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). φLf 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 φLf-sensitive X. campestris pv. campestris P20H a 7-kb DNA fragment required for φLf 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 SmaI 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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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 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 µg 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).
(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, la, 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, la, 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; Mooonenar 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, G509, 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(N10)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′ cartridge insertion was resulted from double crossover, Southern hybridization was performed with the TC7 chromosome digested with BamHI plus SmaI, 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′ 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/m², 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/m² on TC7 was similar to that observed in the Xc17 recA mutant treated with a dosage of 30 J/m² (Lee et al., 1996). This result
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. 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.

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 β-galactosidase gene. Only a basal level of β-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 β-galactosidase levels detected in P20H(pUV7.36) were similar to that present in P20H(pUV7.21). These results

<table>
<thead>
<tr>
<th>Conserved motifs</th>
<th>ID / Sm (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>I</strong></td>
<td></td>
</tr>
<tr>
<td>Ec 32 .H..................F. 48</td>
<td>88.2 / 94.1</td>
</tr>
<tr>
<td>Xc 32 LAKETLLQVTGSGKTTT 48</td>
<td></td>
</tr>
<tr>
<td>Pa 31 .SH..................FS 47</td>
<td>76.5 / 94.1</td>
</tr>
<tr>
<td><strong>Ia</strong></td>
<td></td>
</tr>
<tr>
<td>Ec 59 .M.L.............M... 78</td>
<td>85.0 / 95.0</td>
</tr>
<tr>
<td>Xc 59 PTVMAPKNKTLAAQLYGEFK 78</td>
<td></td>
</tr>
<tr>
<td>Pa 58 ....L.................. 77</td>
<td>95.0 / 100.0</td>
</tr>
<tr>
<td><strong>II</strong></td>
<td></td>
</tr>
<tr>
<td>Ec 329 .A.G...V....... 343</td>
<td>80.0 / 86.7</td>
</tr>
<tr>
<td>Xc 329 LPPDALLVIDEHSV 343</td>
<td></td>
</tr>
<tr>
<td>Pa 328 ....ANS............. 342</td>
<td>73.3 / 93.3</td>
</tr>
<tr>
<td><strong>III</strong></td>
<td></td>
</tr>
<tr>
<td>Ec 390 ...........N...EK 403</td>
<td>78.6 / 85.7</td>
</tr>
<tr>
<td>Xc 390 IYVSATRPGYELRE 403</td>
<td></td>
</tr>
<tr>
<td>Pa 389 .F...........-A. 401</td>
<td>78.6 / 85.7</td>
</tr>
<tr>
<td><strong>IV</strong></td>
<td></td>
</tr>
<tr>
<td>Ec 447 E.............D....E. 468</td>
<td>86.4 / 95.5</td>
</tr>
<tr>
<td>Xc 447 DRLVVTITLKMAENLTYELGE 468</td>
<td></td>
</tr>
<tr>
<td>Pa 445 E.............D....D 466</td>
<td>81.8 / 100.0</td>
</tr>
<tr>
<td><strong>V</strong></td>
<td></td>
</tr>
<tr>
<td>Ec 492 ....E.............G.... 513</td>
<td>90.9 / 95.5</td>
</tr>
<tr>
<td>Xc 492 LRGKFDVVGINLLREALDMP 513</td>
<td></td>
</tr>
<tr>
<td>Pa 490 ....A.A.............G.... 511</td>
<td>86.4 / 86.4</td>
</tr>
<tr>
<td><strong>VI</strong></td>
<td></td>
</tr>
<tr>
<td>Ec 529 ...ER.............V 546</td>
<td>83.3 / 94.4</td>
</tr>
<tr>
<td>Xc 529 LRSTGSLSIQTIGRAARNL 546</td>
<td></td>
</tr>
<tr>
<td>Pa 527 ...ER............. 544</td>
<td>88.9 / 88.9</td>
</tr>
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</table>

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.
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/m²), then the levels of β-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), l-CeuI (2 sites) and Pmel (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
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 *PacI* and *SwaI* site, the *uvrB*-containing *PacI* and *SwaI* fragments from the MPIL chromosome would each be cut into two sub-fragments compared with that from *PacI*- or *SwaI*-digested Xc17 chromosome. *PacI* 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 (*PacI* 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 *PacI* and WBb (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 WBb and PCb (Figure 5), establishing the overlapping relationships of WBb/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...
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, UvrABC, DNA Pol I, and DNA ligase. It is an important DNA repair system, since it recognizes the majority of DNA lesions (Sancar, 1996). In these cases, either the SOS-like genes are not present in bacteria lacking a LexA-binding sequence in their promoters (Fyfe and Davies, 1990; Matsui 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.

<table>
<thead>
<tr>
<th>Table 1. Bacterial strains and plasmids used in this study</th>
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<tr>
<td><strong>Strain or plasmid</strong></td>
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<tr>
<td><strong>Escherichia coli</strong></td>
</tr>
<tr>
<td><strong>Xanthomonas campestris</strong></td>
</tr>
<tr>
<td>Xc17</td>
</tr>
<tr>
<td>TC7</td>
</tr>
<tr>
<td>MPIL</td>
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<tr>
<td><strong>Plasmids</strong></td>
</tr>
<tr>
<td>pOK12</td>
</tr>
<tr>
<td>pUC18</td>
</tr>
<tr>
<td>pRK415</td>
</tr>
<tr>
<td>pFY13-9</td>
</tr>
<tr>
<td>pNEB193</td>
</tr>
<tr>
<td>pBSU151</td>
</tr>
<tr>
<td>pBSU151G</td>
</tr>
<tr>
<td>pMA3106</td>
</tr>
<tr>
<td>pXBSA160</td>
</tr>
<tr>
<td>pBSM427</td>
</tr>
<tr>
<td>pUV7.21</td>
</tr>
<tr>
<td>pUV7.36</td>
</tr>
<tr>
<td>pKN1.4</td>
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<tr>
<td>pMFR9</td>
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</tbody>
</table>
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 32P-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).

**Construction of uvrB Mutant by Gene Replacement**

The P20H uvrB mutant was constructed as the follows. The cloned P20H uvrB gene in plasmid pBSU151, a derivative of pOK12 (Km²) 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 Gm² and Km² 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'-CAGCCCCGCCCTCGAAATGT-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 pFY13-9 (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'-TGACTCGAGCGCCAGGC 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 PstI and cloned into the Stul-PstI 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/m².

**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 PstI and SwaI, 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 KpnI fragment internal to pilY1 gene, which was 1.9 kb upstream from the P20H uvrB gene (Lee, 2000), was cloned into the KpnI site of pN193, a pUC19 derivative (Table 1), resulting in plasmid pKPN1.4. Then, the 3.0-kb PvuII fragment from pUT-Tn5(cpm)CmKm, containing chloramphenicol acetyl transferase and kanamycin phosphotransferase genes with the unique PstI and SwaI sites locating in between, was cloned into pKPN1.4, thus positioning the 3.0-kb PvuII 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


