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Molecular Cloning and Characterization of a *recA*-like Gene Induced by DNA Damage from a Fluorescent *Pseudomonas* sp.

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The *recA* gene plays a central role in genetic recombination and SOS DNA repair in *Escherichia coli* (*E. coli*). We have previously identified a 42 kDa RecA-like protein inducible by a variety of DNA damages from a fluorescent *Pseudomonas* strain sp. and characterized its inducible kinetics. In the present study, we cloned and characterized the gene encoding the RecA-like protein by immunological screening of *Pseudomonas* genomic expression library using polyclonal *E. coli* anti-RecA antibodies as a probe. From 10^5 plaques screened, five putative clones were finally isolated. Southern blot analysis indicated that four clones had the same DNA inserts and the *recA*-like gene was located within the 3.2 kb *Eco*RI fragment of *Pseudomonas* chromosomal DNA. In addition, the cloned *recA*-like gene was transcribed into an RNA transcript approximately 1.1 kb in size, as judged by Northern blot analysis. The cellular level of RNA transcript of the cloned *recA*-like gene was increased to an average of 5.15- fold upon treatment with DNA damaging agents such as ultraviolet (UV)- light, nalidixic acid (NA), methyl methanesulfonate (MMS), and mitomycin-C (MMC). These results suggest that the cloned gene is inducible by DNA damage similarly to the *recA* gene in *E. coli*. However, the cloned gene did not restore the DNA damage sensitivity of the *E. coli recA*-mutant.

The DNA damage-mediated SOS response in *E. coli* is a well-known example of an inducible mutagenesis and a transient mutator pathway (Walker, 1984; Humayun, 1998). The inducible mutagenesis is mediated by the RecA protein, a *recA* gene product that is inducible by DNA damaging agents that either damage DNA or interfere with DNA replication (Walker, 1984; Lloyd and Sharp, 1993). The RecA protein is directly involved in homologous recombination and DNA repair and also promotes homologous sequence-dependent strand exchange *in vitro* (Kowalczykowski and Eggleston, 1994; Mikawa et al., 1998). In addition, the protein cleaves the LexA protein which acts as the repressor of SOS genes, thereby various SOS genes which function in inducible DNA repair and recombination in response to DNA damage are expressed at the elevated level (Walker, 1984; Bazemore et al., 1997).

Many kinds of bacteria show SOS response, which is very similar to that of *E. coli*, when they are exposed to DNA damaging agents. These bacteria include *Prevotella ruminicola* (Aminov et al., 1998), *Bacillus subtilis* (Lovett et al., 1994), *Rhodopseudomonas viridis* (Chen and Michel, 1998), *Thermus aquaticus* (Angov and Camerini-Otero, 1994), and *Thermus thermophilus* (Kato and Kuramitsu, 1993). Eukaryotic organisms also seem to show similar responses. The DMC1, Rad51, and Rad57 from *Saccharomyces cerevisiae* (*S. cerevisiae*) are RecA-like proteins (Angulo et al., 1989; Bishop et al., 1992; Shinohara et al., 1992; Story et al., 1993). The *S. cerevisiae* RecA-like proteins are involved in recombination as well as in the repair of DNA damage (Shinohara et al., 1992; Story et al., 1993). The Rad51 protein promotes DNA strand exchange (Sung, 1994; Sung and Stratton, 1996) and acts similarly to the RecA protein (Kowalczykowski and Eggleston, 1994). Recently, it was demonstrated that the Rad52 protein stimulates DNA strand exchange by targeting the Rad51 protein to a replication protein A (RPA) complex with single-stranded DNA (Anderson and Kowalczykowski, 1998; Benson et al., 1998; New et al., 1998).

In addition, another RecA-like protein has been isolated and characterized from chicken (Bezzubova et al., 1993) and human cells (Yoshimura et al., 1993;

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Benson et al., 1994; Takahashi et al., 1994). These results suggest that the structure and/or function of the RecA protein is conserved throughout prokaryotes and eukaryotes (Ogawa et al., 1993; Story et al., 1993; Bishop, 1994). Based on these results, we have previously characterized the inducible kinetics of the RecA-like protein which is functionally related to the *E. coli* RecA protein from a fluorescent *Pseudomonas* sp. The cellular level of RecA-like protein was immediately increased when DNA damage was challenged to the cell (Kim et al., 1998).

In this study, we attempted to isolate a gene encoding the RecA-like protein from fluorescent *Pseudomonas* cells by using the immunological screening method originally developed by Young and Davis (1983). The cloned *recA*-like gene was located within a 3.2 kb *Eco*RI fragment of *Pseudomonas* chromosomal DNA and transcribed into a 1.1 kb RNA transcript, as determined by Southern and Northern blot analyses, respectively. The cellular level of RNA transcript of *recA*-like gene was markedly increased upon treatment with DNA damaging agents.

Materials and Methods

Bacterial strains and growth conditions

E. coli strains HH49 {*△lacU169 proA*$^+$ *lon araD139 rpsL supF △(srlR-recA)*306::Tn10 (pMC9)} and AB1157 (F$^-$ *thr1 leu6 proA his4 thi1 argE3 lacY galK2 ara14 xyl15 mtl1 tsx33*) were kindly provided by Dr. J. W. Roberts (Section of Biochemistry, Molecular and Cell Biology, Cornell University, U.S.A.). *Pseudomonas* cells were obtained from Dr. Y. Park (Department of Biological Science, Chosun University, Korea). All culture supplies were purchased from Difco Laboratories. *E. coli* cells were cultured in Luria-Bertani (LB; 1% Bactotryptone, 1% NaCl, 0.5% yeast extract) or NZCYM (1% NZ amine, 0.5% NaCl, 0.5% yeast extract, 0.1% casamino acids, 0.2% $MgSO_4 \cdot 7H_2O$) media at 37℃ with vigorous shaking. *Pseudomonas* cells were cultured in King's B medium as described previously (Kho et al., 1995). *E. coli* strains Y1090 {*supF hsdR araD139 △lon △lacU169 rpsL trpC22*::Tn10 (*tet*^{r}) pMC9} and HH49 were used as host cells for the amplification of bacteriophage λgt11 and the immunological screening of the *recA*-like gene, respectively. *E. coli* DH5 {*supE44 △lacU169* (ϕ80 *lacZ △M15*) *hsdR17 recA1 end41 gyrA96 thi-1 relA1*} was used for subcloning of the cloned gene. Bacteriophage λgt11 and the plasmid pGEM3Zf(-) were purchased from Promega.

Enzymes and reagents

Nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) were from Sigma. The polyclonal rabbit anti-*E. coli* RecA antibodies were prepared as described previously (Lee et al., 1994). The secondary antibody and the nitrocellulose paper for Western blot analysis were purchased from Hyclone and Millipore, respectively. Radioactive materials including α-^{32}P-dCTP (spec. act., 3,000 Ci/mol) was obtained from Amersham. Antibiotics such as ampicillin and tetracycline were from Sigma. All restriction enzymes and DNA modifying enzymes including T4 DNA ligase and calf intestinal alkaline phosphatase (CIAP) were from Bethesda Research Laboratories or New England Bio-Labs. The packaging extract for λ phage was purchased from Promega. Reagents for the clonal selection such as isopropyl-β-D-thiogalactoside (IPTG) and 5-bromo-chloro-3-indolyl-β-D-galactoside (X-gal) were from Boehringer Mannheim Biochemicals. Methyl methanesulfonate (MMS), mitomycin-C (MMC), and nalidixic acid (NA) were purchased from Aldrich.

Transformation of E. coli

Transformation of *E. coli* cells was carried out as described by Sambrook et al. (1989).

UV-light irradiation and chemical treatments

Cells grown to mid-exponential phase were harvested, washed with distilled water, and suspended in 50 mM potassium phosphate buffer (pH 7.0). Cells (4×10^7/ml) were exposed to 254 nm UV-light from a mercury germicidal lamp at an incident dose rate of 1.42 J/m^2/sec, as determined by a No. 65 radiometer (Yellow Spring Instrument Co., Yellow Spring, Ohio). Cells were treated with MMS at 37℃ for 2 h at desired concentrations and 10% sodium thiosulfate was added to inhibit the MMS toxicity. After the treatments, approximately 500 - 800 cells were spread onto a culture plate and incubated at 37℃ for a desired time.

Polymerase chain reaction based amplification of insert DNA from putative clones

The primers used for Polymerase chain reaction (PCR) were purchased from Promega. The sequences of λ gt11 forward and reverse primers were 5′-GGTGGCGA CGACTCCAGCCCG-3′ and 5′-TTGACACCAGACCAACT GATG-3′, respectively. PCR was performed by the method originally described by Saiki et al. (1985). Phage lysate was diluted in 20 μl of a solution containing 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 0.1% gelatin), 200 μM of each dNTPs, 50 pmol of each primers, and 1 unit of *Taq* DNA polymerase. After predenaturating at 94℃ for 15 sec, the mixture was subjected to 40 cycles consisting of four serial steps as follows: primer annealing at 62℃ for 10 sec, primer elongation at 72℃ for 60 sec, denaturation at 94℃ for 5 sec, and post-elongation at 72℃ for 5 min. The PCR products were analyzed on a 0.7% agarose gel containing 0.5 μg/ ml of ethidium bromide.

Southern and Northern blot analyses

Southern and Northern blot analyses were carried out as described by Southern (1975) and Yang et al.

(1993), respectively. DNA probes were prepared by the oligolabeling method (Feinberg and Vogelstein, 1984).

Construction of Pseudomonas genomic expression library

Pseudomonas genomic DNA was isolated as described by Cryer et al. (1975) with a minor modification. The genomic DNA (about 20 μg) isolated from the fluorescent *Pseudomonas* cells was partially digested with *Eco*RI and internal *Eco*RI sites were protected by methylation. The degree of partial digestion was monitored on agarose gel and 2 - 8 kb fragments were electro-eluted from the gel. The eluted DNA fragments were ligated with *Eco*RI-cleaved λgt11 vector arms at an end-molar ratio of about 1:1 for 12 h or more at 16℃. The ligated DNA was packaged *in vitro* with packaging extracts. The packaged phages were diluted to 10^{-2} in λ-diluent (10 mM Tris-HCl, pH 7.5, 10 mM $MgSO_4$). One hundred milliliter of the diluted phages was mixed with an equal volume of plating bacteria and incubated at 30℃ for 30 min. Three milliliter of molten LB top agar (45℃) containing 40 μl of X-gal (20 mg/ml in dimethyl formamide) and 4 μl of IPTG (200 mg/ml in distilled water) was added and the mixture was poured onto an LB plate. After standing at room temperature for 5 min, the plate was incubated at 37℃ for 8-10 h. The recombinant frequency was scored by counting blue and colorless plaques.

Immunological screening of the recA-like gene from Pseudomonas expression library constructed in λgt11 vector

The *Pseudomonas* expression library constructed was diluted to make 2,000 plaque forming units (pfu) with SM buffer {50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 8.3 mM $MgSO_4$, 0.01% (w/v) gelatin} and incubated with *E. coli* HH49 cells at 37℃ for 15 min. The infected cells were mixed with 3 ml of top agar and then immediately plated onto NZCYM plates. The plates were incubated at 42℃ for 3.5 h. The NC filters, which had been soaked in 10 mM IPTG solution and air-dried, were placed onto the plates and further incubated at 37℃ for 3.5 h. The filters were removed from the plates, washed with TNT buffer {50 mM Tris-HCl, pH 7.4, 200 mM NaCl, 0.1% (v/v) Triton X-100} and incubated with 3% casein in TBS (20 mM Tris-HCl, pH 7.5, 500 mM NaCl) for 2 h at room temperature. The filters were reacted with the 1,000-fold diluted-polyclonal anti-*E. coli* RecA antiserum for 2 h and rinsed with TBS. Thereafter, the filters were incubated with anti-rabbit IgG conjugated to alkaline phosphatase and the blots were stained with AP solution (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 10 mM $MgCl_2$) containing 165 μg/ml of BCIP and 330 μg/ml of NBT for visualization.

Construction of a plasmid pLK200 harboring Pseudomonas recA-like gene

The isolated C-1 DNA was cleaved with *Eco*RI and the 3.2 kb insert DNA was electro-eluted from the agarose gel. The DNA was ligated to pGEM3Zf(-) vector digested with *Eco*RI using T4 DNA ligase. The ligated DNA was transformed into *E. coli* DH5 cells and recombinant clones were selected on a X-gal/IPTG plate containing 50 μg/ml of ampicillin. The resulting plasmid harboring a 3.2 kb insert DNA was designated as pLK200.

Results and Discussion

Isolation of a recA-like gene from Pseudomonas genomic expression library and identification of insert DNA by PCR

An expression library was constructed in bacteriophage λgt11 for the isolation of *Pseudomonas recA*-like gene. Genomic DNA was isolated from fluorescent *Pseudomonas* cells and partially digested with *Eco*RI restriction enzyme. After monitoring the degree of partial digestion on agarose gel, DNA fragments ranging from 2 to 8 kb in size were electro-eluted and ligated with λgt11 vectors which had been cleaved with *Eco*RI. The DNA ligates were *in vitro* packaged with λ packaging extracts as described in Materials and Methods. The packaged phages were allowed to infect host *E. coli* Y1090 cells and the frequencies of non-recombinants versus recombinant plaques were scored on X-gal/IPTG plates (data not shown). The recombinant frequency of this genomic expression library was more than 90% and the number of recombinants was approximately 6×10^4 pfu, implying that a complete *Pseudomonas* library was constructed.

The *Pseudomonas recA*-like gene was isolated by screening the library for antigens cross-reacting to polyclonal anti-*E. coli* RecA antibodies (Young and Davis, 1983). To enhance specific reaction with a phage-encoded fusion protein, *E. coli* HH49 cells with a total deletion in the *recA* gene (Hurd et al., 1987) were used as plating bacteria for the bacteriophages. From 10^5 plaques screened, 35, 20, and 5 putative clones were isolated by the first-, second-, and third-round of screenings (Fig. 1). Five clones screened were amplified by PCR to identify their insert DNAs using the forward and reverse primers of λgt11. As shown in Fig. 2, all clones contained a 3.2 kb DNA insert.

To examine the sequence homology, the third clones were hybridized with the insert DNA of clone C-1 as a probe (Fig. 3). The phage DNAs isolated from 5 third clones such as C-1, C-2, C-4, C-5, and C-6 were digested with the *Eco*RI restriction enzyme. As shown in Fig. 3A, all clones contained a 3.2 kb insert DNA and a clone C-4 harbored a 6.9 kb DNA insert cleavable to 3.7 kb and 3.2 kb fragments by *Eco*RI digestion (Fig. 3A, lane 3). The cleaved DNAs were electrophoresed on a 0.7% agarose gel (Fig. 3A) and Southern blot analysis was performed with α-^{32}P-labeled 3.2 kb

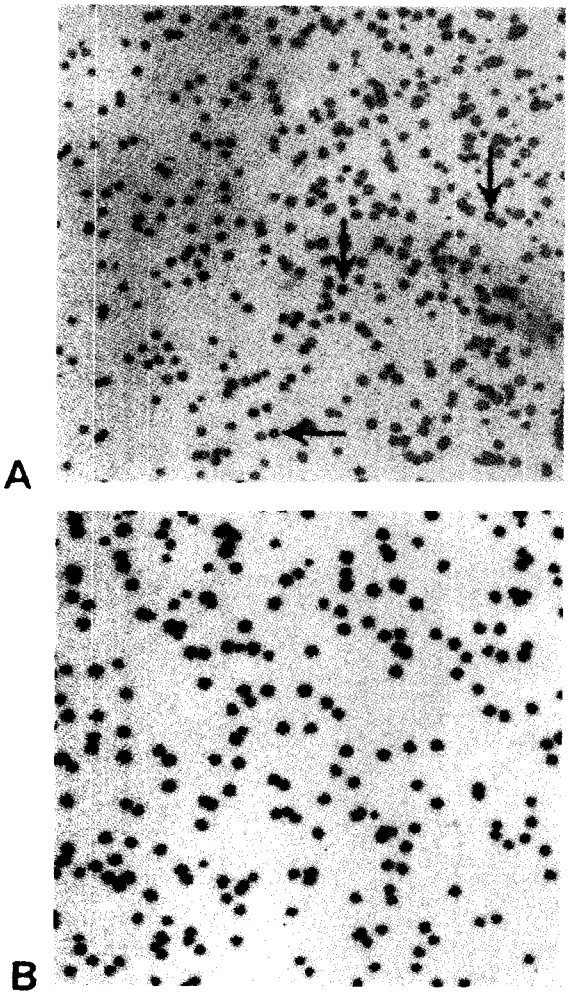

Fig. 1. Immunoscreening of the RecA-like protein-producing plaques from *Pseudomonas* genomic expression library constructed in λgt11. *Pseudomonas* library was transfected into HH49 *E. coli* cells, plated onto LB plates, and incubated for 3.5 h at 42℃. Thereafter, the plates were overlaid with 10 mM IPTG-impregnated NC filter and further incubated at 37℃ for 3.5 h. The filters were removed from the plates and immunoblot analysis was performed with polyclonal anti-*E. coli* RecA antibodies, as described in Materials and Methods. A, First screening. Arrows indicate the putative clones showing positive signal. B, Third screening. Almost all of the clones show positive signals.

C-1 insert DNA as a probe (Fig. 3B). The DNA inserts of C-4, C-5 and C-6 clones were hybridized to the C-1 insert DNA. Of the two *Eco*RI digests of clone C-4 only the 3.2 kb fragment hybridized to the C-1 insert DNA (Fig. 3B, lane 3). These results suggest that C-1, C-4, C-5 and C-6 clones contain the same DNA inserts. However, the insert DNA from clone C-2 hybridized very lightly to that of the C-1 clone (Fig. 3B, lane 2). This result suggests that this clone has low homology to other clones or only small part of the insert DNA of this clone has homology to that of the C-1 clone. Among these third clones, C-1 was selected for further study.

Localization of the cloned recA-like gene by Southern hybridization analysis

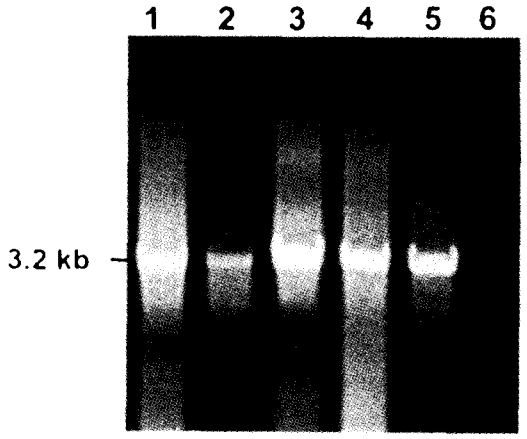

Fig. 2. Identification of inserts from the isolated 5 clones by polymerase chain reaction. Five recombinant clones isolated by the third screening were subjected to PCR with the λgt11 forward and reverse primers as described in Materials and Methods. The PCR products were electrophoresed on a 0.7% agarose gel and stained with 0.5 μg/ml of ethidium bromide. Lanes 1, 2, 3, 4, and 5 were loaded with DNA produced from clones C-1, C-2, C-5, C-6, and C-7, respectively. Lane 6 was loaded with λgt11 DNA.

In order to confirm that the cloned *recA*-like gene is located in the chromosomal DNA of *Pseudomonas*, Southern hybridization analysis was performed (Fig. 4). The genomic DNA isolated from *Pseudomonas* cells was digested with several restriction enzymes such as *Eco*RI, *Bam*HI, *Hin*dIII, and *Pst*I (Fig. 4A) and hybridized with α-^{32}P-labeled 3.2 kb insert DNA of the C-1 clone as a probe (Fig. 4B). As shown in Fig. 4B, a single band 3.2 kb in size was found in *Eco*RI-digested *Pseudomonas* chromosomal DNA. This result indicates that *Pseudomonas* chromosomal DNA contains the same restriction sites as the cloned gene and the *recA*-like gene of *Pseudomonas* is a single copy.

Construction of a plasmid pLK200 harboring Pseudomonas recA-like gene and identification of the RNA transcript by Northern hybridization analysis

The 3.2 kb *Eco*RI fragment of C-1 clone was introduced into the *Eco*RI site of the pGEM3Zf(-) vector to construct a plasmid pLK200 as described in Materials and Methods (data not shown). To determine the transcript size and to analyze the inducibility of the *recA*-like gene against DNA damaging agents, 20 μg each of total RNA was extracted from *Pseudomonas* cells treated with 0.3 μg/ml of MMC for 0, 15, and 150 min, respectively. The RNAs were electrophoresed on an 1.0% formaldehyde agarose gel, transferred onto an NC filter, and hybridized with α-^{32}P-labeled 3.2 kb *Eco*RI fragment of pLK200 under the condition of 50% formamide (Fig. 5). The hybridization was carried out for 18 h at 62℃ and the autoradiogram was developed after exposing for 2 days. As shown in Fig. 5B, the cloned *Pseudomonas recA*-like gene was transcribed into a 1.1 kb RNA transcript and the amount of transcript was markedly increased by MMC treatment. These results indicate that the size of RNA transcript is

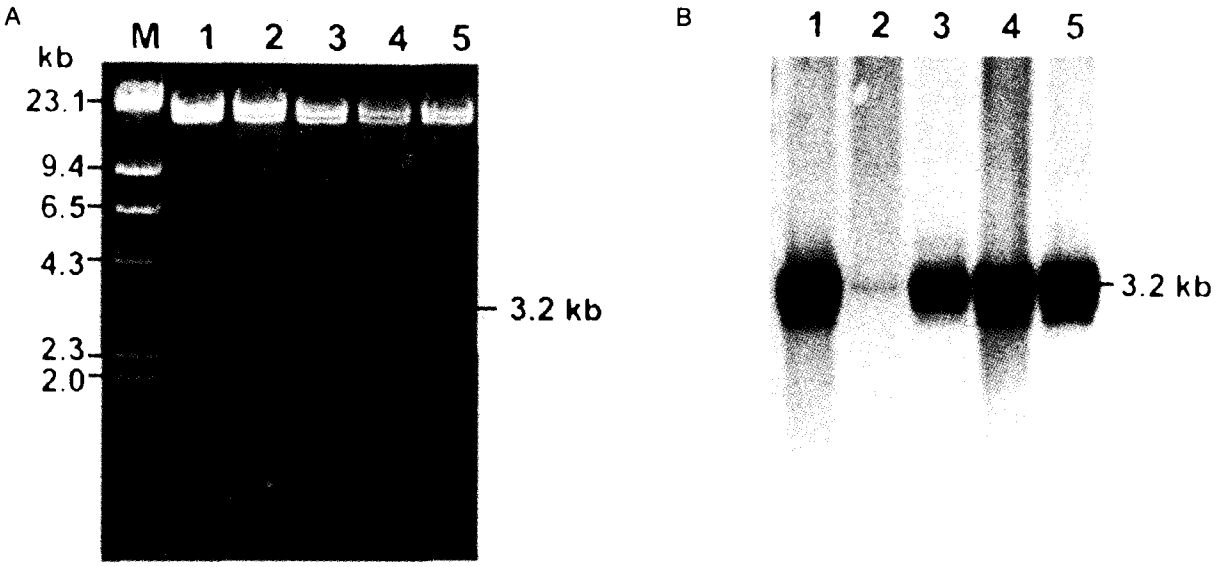

Fig. 3. Cross-hybridization between the isolated clones. Phage DNA was isolated from 5 clones, C-1, C-2, C-3, C-4, and C-5, digested with *Eco*RI, and then electrophoresed on a 0.7% agarose gel (A). The DNA electrophoresed was transferred onto an NC filter and probed with the α-^{32}P-labeled 3.2 kb insert DNA of C-1 clone and autoradiographed on an X-ray film (B). Lanes 1, C-1; 2, C-2; 3, C-4; 4, C-5; 5, C-6.

sufficient to translate into a 42 kDa protein and that the *recA*-like gene is inducible by DNA damage. These results are also well matched to those of our previous observation, in that the 42 kDa *Pseudomonas* RecA-like protein, which is structurally and functionally related to the *E. coli* RecA protein, has an inducible characteristics by DNA damaging agents (Kim et al., 1998).

Inducibility of the RNA transcript of the cloned recA-like gene of Pseudomonas by various DNA damaging agents

The DNA damage inducible properties of *Pseudomonas recA*-like gene were further studied by slot-blot Northern hybridization analysis (Fig. 6). Exponentially growing cells were treated with 30 μg/ml of NA and 0.3 μg/ml of MMC for 150 min. UV-light was irradiated at a dose of 10 J/m^2. MMS was treated to the cells at a final concentration of 5 mM for 2 h. From the damaged cells, total RNA was isolated, slot-blotted onto an NC filter,

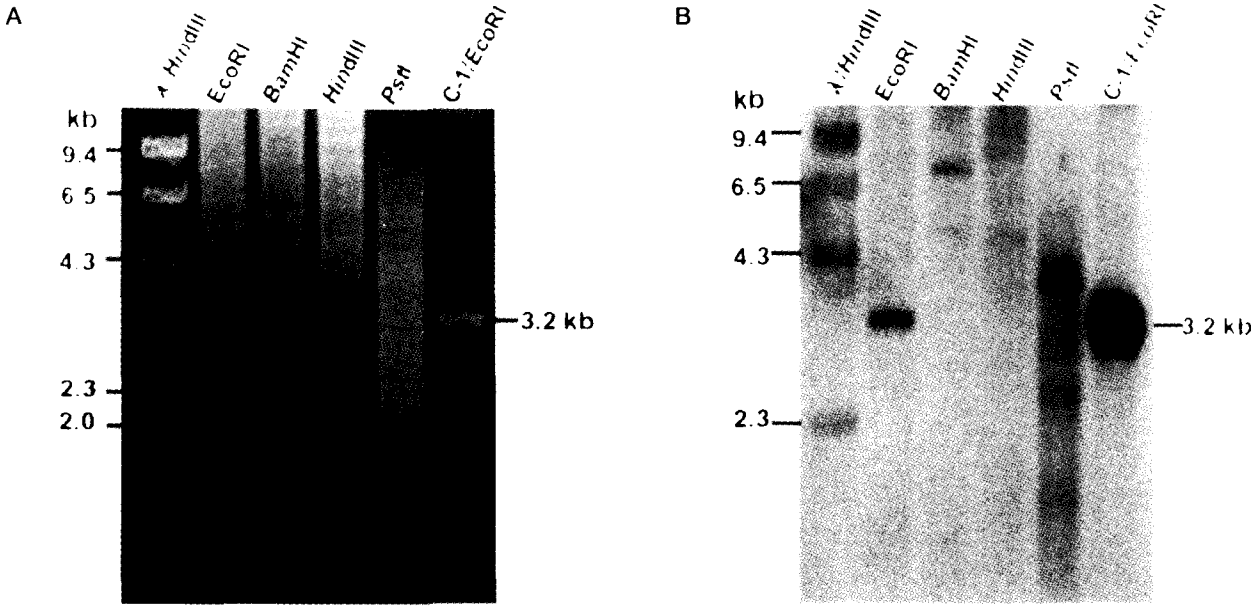

Fig. 4. Southern hybridization analysis of *Pseudomonas* genomic DNA with the insert DNA of clone C-1. Genomic DNA was isolated from *Pseudomonas* cells and digested with several restriction enzymes as indicated. Each 5 μg of the digested DNA was electrophoresed on a 0.7% agarose gel, transferred onto an NC filter, and then hybridized with a 3.2 kb C-1 insert DNA labeled with α-^{32}P-dCTP as a probe. λ/*Hin*dIII, λ bacteriophage DNA digested with *Hin*dIII. C-1/*Eco*RI, C-1 DNA digested with *Eco*RI.

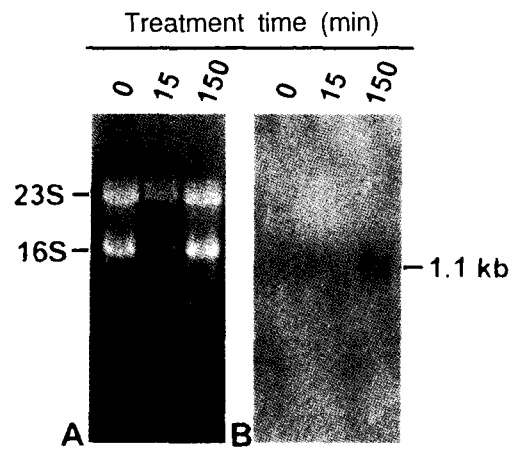

Fig. 5. Northern hybridization analysis of the RNA transcript of *Pseudomonas recA*-like gene. *Pseudomonas* cells grown to an exponential growth phase were treated with 0.3 μg/ml of MMC for the indicated time points and then total cellular RNA was extracted. The RNA was electrophoresed on an 1.0% formaldehyde gel, transferred onto an NC filter, and then hybridized with 3.2 kb DNA insert of pLK200 labeled with α-^{32}P-dCTP. A, Agarose gel stained with 0.5 μg/ml of ethidium bromide. B, The autoradiogram of panel A.

and probed with α-^{32}P-labeled 3.2 kb DNA insert of pLK200. Autoradiography was performed on an X-ray film and the intensity of autoradiogram was measured by densitometric scanning. The level of RNA transcripts of *Pseudomonas recA*-like gene was increased to 5.5-, 6.3-, 4.3-, and 4.5-folds by the NA, MMC, UV-light, and MMS treatments, respectively. Taken together, the transcription level of the *recA*-like gene was increased to an average of 5.15-fold by treatment with the DNA damaging agents tested. This is in good accordance with the result of Northern analysis in Fig. 5. These

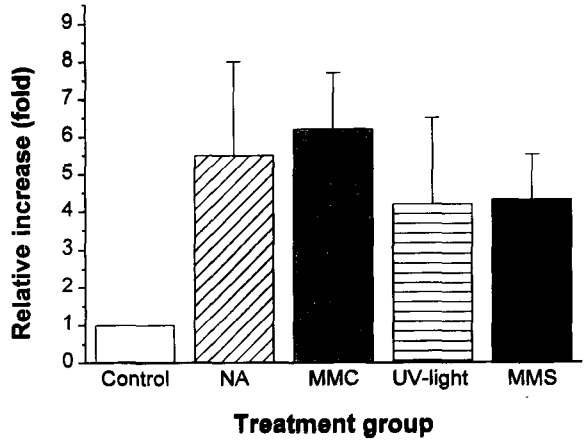

Fig. 6. Inducibility of the RNA transcript of *Pseudomonas recA*-like gene by various DNA damaging agents. Exponentially growing cells were treated with 30 μg/ml of nalidixic acid (NA) and 0.3 μg/ml of mitomycin-C (MMC) for 150 min. Methyl methanesulfonate (MMS) was treated at a final concentration of 5 mM for 2 h. UV-light was irradiated to a dose of 10 J/m^2. From the damaged cells, total RNA was isolated, slot-blotted onto an NC filter, and probed with α-^{32}P-labeled 3.2 kb DNA insert of pLK200. Autoradiography was performed on an X-ray film and the intensity of the autoradiogram was measured by densitometric scanning. The degree of inducibility was expressed as a relative increase (mean ± S.D.) in fold compared to that of the control from three independent experiments.

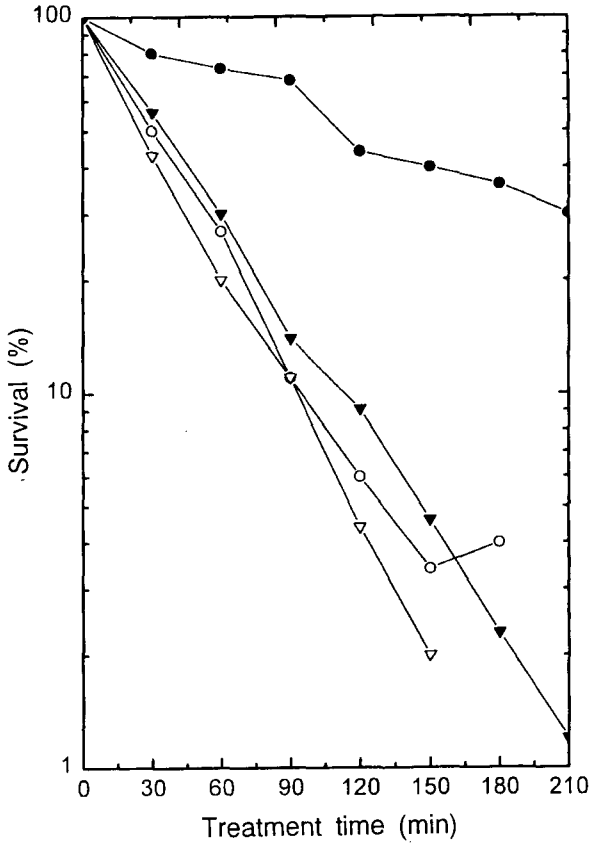

Fig. 7. DNA damage sensitivity of *E. coli recA* mutant transformed with pLK200 plasmid. The wild type *E. coli* (AB1157) and the *recA* mutant (HH49) cells which had been transformed with the plasmid pLK200 harboring the cloned *recA*-like gene were treated with 0.3 μg/ml of MMC for the indicated time and plated onto the LB plate. After incubating overnight at 37℃, colonies were counted and expressed as the rate of survival in percent. ●, *E. coli* AB1157 (*recA*$^+$); ▽, *E. coli* HH49 (*recA*$^-$); ▼, *E. coli* HH49 harboring the cloned *recA*-like gene; ○, *E. coli* HH49 harboring the plasmid pGEM3Zf(-) vector only.

results suggest that the *recA*-like gene of *Pseudomonas* is highly inducible to DNA damage like other bacterial *recA* genes (Walker, 1984; Angov and Camerini-Otero, 1994; Chen and Michel,1998).

Complementation of the cloned recA-like gene to the DNA damage sensitivity of E. coli recA mutant

As mentioned previously, the structure and function of *recA* gene is conserved in many bacteria (Walker, 1984; Kato and Kuramitsu, 1993; Lovett et al., 1994; Chen and Michel, 1998). In this study, we tested whether the cloned gene can complement the DNA damage sensitivity of the *E. coli recA* mutant (Fig. 7). *E. coli* HH49 in which *recA* gene is totally deleted was transformed with the pLK200 plasmid and the transformed cells were treated with 0.3 μg/ml of MMC for the indicated time. As shown in Fig. 7, the *Pseudomonas recA*-like gene did not restore the DNA damage sensitivity of the *E. coli recA* mutant. This result suggests a possibility that the promoter of cloned *Pseudomonas recA*-like gene can not operate in *E. coli*.

The data presented in this study have demonstrated that an SOS-like response triggered by a *recA*-like gene product is also conserved in fluorescent *Pseudomonas* sp. cells. The *recA*-like gene of *Pseudomonas* was expressed markedly by various DNA damaging agents including nalidixic acid, methyl methanesulfonate, mitomycin-C, and UV-light. This is a typical property in all kinds of *recA* genes found in bacteria (Cox and Lehman, 1987; Ewing, 1995; Rashid et al., 1997) and in some eukaryotic cells (Shinohara et al., 1992; Lovett et al., 1994).

Therefore, it would be interesting to know whether the cloned *recA*-like gene also has the same regulatory function to cause SOS response in *Pseudomonas* cells and if expression of the *recA*-like gene can also be regulated by the LexA-like protein in the normal state as in *E. coli* (Lovett et al., 1994). The nucleotide sequence of the cloned *recA*-like gene is now being analyzed to reveal a structural similarity to other bacterial *recA* genes and to examine the existence of an SOS box in its 5′-flanking region (Rashid et al., 1996; Sandler et al., 1996; Anderson and Kowalczykowski, 1998).

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