of the SOS regulon is under the control of the RecA and LexA proteins: the LexA protein acts as the common repressor of all SOS genes by binding to a consensus sequence known as the SOS box (Walker, 1984) and the presence of damaged DNA causes conformational changes in the RecA protein which mediates autocleavage of LexA and results in the derepression of the SOS regulon (Friedberg *et al.*, 1995).

The *recA* and *lexA* homologs from *X. campestris* have been studied (Lee *et al.*, 1996; Yang and Wu, 1999; Yang *et al.*, 2000); however, the other genes which should be involved in DNA repair and whether an SOS-like system is present in xanthomonads remain unknown. In this study, we determined the nucleotide sequence of the *uvrB* gene from P20H, isolated an *uvrB* mutant for testing the UV sensitivity, and analyzed its transcription activity. In addition, to improve the use of the *X. campestris* pv. *campestris* 17 chromosome map constructed previously (Tseng *et al.*, 1999), the location of the *uvrB* gene was determined.

## Results

### Cloning and Sequencing of the P20H *uvrB* Gene

Plasmid pSMA3106 contained an inserted *Sma*I fragment of 10.6 kb cloned from the *X. campestris* pv. *campestris* P20H chromosome. It was known prior to this study that the upstream two thirds of this fragment contained the *pil* gene cluster required for the biogenesis of type IV pilus (Lee, 2000). In this study, the downstream region of the pSMA3106 insert was subcloned into pUC18 and pUC19, and the nucleotide sequences of both strands were determined. Sequence analysis of this region (2,432 bp) revealed an open reading frame (*orf673*) of 2,022 nt, spanning nt 156 to 2,177, capable of coding for a

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(A)

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PpuMI (orf183) pilZ      ctctccggttggtgagtagcagtcctctgcggtgattgctgggtgcagc 70
TCCAGGACAGACGTAgcgcgggagaggcaaccgacctcatcgtcagagacgccactaacgcaccacgtcg
P G T Q M                                     Tss →
agggggctttggcctggcgtgctgtcaggtttctaccttcaaagacctccggttgtttcgtactgtcctcc 140
tcccccgaaaccggaccgcacgacagtcctaaagatggaagtttctggaggcaacaaagcatgacaggagg

S/D      uvrB (orf673)
cccgagagttgctccATGACCGACCGCTTTGAGCTTGTATCCCCGTATTCCCCAGCCGGCGACCAACCTG 210
gggctctc      M T D R F E L V S P Y S P A G D Q P A 19

CGCCATCGACAAGTTGGTGGCCAACTTCGAGGCGGGGCTGGCCAAGCAGACGCTGCTGGGGGTGACGGG 280
A I D K L V A N F E A G L A K Q T L L G V T G 42

TTCGGGTAAGACCTACACCATCGCCAACGTCGTGCAGCAGGTGCAGAAGCCGACGCTAGTGATGGCGCCG 350
S G K T Y T I A N V V Q Q V Q K P T L V M A P 65

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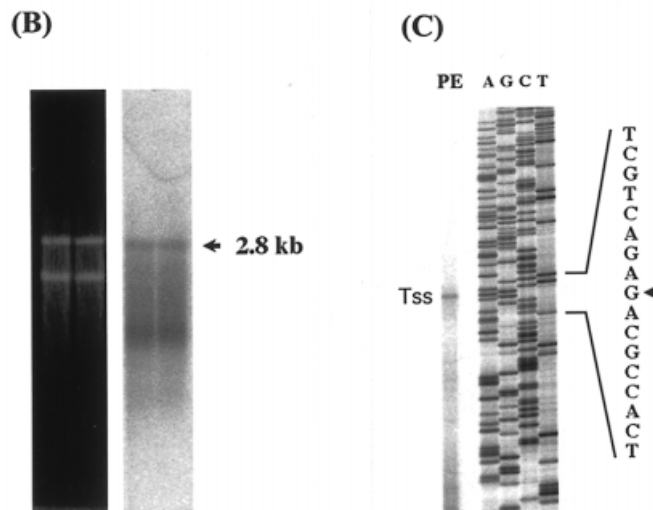


Figure 1. (A) Nucleotide and deduced amino acid sequences of the upstream region of *X. campestris* pv. *campestris* P20H *uvrB* gene. Shown are the N-terminal portion of *pilZ* in the opposite strand, the intergenic region, and the N-terminus of *uvrB* (65 amino acid residues). Tss stands for the transcriptional start site determined by primer extension and S/D represents the Shine-Dalgarno sequence. The underlined region is the sequence complementary to the oligonucleotides used for primer extension. (B) Northern hybridization of the P20H *uvrB* transcript. About 1.0  $\mu$ g of total RNA was loaded on the gel. The same oligonucleotides as that for primer extension was end-labeled and used as the probe for hybridization. (C) Determination of transcriptional start site of the P20H *uvrB* gene. PE is the primer extension product. A sequencing gel using the same primer and template was run in parallel. The sequence listed on the right is complementary to that in (A).

polypeptide of 673 amino acid residues with a calculated molecular mass of 75.9 kDa. Running in the opposite direction to *pilZ*, the last gene of the *pil* cluster (Lee, 2000), *orf673* started with ATG 140 nt apart from the *pilZ* start codon. This ATG was seven nt downstream from a consensus Shine-Dalgarno sequence (S/D) 5'-GAGAG-3' (Figure 1A), complementary to the 3'-end of the *X. campestris* pv. *campestris* 16S rRNA (Lin and Tseng, 1997). The ATG and the predicted S/D, consistent with the structures predicted for translational initiation in bacteria, were the only sequences found in the upstream region. No sequence consensus to an *E. coli*-type promoter was found in the 140-nt intergenic region, although this region exhibited promoter activity in reporter assay (see below). After *orf673*, a sequence of 255 nt was also determined,

in which no homology to any known gene was identified.

*Orf673* had a G+C content of 62.8%, similar to that of the *X. campestris* pv. *campestris* chromosome (Bradbury, 1984). Consistent with this is that 85.5% of the triplets had G or C at their third positions. These properties are typical of *Xanthomonas* genes.

#### Sequence Conservation of UvrB

Consisting of 673 amino acid residues, the predicted protein product of *orf673* had a size similar to those of bacterial UvrB proteins (Figure 2). The deduced amino acid sequence shared high degree of identity with the UvrB proteins from *Xylella fastidiosa* (83%), *Pseudomonas aeruginosa* (71%), *E. coli* (67%), *Haemophilus influenzae* (66%), *Neisseria meningitidis* (62%), and *N. gonorrhoeae*

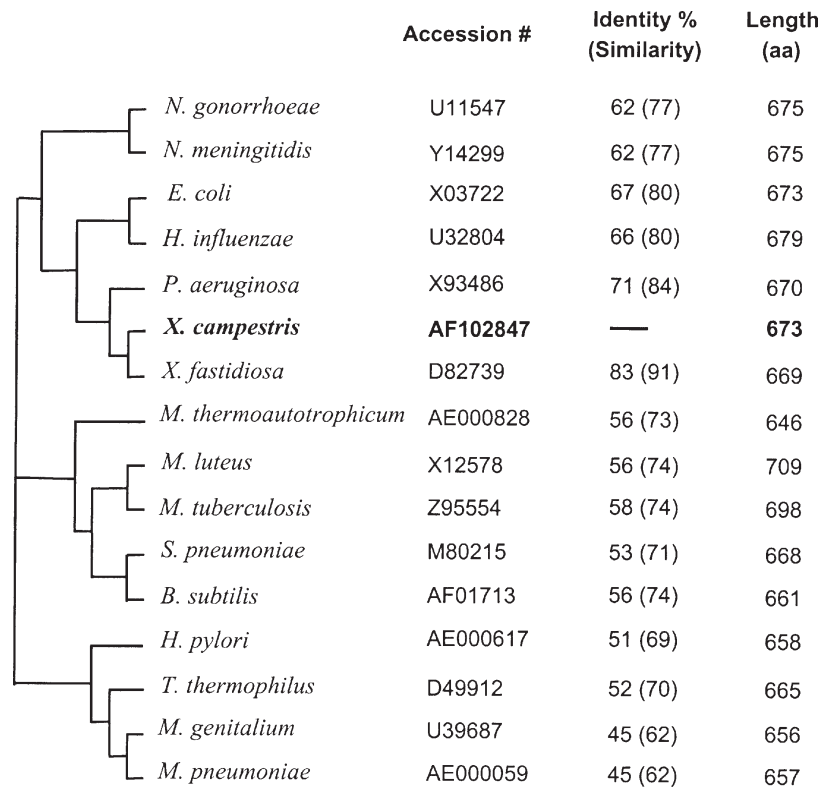


Figure 2. Dendrogram showing relatedness of bacterial UvrB proteins. The accession number, percentages of identity and homology (in parentheses) with the *X. campestris* pv. *campestris* P20H sequence, and the protein sizes are indicated on the right.

(62%). A dendrogram was constructed by clustering with these and nine other related UvrB sequences, and the closest relatedness found was consistent with the percent identity obtained by sequence alignment (Figure 2). Based on these characteristics, we thus identified *orf673* as the *X. campestris* pv. *campestris* *uvrB* gene.

Biochemical and genetic studies have revealed several important features of *E. coli* UvrB. Most of these features were also found in the predicted P20H UvrB protein, including i) an ATP/GTP binding site, called Walker box A (Walker, 1984; Sancar and Tang, 1993), at aa 39-46, and ii) seven helicase motifs conserved in a subfamily of proteins with helicase activities, including the *E. coli* UvrB. These motifs, designated as I, Ia, II, III, IV, V and VI, were found in aa 32-48, 59-78, 329-343, 390-403, 447-468, 492-513 and 529-546, respectively, of the P20H UvrB (Figure 3). While motifs I, Ia, IV, V and VI showed a high degree of identity (83-91%) to the *E. coli* UvrB, motifs II and III were slightly less conserved (79-80% identity). In addition to these features, 12 amino acid residues important for UvrB function in *E. coli* have been identified. Ten of them are E99, E266, D338, E339, F366, F497, G509, D511, E514, and R544 (Lin *et al.*, 1992; Moolenaar *et al.*, 1994; Hsu *et al.*, 1995), and each of these was also found in the predicted P20H UvrB as E99, E266, D338, E339, F366, F497, A509, D511, E514, and R544, respectively (Figure 3). The other two important residues in *E. coli* UvrB, D479 and E640, were present in P20H UvrB as the conservative substitutions D479 and E642, respectively (Figure 3).

In *E. coli*, SOS box is a consensus palindromic operator sequence CTG(N<sub>10</sub>)CAG located upstream of most, or all, SOS genes for the binding of the LexA repressor (Walker, 1984). However, no sequence similar to the *E. coli* SOS box was found in upstream region of the P20H *uvrB*.

#### UV Sensitivity of P20H *uvrB* Mutant

A P20H *uvrB* mutant was constructed by gene replacement and designated as TC7. To verify that the Gm<sup>r</sup> cartridge insertion was resulted from double crossover, Southern hybridization was performed with the TC7 chromosome digested with *Bam*HI plus *Sma*I, using the probe prepared from pBSM427 carrying the P20H *uvrB* gene. A 2.7-kb fragment was detected in P20H, whereas a 3.5-kb fragment was detected in TC7, indicating that a single copy of Gm<sup>r</sup> cartridge (0.85 kb) indeed had been inserted by double crossover (data not shown). In UV sensitivity test with 302-nm irradiation, cells of TC7 were rapidly killed and the survival rate decreased drastically with increasing dosage of UV. As shown in Figure 4, at a dosage higher than 10 J/m<sup>2</sup>, practically no survivor of TC7 was detectable; in contrast, about 70% of P20H and the complemented cells, TC7(pBSM427), were recovered at the same dosage. It was noted that the survival rates of P20H and TC7(pBSM427) were about the same, which were similar to those observed for Xc17 and the complemented *recA* mutant; however, the killing effect of UV at 10 J/m<sup>2</sup> on TC7 was similar to that observed in the Xc17 *recA* mutant treated with a dosage of 30 J/m<sup>2</sup> (Lee *et al.*, 1996). This result

		Conserved motifs	ID / Sm (%)
I	Ec	32 ..H.....F. 48	88.2 / 94.1
	Xc	32 LAKZTLLGVTGSGKTYT 48	
	Pa	31 .SH.....FS 47	76.5 / 94.1
Ia	Ec	59 ..M.L.....M... 78	85.0 / 95.0
	Xc	59 PTLVMAPNKTLAAQLYGEFK 78	
	Pa	58 ....L..... 77	95.0 / 100.0
II	Ec	329 ..A.G...V..... 343	80.0 / 86.7
	Xc	329 LPPDALLVIDESHVT 343	
	Pa	328 ..ANS..... 342	73.3 / 93.3
III	Ec	390 .....N...EK 403	78.6 / 85.7
	Xc	390 IYVSATPGPYELRE 403	
	Pa	389 .F.....-A. 401	78.6 / 85.7
IV	Ec	447 E.....D.....E. 468	86.4 / 95.5
	Xc	447 DRVLVTTLTKRMAENLTEYLGE 468	
	Pa	445 E.....D..D...D 466	81.8 / 100.0
V	Ec	492 ....E.....G.... 513	90.9 / 95.5
	Xc	492 LRLGKFDVLVGINLLREALDMP 513	
	Pa	490 ..A.A.....G.... 511	86.4 / 86.4
VI	Ec	529 ...ER.....V 546	83.3 / 94.4
	Xc	529 LRSTGSLIQTIGRAARNL 546	
	Pa	527 ...ER..... 544	88.9 / 88.9

Figure 3. Similarities between the seven helicase motifs predicted for *E. coli* UvrB (Gorbalenya *et al.*, 1989) and the corresponding segments from the deduced *X. campestris* pv. *campestris* P20H UvrB. Similarity (%Sm) and identity (%ID) are shown to the right.

suggested the *uvrB* mutant to be more sensitive than the *recA* mutant.

#### Transcriptional Analysis of P20H *uvrB* Gene

Using the same oligonucleotide primer used in primer extension (Experimental Procedures) as the probe for Northern hybridization with the total RNA prepared from an overnight culture of P20H, the hybridization signal was associated with a single band of 2.8 kb (Figure 1B). The size was about 600 nt larger than the *uvrB* coding region. The blotting experiment was repeated several times with freshly prepared RNA (ca. 1.0 µg) and hybridization signals of similar intensity were readily detectable (data not shown), suggesting that *X. campestris* pv. *campestris* retained high levels of *uvrB* mRNA.

In primer extension, the signal obtained was a C located 107 nt upstream from the predicted translational initiation codon (Figure 1B). A 5' untranslated region of 107 nt was longer than those previously observed for other *Xanthomonas* genes. Subtracting the 5' 107 nt and the

size of *uvrB*, there was still about 500 nt at the 3' end, indicating that the transcript may contain either a downstream gene or a long 3' untranslated region.

To assay for promoter activity, a PCR-amplified 214-bp fragment encompassing -272 to -59 relative to the *uvrB* translational initiation codon was cloned into the promoter-probing vector pFY13-9 (Lee *et al.*, 2001). The resultant plasmid, pUV7.21, carried the putative *uvrB* promoter running in the same direction as the reporter  $\beta$ -galactosidase gene. Only a basal level of  $\beta$ -galactosidase activity (less than 10 Miller units) was detectable in P20H containing vector pFY13-9 only. In contrast, enzyme activity detected in P20H(pUV7.21) ranged from 1,160 to 2,985 U at different time points during a growth period of 12 hr. We also tested the promoter activity of the 356-bp fragment (-59 to -415 relative to the *uvrB* initiation codon), which included the pUV7.21 insert. The plasmid containing the 356-bp insert was designated as pUV7.36. The  $\beta$ -galactosidase levels detected in P20H(pUV7.36) were similar to that present in P20H(pUV7.21). These results

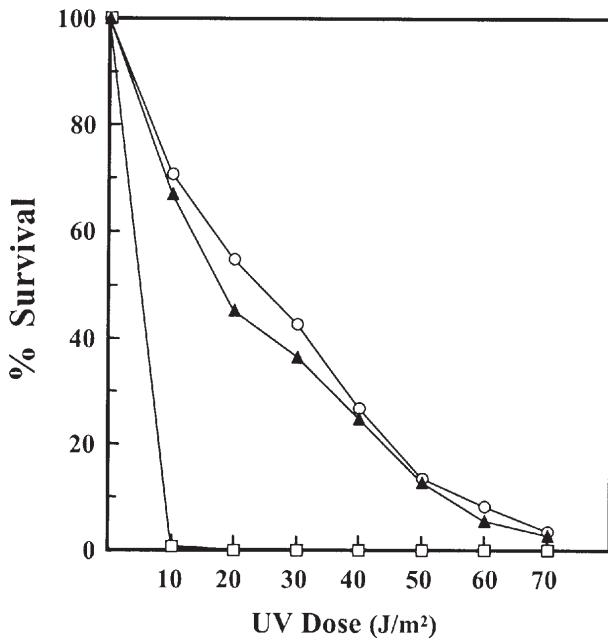


Figure 4. UV sensitivity of *X. campestris* pv. *campestris* P20H (circles), *uvrB* mutant, TC7 (squares), and TC7 containing pBSM427 (triangles).

indicated that the 214-bp upstream fragment indeed contained the whole promoter region. To test for inducibility of the *uvrB* promoter, cells of P20H(pUV7.21) and P20H(pUV7.36) were irradiated with UV light (20 J/m<sup>2</sup>), then the levels of  $\beta$ -galactosidase were measured at intervals of 30 min following growth for 6 hr. Similar levels of the enzyme activity, 1,964 to 2980 units, were detected in induced and non-induced cells. This data was consistent with the observation that high levels of *uvrB* transcript were present in *X. campestris* pv. *campestris* cells.

Taken these transcriptional analysis results together, it appeared that expression of the *X. campestris* pv. *campestris uvrB* gene is constitutive and not induced by treatments causing DNA damage.

**Location of *uvrB* Gene on Xc17 Chromosome Map**

We have previously constructed a physical map of Xc17 chromosome (4.8 Mb) bearing restriction sites for the rare-cutting enzymes *SwaI* (6 sites), *PacI* (5 sites), *I-CeuI* (2 sites) and *PmeI* (2 sites), and determined the chromosomal location for 22 genetic loci including *recA* (Tseng *et al.*, 1999). In this study, the Xc17 chromosome was tagged via single crossover with pMFR9, a pNEB193-derived plasmid carrying a fragment internal to the P20H *pilY1* together with one additional site for *PacI* and *SwaI*, giving rise to mutant strain MPIL as described in Materials and

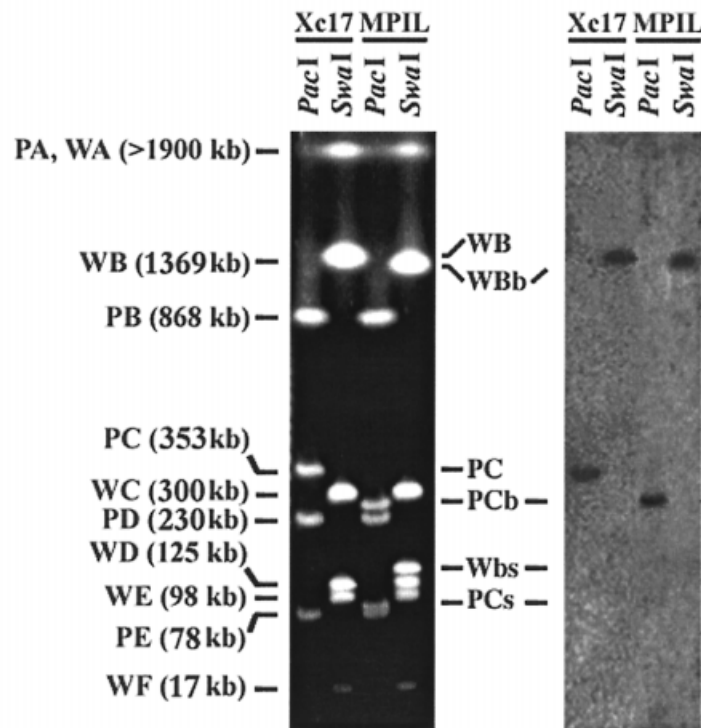


Figure 5. Pulsed-field gel electrophoresis of the *SwaI* and *PacI* fragments from *X. campestris* pv. *campestris* 17 and MPIL (left panel) and Southern hybridization of the DNA fragments, after being transferred from the gel onto a nylon membrane, with the probe prepared from pXBAS160 (right panel).

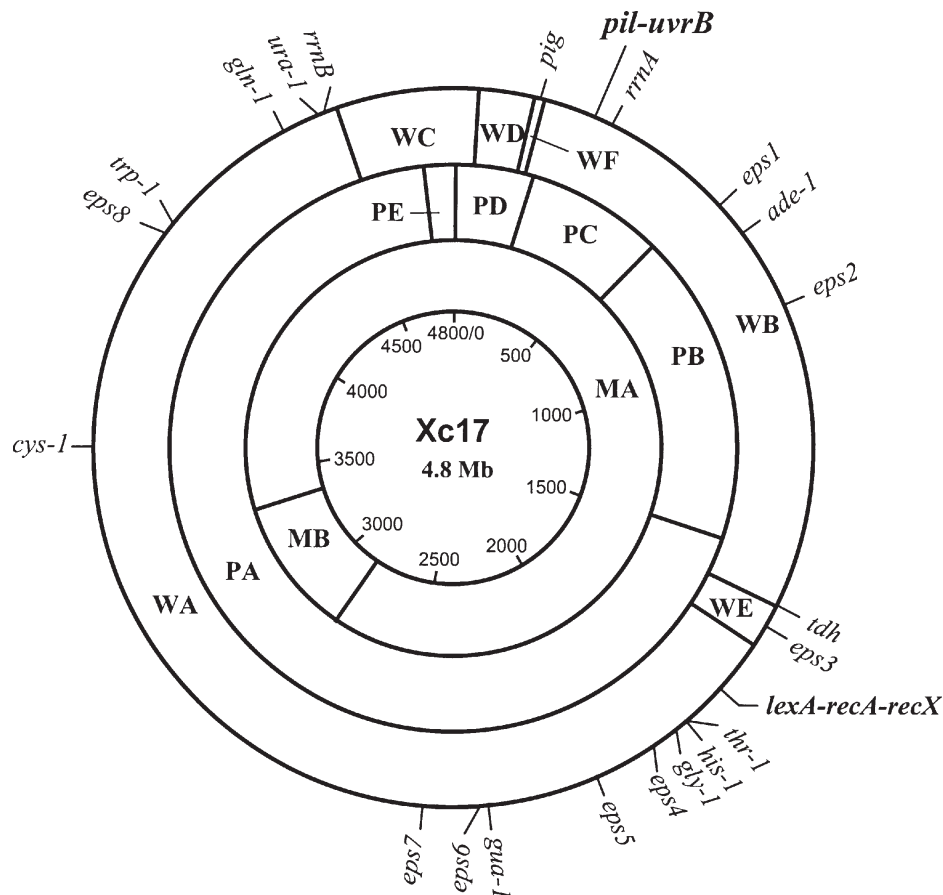


Figure 6. Location of *pil-uvrB* region on *X. campestris* pv. *campestris* 17 chromosome map. The three concentric circles represent cleavage maps (outer to inner) for *SwaI*, *PaeI* and *PmeI*, which cut the chromosome into 5, 6 and 2 fragments, respectively. The PA/PE junction is set as 12 o'clock. The position of *pil-uvrB* and *recA* are shown by bold-faced letters. The other markers were determined previously (Tseng et al., 1999).

**Methods.** Southern hybridization was performed, using the probe prepared from pNEB193, to verify that pMFR9 had been integrated by single crossover and only one copy of pMFR9 was present. Since integration of pMFR9 introduced an additional *PaeI* and *SwaI* site, the *uvrB*-containing *PaeI* and *SwaI* fragments from the MPIL chromosome would each be cut into two sub-fragments compared with that from *PaeI*- or *SwaI*-digested Xc17 chromosome.

*PaeI* and *SwaI* cut the Xc17 chromosome into five and six fragments, respectively (Figure 5). Southern hybridization showed that the probe prepared from plasmid pXBAS160 hybridized to fragments PC (*PaeI* fragment C, 353 kb) and WB (*SwaI* fragment B, 1369 kb) from the Xc17 chromosome, indicating that *uvrB* gene located within the PC/WB overlapping region (Figure 5). Due to the presence of inserted pMFR9, fragments PC and WB from the MPIL chromosome were cut into PCb (273 kb) plus PCs (80 kb) by *PaeI* and Wb (1,250 kb) plus WBs (120 kb) by *SwaI*, respectively (Figure 5). Southern hybridization was performed using the probe prepared from pXBAS160, containing the 4.0-kb *SmaI*-*XbaI* fragment locating 4.5 kb upstream from *uvrB*. Hybridization signals were shown to associate with fragments Wb and PCb (Figure 5), establishing the overlapping relationships of Wb/PCb and

WBs/PCs. In other words, WBs and PCs were on the same side relative to the inserted pMFR9. Since the size of WBs was similar to that of PCs plus the size of the PD/WB overlapping region (40 kb), these data indicated that the *pil-uvrB* region located ca. 80-kb from the PC/PD junction (Figure 6). Setting the PA/PE junction as 12 o'clock, the *pil-uvrB* region was close to *rrnA* operon near 1 o'clock, far apart from *recA* gene previously determined to be near 5 o'clock (Figure 6; Tseng et al. 1999). Recently, we have sequenced the *recA* region and found that *recA* is linked with *lexA* and *recX*, *lexA-recA-recX*, on the *X. campestris* pv. *campestris* chromosome (Lee and Tseng, unpublished data).

## Discussion

In this study, *orf673* was identified to be the *X. campestris* pv. *campestris* *uvrB* gene. Several lines of evidence in support of the identification were obtained. First, the deduced protein product has a high degree of sequence similarity and motif conservation to those of other bacterial UvrB. Second, the *uvrB* mutant (TC7), isolated by gene replacement, is extremely sensitive to UV irradiation and the cloned *uvrB* gene can partially complement the mutation. Following our report on *recA* (Lee et al., 1996), it

Table 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
<i>Escherichia coli</i> DH5 $\alpha$	F <sup>+</sup> supE44 $\Delta$ lacU169 ( $\phi$ 80lacZ $\Delta$ M15) hsdR17 recA1 endA1 gyrA96 thi <sup>-1</sup> relA1	Hanahan, 1983
<i>Xanthomonas campestris</i> pv. campestris		
Xc17	Wild-type strain isolated in Taiwan, Ap <sup>r</sup>	Yang and Tseng, 1988
P20H	Non-mucoid mutant of Xc11A, Ap <sup>r</sup>	Yang <i>et al.</i> , 1988
TC7	<i>uvrB</i> mutant derived from P20H by insertion of Gm <sup>r</sup> cartridge, Ap <sup>r</sup>	This study
MPIL	Xc17 derivative with pMFR9 integrated in <i>pilY1</i> gene immediately upstream of <i>uvrB</i>	This study
Plasmids		
pOK12	<i>E. coli</i> general cloning vector, P15A <i>ori</i> , <i>lacZ</i> $\alpha$ fragment	Vieira and Messing, 1991
pUC18	<i>E. coli</i> general cloning vector, <i>lacZ</i> $\alpha$ fragment	Yanisch-Peron <i>et al.</i> , 1985
pRK415	Broad-host-range vector, RK2 <i>ori</i> , Tc <sup>r</sup>	Keen <i>et al.</i> , 1988
pFY13-9	Promoter-probing vector, derived from pRK415, <i>lacZ</i> as reporter, Tc <sup>r</sup>	Lee <i>et al.</i> , 2001
pNEB193	pUC19 derivative carrying unique sites for <i>Ascl</i> , <i>Pacl</i> and <i>Pmel</i>	New England Biolabs
pBSU151	pOK12 derivative with a 5.1-kb <i>Bam</i> HI- <i>Stul</i> fragment containing <i>uvrB</i> gene cloned from P20H chromosome	This study
pBSU151G	pBSU151 derivative carrying Gm <sup>r</sup> cartridge inserted into <i>Scal</i> site within <i>uvrB</i>	This study
pSMA3106	pUC18 derivative with a 10.6-kb <i>Sma</i> I fragment containing <i>uvrB</i> gene cloned from P20H chromosome	This study
pXBAS160	pOK12 carrying a 4.0-kb <i>Sma</i> I- <i>Xba</i> I fragment 4.5 kb upstream from P20H <i>uvrB</i> gene	This study
pBSM427	pRK415 derivative carrying the 2.7-kb <i>Bam</i> HI- <i>Sma</i> I fragment containing complete P20H <i>uvrB</i>	This study
pUV7.21	pFY13-9 derivative carrying a 214-bp PCR fragment amplified from P20H <i>uvrB</i> upstream region	This study
pUV7.36	pFY13-9 derivative carrying a 356-bp PCR fragment amplified from P20H <i>uvrB</i> upstream region	This study
pKPN1.4	pNEB193 derivative with a 1.4-kb insert internal to P20H <i>pilY1</i>	This study
pMFR9	pKPN1.4 derivative constructed by cloning the Cm <sup>r</sup> and Km <sup>r</sup> cartridges together with the restriction sites <i>Swa</i> I and <i>Pacl</i> from pUT-Tn5(pfm)CmKm	This study

is the second gene characterized for DNA repair system of this plant pathogenic bacterium.

The nucleotide excision repair (NER) pathway in bacteria depends on six proteins, UvrABCD, DNA Pol I, and DNA ligase. It is an important DNA repair system, since it recognizes the majority of DNA lesions (Sancar, 1996). As a central protein in the NER for DNA damage recognition and excision processes, UvrB proteins are highly conserved. In the *X. campestris* pv. *campestris* UvrB, the most conserved regions are the ATP/GTP binding site in motif I, required for complexing with UvrA and together to perform ATP-depend helicase activity, and the helicase motifs V and VI. In addition, 11 out of the 12 important amino acid residues are identical. Sequence similarity and motif conservation found in the *X. campestris* pv. *campestris* UvrB provide additional evidence for structural features necessary for UvrB function.

In *E. coli*, SOS genes are repressed under non-inducing conditions, with the repression being mediated by binding of LexA protein to the conserved palindromic sequences in SOS promoters (SOS box) preventing their transcription. Many bacteria appear to have SOS-like responses similar to that of *E. coli*, as revealed by the presence of sequences resembling SOS box in the promoters of *recA* and *uvrB* (Fyfe and Davies, 1990; Rivera *et al.*, 1996). However, some DNA-damage-inducible genes in bacteria lack a LexA-binding sequence in their promoters (Fyfe and Davies, 1990; Matsui *et al.*, 1993; Riera *et al.*, 1994). In these cases, either the SOS-like genes are not co-regulated as part of an SOS response, or regulation may be mediated by binding of the repressor to a different binding site. Therefore, the mechanism of regulation of the DNA-damage-inducible genes observed in *E. coli* may not

be applicable to all other bacteria. For examples, SOS-like system is not found in *N. gonorrhoeae* (Black *et al.*, 1998) and the *P. aeruginosa uvrB* gene that lacks an *E. coli*-like operator is not DNA damage inducible (Rivera *et al.*, 1996). We suggest the expression of *X. campestris* pv. *campestris uvrB* gene to be regulated by a mechanism different from that in *E. coli*, based on the results that (1) no sequence resembling an SOS box is present in the *uvrB* promoter region, (2) high levels of *uvrB* transcript are detectable, indicating a situation of constitutive expression, and (3) UV treatments cause no further induction of the transcription. Furthermore, it is worth noting that SOS box is also absent from the promoter region of the Xc17 *recA* gene (Lee *et al.*, 1996), another gene which might be expected to be one of the members of an SOS-like system in *X. campestris* pv. *campestris*. These observations together suggest that the SOS-like system of *X. campestris* pv. *campestris* is operating in a manner different from that of *E. coli*.

We have previously used rare-cutting restriction enzymes to construct a physical map for Xc17 chromosome, on which 22 genetic loci, including *recA*, were determined (Tseng *et al.*, 1999). Some of the loci were located by the gene-tagging strategy, which is suitable for localization of genes with known sequences. Using this strategy, *pil* cluster-*uvrB* was located on the PC/WB overlapping region, about 80-kb from the PC/PD interface. This location is close to *rrnA* operon near 1 o'clock, and far apart from *recA* which is near 5 o'clock. Recently, we have obtained sequence data showing that *lexA* and *recX* homologues are clustered with *recA* gene in Xc17, *lexA-recA-recX* (Lee and Tseng, unpublished data). From these observations, it appears that in *X. campestris* pv.

campestris some of the DNA repair genes are organized into clusters but the clusters are dispersed in the chromosome. This situation is similar to that in *E. coli* and several other bacteria (Walker *et al.*, 1984; Simpson *et al.*, 2000). It will be interesting to know the locations for the other genes involved in DNA repair. Finally, localization of *pil* cluster-*uvrB* has increased the use of the Xc17 chromosome map.

## Experimental Procedures

### Bacterial Strains, Plasmids, and Growth Conditions

The bacterial strains and plasmids used in this study are listed in Table 1. Luria-Bertani broth and LB agar were used as the media for growing *X. campestris* pv. *campestris* (28°C) and *E. coli* (37°C). Antibiotics used were: kanamycin (50 µg/ml), tetracycline (15 µg/ml), and gentamycin (15 µg/ml).

### DNA Techniques

Restriction endonucleases and other enzymes were purchased from New England Biolabs (Beverly, MA) and used according to the instructions provided by the supplier. The procedures described by Sambrook *et al.* (1989) were used for preparation of chromosomal, plasmid and phage DNA's, agarose gel electrophoresis, end-labeling of oligonucleotides, preparation of <sup>32</sup>P-labeled probes by either random priming or nick translation, Southern hybridization, and transformation of *E. coli*. *X. campestris* pv. *campestris* was transformed by electroporation (Wang and Tseng, 1992). Nucleotide sequence was determined by the dideoxy chain termination method of Sanger (1977). Sequence analysis was performed using the Genetics Computer Group (GCG, Madison, WI) package. Dendrogram was constructed using SeqWeb program. The BLAST algorithms of National Center for Biotechnology Information was used for sequence searches. The nucleotide sequence presented here has been registered in the GenBank under the accession number AF102847.

### Construction of *uvrB* Mutant by Gene Replacement

The P20H *uvrB* mutant was constructed as follows. The cloned P20H *uvrB* gene in plasmid pBSU151, a derivative of pOK12 (Km<sup>r</sup>) was interrupted by inserting a 0.85-kb DNA fragment specifying gentamycin resistance (Schweizer, 1993) into the unique *Scal* site. The resultant plasmid, designated pBSU151G, was transformed into P20H by electroporation. Since this plasmid, having a P15A origin of replication (Vieira and Messing, 1991), could not be maintained in *X. campestris* pv. *campestris*, the Gm<sup>r</sup> and Km<sup>s</sup> phenotype of the transformants indicated replacement of the wild-type gene by the interrupted gene through double crossover. This was verified by Southern hybridization. One of the mutants obtained was designated as TC7.

### UV Sensitivity Test

UV sensitivity test was carried out as described previously for testing the *X. campestris* pv. *campestris* *recA* mutant (Lee *et al.*, 1996) by irradiating the cells spread on LB agar plates with different doses of UV light (302 nm) and the survival rates were calculated.

## Transcriptional Analyses

The previously described methods were used for preparation of total RNA, Northern hybridization and primer extension (Lin *et al.*, 1999). The oligonucleotide used for primer extension was a 18-mer 5'-CAGCCCCGCC TCGAAGTT-3' with a sequence complementary to nt 79-96 relative to the *uvrB* translational initiation codon. The same oligonucleotide was used as the probe for Northern hybridization.

For promoter activity assay, two overlapping fragments containing the *uvrB* promoter region were amplified by PCR and cloned into pYF13-9 (Lee *et al.*, 2001), generating pUV7.21 and pUV7.36. The pUV7.21 insert was a fragment containing nt -272 to -59 (214 bp) relative to *uvrB* translational start codon amplified by using a 18-mer (5'-CAACCGCTGCCGCTCGAT-3') corresponding to nt -272 to -255 and a 21-mer (5'-TGACTGCAGGCCAGGC CAAAG-3') complementary with nt -39 to -59. The pUV7.36 insert was a fragment containing nt -415 to -59 (356 bp) relative to *uvrB* translational start codon amplified by using a 18-mer (5'-CAACCGCTGCCGCTCGAT-3') corresponding to nt -272 to -255 and the same 21-mer used for amplifying the 214-bp fragment. The amplified fragments were filled in with Klenow enzyme and then cut with *Pst*I and cloned into the *Stul*-*Pst*I sites of the promoter-probing vector pFY13-9. pUV7.21, pUV7.36 and pFY13-9 were separately electroporated into P20H. The cells to be assayed were grown overnight and then diluted 20-fold into fresh LB medium. Aliquots of the cultures were taken at intervals and assayed for β-galactosidase activity as described by Miller (1972). For UV induction, the cells of P20H(pUV7.36) were treated as in UV sensitivity test prior to promoter activity assay, except that the dosage was 20 J/m<sup>2</sup>.

### Determination of Chromosomal Location of *uvrB* Gene

The procedures described by Tseng *et al.* (1999) were used for preparation of intact chromosomes, in-gel digestion of the chromosomes with *Pac*I and *Swa*I, and pulsed-field gel electrophoresis in a CHEF-DR III machine from Bio-Rad (Richmond, CA). Chromosomal location of the Xc17 *uvrB* gene was determined by the gene-tagging method (Tseng *et al.*, 1999), suitable for localizing DNA fragments with known sequences. The 1.4-kb *Kpn*I fragment internal to *pilY1* gene, which was 1.9 kb upstream from the P20H *uvrB* gene (Lee, 2000), was cloned into the *Kpn*I site of pNEB193, a pUC19 derivative (Table 1), resulting in plasmid pKPN1.4. Then, the 3.0-kb *Pvu*II fragment from pUT-Tn5(cfm)CmKm, containing chloramphenicol acetyl transferase and kanamycin phosphotransferase genes with the unique *Pac*I and *Swa*I sites locating in between, was cloned into pKPN1.4, thus positioning the 3.0-kb *Pvu*II fragment next to the *pilY1* sequence. The generated plasmid pMFR9 was electroporated into Xc17 and allowed for integration into the chromosome through the homologous regions. The strain obtained was designated as MPIL.

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## References

- Black, C.G., Fyfe, J.A.M. and Davies, J.K. 1998. Absence of an SOS-like system in *Neisseria gonorrhoeae*. *Gene*. 208: 61-66.
- Bradbury, J.F. 1984. Bergey's Manual of systematic bacteriology. 1999. In N. R. Krieg and J. G. Holt ed, Williams and Wilkins. Baltimore.
- Friedberg, E.C., Walker, G.C., and Siede, W. 1995. DNA repair and mutagenesis. ASM Press, Washington, D. C.
- Fyfe, J.A. and Davies, J.K. 1990. Nucleotide sequence and expression in *Escherichia coli* of the *recA* gene of *Neisseria gonorrhoeae*. *Gene*. 93: 151-156.
- Gorbalenya, A.E., Koonin, E.V., Donchenko, A.P. and Blinov, V.M. 1989. Two related superfamilies of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. *Nucleic Acids Res.* 17: 4713-4730.
- Hanahan, D. 1983. Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* 166: 557-580.
- Hsu, D.S., Kim, S.T., Sun, Q. and Sancar, A. 1995. Structure and function of the UvrB protein. *J Biol Chem.* 270: 8319-8327.
- Keen, N.T., Tamaki, S., Kobayashi, D., and Trollinger, D. 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. *Gene*. 70: 191-197.
- Lee, T.C. 2000. Ph.D dissertation. National Chung Hsing University. Taichung, Taiwan.
- Lee, T.C., Lin, N.T. and Tseng, Y.H. 1996. Isolation and characterization of the *recA* gene of *Xanthomonas campestris* pv. *campestris*. *Biochem Biophys Res Commun.* 221: 459-465.
- Lee, T.C., Chen, S.T., Lee, M.C., Chang, C.M., Chen, C.H., Weng, S.F., and Tseng, Y.H. 2001. The Early Stage of Filamentous Phage  $\phi$ Lf Infection Require the Host Transcription Factor, Clp. *J. Mol. Microbiol. Biotechnol.* 3: 471-481.
- Lee, T.C., and Tseng, Y.H. 1999. Type IV pilin genes of *Xanthomonas campestris* pv. *campestris* are involved in filamentous phage  $\phi$ Lf infection, abstr. M-10, p. 444. In: Abstracts of the 99th General Meeting of the American Society for Microbiology 1999. American Society for Microbiology, Chicago, Ill.
- Lin, J.J., Phillips, A.M., Hearst, J.E., Sancar, A. 1992. Active site of (A)BC excinuclease. II. Binding, bending, and catalysis mutants of UvrB reveal a direct role in 3' and indirect role in 5' incision. *J. Biol. Chem.* 267: 17693-17700.
- Lin, N.T. and Tseng, Y.H. 1997. Sequence and copy number of the *Xanthomonas campestris* pv. *campestris* gene encoding 16S rRNA. *Biochem Biophys Res Commun.* 235: 276-280.
- Lin, N.T., Liu, T.J., Lee, T.C., You, B.Y., Yang, M.H., Wen, F.S. and Tseng, Y.H. 1999. The adsorption protein genes of *Xanthomonas campestris* filamentous phages determining host specificity. *J Bacteriol.* 181: 2465-2471.
- Little, J.W. and Mount, D.W. 1982. The SOS regulatory system of *Escherichia coli*. *Cell.* 29: 11-22.
- Matsui, H., Sano, Y., Ishihara, H. and Shinomiya, T. 1993. Regulation of pyocin genes in *Pseudomonas aeruginosa* by positive (*prtN*) and negative (*prtR*) regulatory genes. *J Bacteriol.* 175: 1257-1263.
- Miller, J.H. 1972. Experiments in molecular genetics. p:352-355. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
- Moolenaar, G.F., Visse, R., Ortiz-Buysse, M., Goosen, N. and van de Putte, P. 1994. Helicase motifs V and VI of the *Escherichia coli* UvrB protein of the UvrABC endonuclease are essential for the formation of the preincision complex. *J Mol Biol.* 240: 294-307.
- Radman, M. 1975. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis. *Basic Life Sci.* 5A: 355-367.
- Riera, J., Fernandez de Henestrosa, A.R., Garriga, X., Tapias, A. and Barbe, J. 1994. Interspecies regulation of the *recA* gene of gram-negative bacteria lacking an *E. coli*-like SOS operator. *Mol Gen Genet.* 245: 523-527.
- Rivera, E., Vila, L. and Barbe, J. 1996. The *uvrB* gene of *Pseudomonas aeruginosa* is not DNA damage inducible. *J Bacteriol.* 178: 5550-5554.
- Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular Cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
- Sancar, A. and Tang, M.S. 1993. Nucleotide excision repair. *Photochem Photobiol.* 57: 905-921.
- Sancar, A. 1996. DNA excision repair [published erratum appears in *Annu Rev Biochem* 1997;66:VII]. *Annu Rev Biochem.* 65: 43-81.
- Sandford, P.A., and Baird, J. 1983. Industrial utilization of polysaccharides. 411-490. The polysaccharide. In G. O. Aspinall (ed.). Academic Press. New York.
- Sanger, F., Nicklen, S. and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A.* 74: 5463-5467.
- Schweizer, H.D. 1993. Small broad-host-range gentamicin resistance gene cassettes for site-specific insertion and deletion mutagenesis. *Biotechniques.* 15: 831-834.
- Simpson, A.J., Reinach, F.C., Arruda, P., Abreu, F.A., Acencio, M., Alvarenga, R. *et al.*, 2000. The genome sequence of the plant pathogen *Xylella fastidiosa*. The *Xylella fastidiosa* Consortium of the Organization for Nucleotide Sequencing and Analysis. *Nature.* 406: 151-157.
- Tseng, Y.H., Lo, M.C., Lin, K.C., Pan, C.C. and Chang, R.Y. 1990. Characterization of filamentous bacteriophage phi Lf from *Xanthomonas campestris* pv. *campestris*. *J Gen Virol.* 71: 1881-1884.
- Tseng, Y.H., Choy, K.T., Hung, C.H., Lin, N.T., Liu, J.Y., Lou, C.H., Yang, B.Y., Wen, F.S., Wen, S.F. g and Wu, J.R. 1999. Chromosome map of *Xanthomonas campestris* pv. *campestris* 17 with locations of genes involved in xanthan gum synthesis and yellow pigmentation. *J Bacteriol.* 181: 117-125.
- Vieira, J. and Messing, J. 1991. New pUC-derived cloning vectors with different selectable markers and DNA replication origins. *Gene.* 100: 189-194.
- Walker, G.C. 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol Rev.* 48: 60-93.

- Wang, T.W. and Tseng, Y.H. 1992. Electrotransformation of *Xanthomonas campestris* by RF DNA of filamentous phage phi Lf. *Lett Appl Microbiol.* 14: 65-68.
- Williams, P.H. 1980. Black rot: a continuing threat to world crucifers. *Plant Dis.* 64: 736-742.
- Yang, B.Y., Tsai, H.F., and Tseng, Y.H. 1988. Broad host range cosmid pLAFR1 and non-mucoid mutant XCP20H provide a suitable vector-host system for cloning genes in *Xanthomonas campestris* pv. *campestris*. *Chin. J. Microbiol. Immunol.* 21: 40-49.
- Yang, B.Y., and Tseng, Y.H. 1988. Production of exopolysaccharide and levels of protease and pectinase activity in pathogenic and non-pathogenic strains of *Xanthomonas campestris* pv. *campestris*. *Bot. Bull. Acad. Sci.* 29: 93-99.
- Yang, M.K. and Wu, P.I. 1999. Identification of the promoter region of the *Xanthomonas campestris* pv. *citri* *recA* gene responsible for induction by DNA-damaging agents. *FEMS Microbiol Lett.* 176: 57-65.
- Yang, M.K., Wu, P.I. and Yang, Y.C. 2000. Identification of a *lexA* gene in, and construction of a *lexA* mutant of, *Xanthomonas campestris* pv. *citri*. *Curr Microbiol.* 40: 233-238.
- Yanisch-Perron, C., Vieira, J., and Messing, J. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene.* 33: 103-119.