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Nucleotide Sequence of the *Porphyromonas gingivalis* W83 *recA* Homolog and Construction of a *recA*-Deficient Mutant

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Degenerate oligonucleotide primers were used in PCR to amplify a region of the *recA* homolog from *Porphyromonas gingivalis* W83. The resulting PCR fragment was used as a probe to identify a recombinant λ DASH phage (L10) carrying the *P. gingivalis recA* homolog. The *recA* homolog was localized to a 2.1-kb *Bam*HI fragment. The nucleotide sequence of this 2.1-kb fragment was determined, and a 1.02-kb open reading frame (341 amino acids) was detected. The predicted amino acid sequence was strikingly similar (90% identical residues) to the RecA protein from *Bacteroides fragilis*. No SOS box, characteristic of LexA-regulated promoters, was found in the 5′ upstream region of the *P. gingivalis recA* homolog. In both methyl methanesulfonate and UV survival experiments the *recA* homolog from *P. gingivalis* complemented the *recA* mutation of *Escherichia coli* HB101. The cloned *P. gingivalis recA* gene was insertionally inactivated with the *ermF-ermAM* antibiotic resistance cassette to create a *recA*-deficient mutant (FLL33) by allelic exchange. The *recA*-deficient mutant was significantly more sensitive to UV irradiation than the wild-type strain, W83. W83 and FLL33 showed the same level of virulence in in vivo experiments using a mouse model. These results suggest that the *recA* gene in *P. gingivalis* W83 plays the expected role of repairing DNA damage caused by UV irradiation. However, inactivation of this gene did not alter the virulence of *P. gingivalis* in the mouse model.

Porphyromonas gingivalis, a black-pigmented, gram-negative anaerobe, has been implicated as an important etiological agent in adult periodontitis. This organism possesses several putative virulence factors (e.g., hydrolytic enzymes, fimbriae, hemagglutinin, capsule, and lipopolysaccharide) that can directly affect the periodontium or elicit host functions that result in destruction typical of advanced periodontitis (12, 23, 35). In addition, colonization of the inflammatory microenvironment of the periodontal pocket by this organism requires an ability to overcome oxidative stress resulting from bactericidal metabolites generated from neutrophils (1) and occasional exposure to air (2). Reactive oxygen metabolites such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH) are major components of the bactericidal activity of polymorphonuclear leukocytes (5, 32). The toxic oxygen metabolites can be neutralized by superoxide dismutase, catalase, and peroxidase, all of which are generally expressed by aerobic and many anaerobic bacteria (3, 31). Although *P. gingivalis* is oxygen tolerant (2) and expresses superoxide dismutase activity (6, 25, 26), it is missing catalase and peroxidase activity (2).

DNA damage is one of the lethal effects of oxygen metabolites. The *recA* gene product is a key protein in repair of DNA. In addition to being involved in homologous recombination, it is a regulatory protein that is induced by DNA damage and mediates, in *Escherichia coli*, the expression of more than 20 genes in the SOS regulatory network (reviewed in reference 24). Effects of SOS induction are many and include increased DNA repair, prophage induction, and/or an increased rate of mutagenesis (reviewed in reference 39). Since an important source of spontaneous DNA damage is attack by reactive oxygen species (15), it is reasonable to assume that DNA repair ability may play a role in the virulence of *P. gingivalis*. DNA repair ability may also be important for survival of *P. gingivalis* in the periodontal pocket, where a high level of inflammation and neutrophil infiltration occurs (19).

Here we report the nucleotide sequence determination of a *recA* homolog from *P. gingivalis* W83. This gene complemented the *recA* mutation of *E. coli* HB101. Using the cloned *recA* homolog, a *recA*-defective mutant was constructed by allelic exchange. This strain demonstrated an increased sensitivity to UV irradiation. Further, we found that the *recA* mutation did not affect the virulence of that mutant in a mouse model. This *recA*-defective strain of *P. gingivalis* W83 will facilitate genetic complementation analysis and vaccine development.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *P. gingivalis* W83 was grown in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) supplemented with hemin (5 μg/ml), vitamin K (0.5 μg/ml), and cysteine (1%). *E. coli* strains were grown in Luria-Bertani (LB) broth (33). Unless otherwise stated, all cultures were incubated at 37°C. *P. gingivalis* was maintained in an anaerobic chamber (Coy Manufacturing, Ann Arbor, Mich.) in 10% H_2, 10% CO_2, and 80% N_2. Growth rates for *P. gingivalis* strains grown in BHI broth supplemented with vitamin K and hemin were determined spectrophotometrically (optical density at 660 nm).

DNA isolation and analysis. *P. gingivalis* chromosomal DNA was prepared by the method of Marmur (22). Plasmid DNA extraction followed the alkaline lysis procedure of Birnboim and Doly (4). Plasmids were purified by cesium chloride-ethidium bromide centrifugation as described by Sambrook et al. (33). λ DNA was prepared as described by Sambrook et al. (33). DNA was digested with restriction enzymes as specified by the manufacturer (GIBCO BRL, Gaithersburg, Md.). Southern blot transfer was done according to the method of Smith and Summers (37). DNA hybridization was done at 42°C with 50% formamide to allow a stringency of 80%. The blot was washed with 0.1% SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7]) and 0.1% sodium dodecyl sulfate. DNA labeling and autoradiography were done as reported previously (21).

DNA sequencing. DNA from the 2.1-kb *Bam*HI fragment containing the *recA* homolog from L10 was subcloned into pUC19. Nucleotide sequences were determined by the dideoxy chain termination method (34) with a Perkin-Elmer (Foster City, Calif.) DNA sequencing kit using M13 forward (−40) and M13

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reverse (−48) primers (United States Biochemical Corporation, Cleveland, Ohio) and analyzed on an Applied Biosystems (Foster City, Calif.) model 373A DNA sequencing system at the DNA core facilities of Virginia Commonwealth University (Richmond, Va.) and Loma Linda University (Loma Linda, Calif.). Overlapping oligonucleotide primers were synthesized at the DNA core facilities of Virginia Commonwealth University and Loma Linda University. Nucleotide sequences were analyzed with the Genetics Computer Group sequence analysis software package (8) and the MacVector sequence analysis software package for the Macintosh (International Biotechnologies, Inc., New Haven, Conn.).

PCR conditions. Degenerate oligonucleotide primers were designed as previously described (9). PCR amplification was performed with a Perkin-Elmer Cetus DNA thermal cycler (Perkin-Elmer Corporation, Norwalk, Conn.) as described by Sambrook et al. (33). The reaction mixture (100 μl) contained 700 ng of each primer, 70 ng of template DNA, and 1 μl of Vent Polymerase (New England Biolabs, Beverly, Mass.). PCR consisted of 28 cycles with a temperature profile of 1 min at 94°C, 2 min at 48°C, and 3 min at 72°C. Total DNA from *P. gingivalis* W83 was used as a template. The PCR-amplified DNA product was purified by phenol-chloroform extraction and ethanol precipitation and was identified by agarose gel electrophoresis. PCR fragments used in further manipulations were isolated from 0.7% agarose gels run in Tris-borate-EDTA buffer (33). Desired fragments were purified by a phenol freeze-thaw method followed by ethanol precipitation (33).

UV and MMS sensitivity measurements. Samples (0.1 ml) of exponentially growing cells at a 10^{-5} dilution were spread on LB or BHI agar. The cells were then irradiated with increasing doses of UV with the Stratalinker 2400 (Stratagene, La Jolla, Calif.). Plates were incubated at 37°C for 24 h and examined for growth. For methyl methanesulfonate (MMS) sensitivity experiments, samples (0.1 ml) of exponentially growing cells at a 10^{-4} dilution were plated on LB agar containing various concentration of MMS (Sigma, St. Louis, Mo.). Plates were incubated at 37°C for 24 h and examined for growth.

Mutagenesis of the cloned *P. gingivalis recA* homolog. pFLL23 was constructed by subcloning a 1.8-kb *Eco*RI-*Pst*I fragment carrying the *recA* homolog into pUC19 digested with *Eco*RI and *Pst*I. The *ermF-ermAM* cassette was purified from pVA2198 digested with *Eco*RI and *Bam*HI (11) and treated with Klenow to fill in the single-stranded ends. pFLL23 was linearized at a unique *Hin*cII site located within codon 147 of the *recA* open reading frame and ligated with the *ermF-ermAM* cassette (11). After transformation into *E. coli* DH5α and selection on erythromycin (300 μg/ml) plates, a single colony (FLL24) was chosen for further study.

Electroporation of *P. gingivalis*. Electroporation of cells was performed as previously reported (11). One milliliter of an actively growing culture of *P. gingivalis* was used to inoculate 10 ml of BHI broth supplemented with hemin and vitamin K, which then was incubated overnight at 37°C. Seventy milliliters of warmed medium (37°C) then was inoculated with 3 ml of the overnight culture and incubated for an additional 4 h. The cells were harvested by centrifugation at 2,600 × *g* for 7 min at 4°C and washed in 70 ml of electroporation buffer (10% glycerol and 1 mM $MgCl_2$ filter sterilized and stored at 4°C), and the pellet was suspended in 0.5 ml of electroporation buffer. A 100-μl sample of cells, to which 1 μg of DNA was added, was placed in a sterile electrode cuvette (0.2-cm gap). The cells were pulsed with a Bio-Rad gene pulser for 4.2 ms at 2,500 V (12.5 kV/cm) and then incubated on ice for 3 min. The cell suspension was added to 0.5 ml of BHI broth supplemented with hemin and vitamin K and incubated for approximately 16 h. A 100-μl sample was plated on solid medium containing clindamycin (5 μg/ml) and incubated anaerobically at 37°C for 7 to 10 days.

Virulence testing. *P. gingivalis* W83 and the mutant strain FLL33 were tested for invasiveness in a mouse model, as previously described (11). One milliliter of an actively growing culture of *P. gingivalis* was used to inoculate 15 ml of tryptic soy broth supplemented with hemin (1 μg/ml), vitamin K (1 μg/ml), and dithiothreitol (0.5 μg/ml), which then was incubated overnight at 37°C. Eighty-five milliliters of warmed medium (37°C) was then inoculated with 15 ml of the overnight culture and incubated for an additional 4 h. This culture was then used to inoculate 900 ml of tryptic soy broth, and the mixture was incubated for 24 h at 37°C. The cells were centrifuged, washed in sterile phosphate-buffered saline (0.147 M NaCl, 0.01 M sodium phosphate [PBS]) under anaerobic conditions, and adjusted to the desired concentration in PBS. All mice were challenged by subcutaneous injections of 0.1 ml of bacterial suspension at two sites on the dorsal surface. Mice were then examined daily to assess their general health status, as well as the presence and location of lesions. Body weights were determined for all surviving mice. These experiments were performed under authorization of an institutionally approved animal use protocol (20).

Nucleotide sequence accession number. The nucleotide sequence reported here may be found under GenBank accession no. U70054.

RESULTS

Cloning of the *recA* homolog from *P. gingivalis* W83. PCR was used to amplify a 320-bp fragment of the *recA* sequence of *P. gingivalis* W83. This PCR fragment was ^{32}P labeled and used to screen a λ DASH recombinant phage bank of *P. gingivalis* W83 genomic DNA (10) for the presence of hybridizing clones. Ten of 1.0×10^3 phage clone plaques (1.0%) hybridized with the probe.

The hybridizing phage plaques were amplified and absorbed onto maltose-grown *E. coli* DH5α cells. DNA from the phage clones was isolated with the Promega Lambda Wizard DNA purification system. *Not*I-*Bam*HI cleavage of purified DNA from two of the recombinants (L2 and L10) revealed that the phage clones had different restriction fragment patterns (data not shown), although L2 and L10 each contained a 2.1-kb fragment (data not shown). L2 also contained additional fragments of 8.0 and 6.5 kb, in contrast to L10, which contained fragments of 11, 5.8, and 0.3 kb. These data indicate that these were independent clones and not siblings from a single cloning event. L10 was chosen for further study.

Southern blot hybridization with the ^{32}P-labeled 0.3-kb PCR fragment of the *recA* gene used as a probe identified the hybridizing fragment. Plasmid pUC19 was used to subclone a 2.1-kb hybridizing *Bam*HI fragment from L10 (designated pFLL26).

Nucleotide sequencing of the *recA* homolog. Both strands of the 2.1-kb *Bam*HI fragment carried on pFLL26 were sequenced, and one 1.02-kb open reading frame corresponding to a 36-kDa protein was detected (Fig. 1). There was a start codon at base 774. A purine-rich sequence found in *E. coli* ribosome binding sites was also seen three bases upstream from the initiation site (Fig. 1). Sequences resembling procaryotic −10 and −35 promoter regions were detected at bases 749 and 729, respectively. The calculated G+C ratio for the *recA* homolog was 50%, which is close to the ratio of 46 to 48% previously reported for genomic *P. gingivalis* DNA (36). No SOS box (39) was found in the 5′ upstream region of the *P. gingivalis recA* homolog. A comparison of the amino acid sequence of this gene with the National Center for Biotechnology Information genetic sequence data bank revealed the highest similarities, of approximately 90, 86, and 82%, to the RecA proteins from *Bacteroides fragilis*, *Prevotella ruminicola*, and *Mycobacterium smegmatis*, respectively (Fig. 2). Furthermore, regions from amino acids 68 to 81 and 266 to 288 revealed conserved ATP binding domains (14, 17, 38).

Complementation of an *E. coli recA*-deficient mutation by the *P. gingivalis recA* homolog. *E. coli* HB101 transformed with plasmids carrying the *recA* gene from *P. gingivalis* was irradiated with increasing doses of UV (Table 1). HB101 strains carrying pFLL26 and pFLL23 (with the intact *recA* gene in the opposite orientation to that of pFLL26) were more resistant than the wild-type strain to UV exposure. Further, UV sensitivity was similar to the level of the wild-type HB101 strain in cells that carried pFLL24, in which the *recA* gene was interrupted with the *ermF-ermAM* cassette (Table 1). On LB medium containing MMS, only HB101 cells that carried the intact *recA* gene were able to grow at a concentration of >0.04% (Table 1).

Construction of a *recA*-defective mutant in *P. gingivalis* W83. An isogenic *recA*-defective mutant of *P. gingivalis* W83 was constructed by allelic exchange mutagenesis (Fig. 3). The nucleotide sequence of the cloned *recA* fragment revealed a unique *Hin*cII restriction site at bp 435 of the open reading frame. To utilize this site, a 1.8-kb *Eco*RI-*Pst*I fragment containing the intact *recA* gene was subcloned into *Eco*RI–*Pst*I-cleaved pUC19. The resulting plasmid, pFLL23, was digested with *Hin*cII and ligated with the 2.1-kb *ermF-ermAM* cassette from pVA2298. The recombinant plasmid, pFLL24, was used as donor DNA in electroporation of *P. gingivalis* W83. Since the plasmid was unable to replicate in *P. gingivalis*, clindamycin-resistant transformants could arise only as a result of an integration into the wild-type gene on the chromosome. We

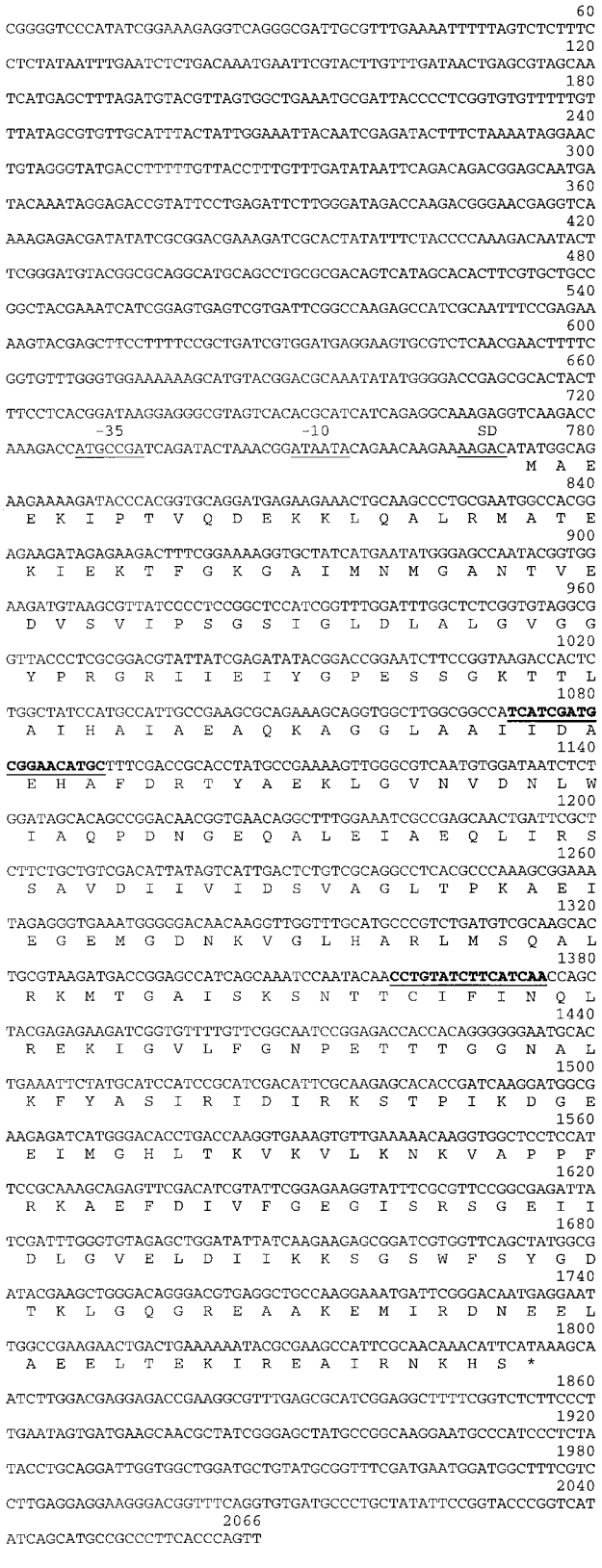

FIG. 1. Nucleotide sequence of the *recA* gene and its flanking regions from *P. gingivalis* W83. The deduced amino acid sequence of the open reading frame is indicated below the nucleotide sequences. Potential procaryotic −35 and −10 promoter sequences, as well as a possible Shine-Dalgarno (SD) sequence, are underlined. The stop codon (TAG) is indicated by an asterisk. Sequences which served as primer-binding sites for PCR amplification are in underlined boldface.

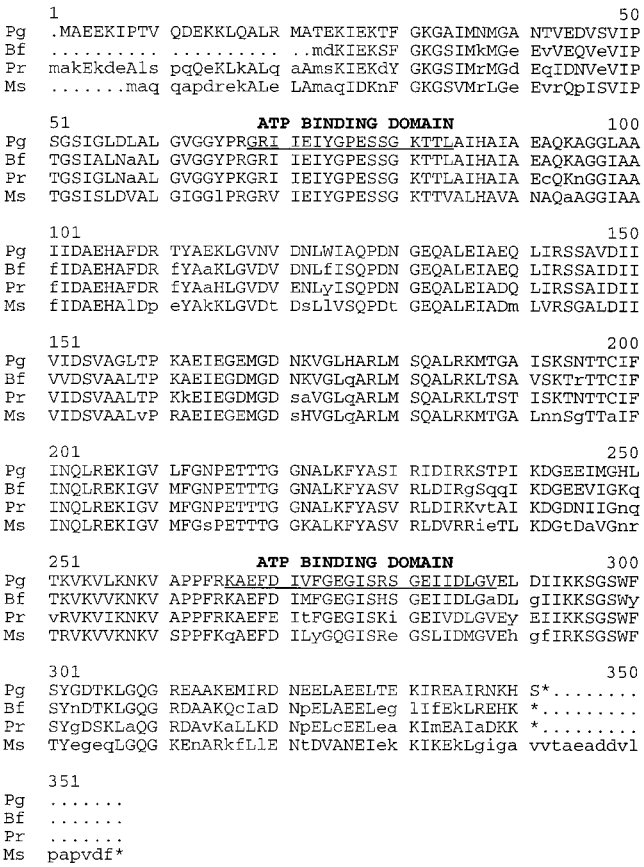

FIG. 2. A comparison of amino acid sequences of the *P. gingivalis* W83 RecA homolog and other RecA proteins of procaryotic origin. Uppercase letters indicate conserved amino acids and/or amino acids with conservative replacements according to the following groupings: ILVM, PAGST, HKR, and QNED. Abbreviations: Pg, *P. gingivalis* W83 RecA homolog; Bf, *B. fragilis* (14); Pr, *P. ruminicola* (accession no. U21227); Ms, *M. smegmatis* (accession no. X99208).

predicted that two double crossover events between the regions flanking the *erm* marker and the wild-type gene on the chromosome would result in replacement of a segment of the wild-type gene with a fragment conferring clindamycin resistance.

Following electroporation and plating on selective medium, we detected 15 clindamycin-resistant colonies after a 7-day incubation period. These colonies were replica plated onto selective medium and exposed to UV to determine their sen-

TABLE 1. Complementation of the *E. coli* HB101 *recA* mutation with the plasmid carrying the *P. gingivalis recA* homolog

Plasmid	Phenotype	MIC of MMS (%)[a]	UV radiation dose (μJ) for 0% survival[b]
None	RecA$^-$	0.01	≤500
pUC19	RecA$^-$	0.01	≤500
pFLL26	RecA$^+$	>0.04	>4,000
pFLL23	RecA$^+$	>0.04	>2,000
pFLL24	RecA$^-$	0.01	≤500

[a] MMS sensitivity was determined by spreading exponentially growing cells on LB plates containing appropriate concentrations of MMS (0.001 to 0.04%).

[b] UV sensitivity was determined by spreading exponentially growing cells on LB plates and then subjecting them to irradiation with increasing doses (0, 500, 1,000, 2,000, and 4,000 μJ) of UV with the Stratalinker 2400 (Stratagene).

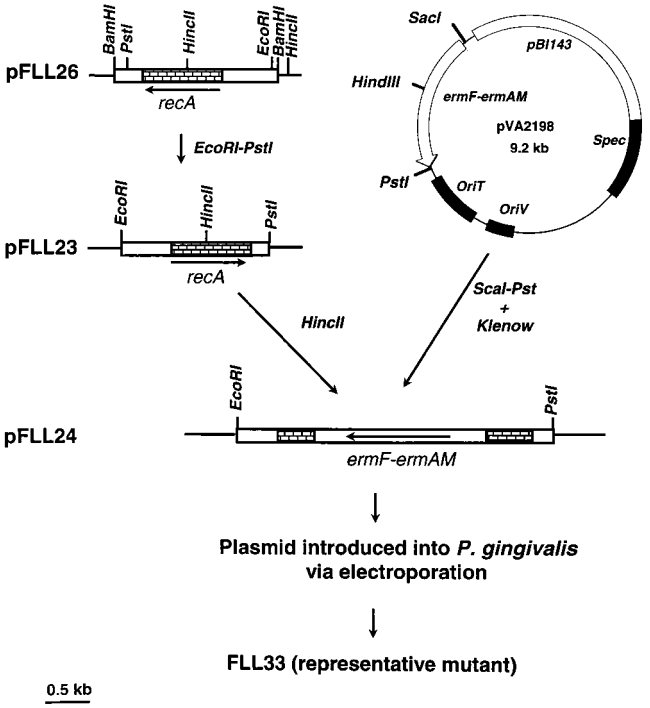

FIG. 3. Construction of a site-specific mutant by allelic exchange. pFLL24 contained the *recA* gene interrupted by an *ermF-ermAM* cassette (*ermF* confers Em^r in *P. gingivalis*, and *ermAM* confers Em^r in *E. coli* [11]). The plasmid was introduced into *P. gingivalis* W83 by electroporation. A reciprocal recombination event between areas of homology on the target cell's chromosome and regions flanking the Em^r cassette of pFLL24 replaced the *recA* gene with a fragment containing *ermF-ermAM*. *P. gingivalis* cells were incubated for about 16 h post-electroporation and plated on supplemented BHI agar with clindamycin (0.5 μg/ml) for 7 to 10 days at 37°C. The cloning vector used was pUC19.

sitivity. Four UV-sensitive colonies were chosen from the unexposed plate for further study. To confirm the presence of the *ermF-ermAM* cassette in the predicted location, chromosomal DNA from the transformants was probed with ^{32}P-labeled pFLL26 and pVA2198. If the DNA was digested with *Bam*HI, a predicted 2.1-kb fragment should have been seen in the wild-type. Since the *ermF-ermAM* cassette is missing a *Bam*HI site, a 4.2-kb fragment should have been seen in the clindamycin-resistant mutants. As shown in Fig. 4A, the predicted 2.1-kb fragment was seen in W83 (lane A). A 4.2-kb fragment was present in the clindamycin-resistant mutants of W83 (lanes B to E) when probed with ^{32}P-labeled pFLL26, which carried the *recA* gene. A similar blot probed with pVA2198, which carried the *ermF-ermAM* cassette, revealed an identical 4.2-kb hybridizing fragment present only in the clindamycin-resistant mutants (Fig. 4B, lanes B to E). No hybridizing sequences were seen in W83 (lane A). pUC19 vector sequences did not hybridize with W83 or any of the clindamycin-resistant mutants (data not shown). These data indicate that the predicted recombination had occurred, resulting in the wild-type *recA* gene being interrupted by the *ermF-ermAM* cassette.

Characterization of *P. gingivalis* W83 *recA* mutants. *recA* mutants plated on brucella blood agar plates (Anaerobic Systems Inc., San Jose, Calif.) displayed phenotypes similar to the wild-type strain, which was beta-hemolytic and black pigmented (data not shown). One strain from this group, designated FLL33, was chosen for further study. A generation time of 3 h was determined for W83, in contrast to 3.5 h for FLL33.

To reconfirm the loss of activity of the *P. gingivalis* RecA protein, we assayed the sensitivities of the wild-type and *recA* strains to UV irradiation. There was 80% survival of the wild-type strain, W83, to 1,000 μJ of UV irradiation, in contrast to 18% survival for FLL33. At 2,000 μJ there was 40% survival of the wild-type cells, compared to 0% survival for the *recA*-defective mutant. These data indicate that the *recA* gene plays an important role in *P. gingivalis* W83 survival following DNA damage by UV irradiation.

Virulence testing of FLL33 in a mouse model. At 24 h, two of five mice challenged with *P. gingivalis* W83 (wild type) at a dose of 10^{10} bacteria/animal died. Surviving animals appeared cachectic and hunched, with ruffled hair. Although the mice did not display lesions at the dorsal surface site of injection, all had developed spreading ulcerative abdominal lesions. By 48 h, all animals had died. When mice were challenged with a similar dose of strain FLL33 (*recA* mutant), one of five died after 24 h. Surviving animals appeared cachectic and hunched, with ruffled hair. None of the mice in this group displayed any lesions at the dorsal surface site of injection but all had developed spreading ulcerative abdominal lesions. Three of the surviving mice had died by 48 h, and the fourth had died by day 4.

When mice were challenged with W83 at a dose of 5×10^9 bacteria/animal, one of five died and four of five developed secondary-site lesions after 24 h. By 48 h, three of five mice in this group had died, and by day 4 only one of five survived. All had developed ulcerated abdominal lesions. The lesion in the surviving animal was resolving at day 14. In a challenge with a similar dose of FLL33, one of six mice died and five of six mice developed secondary-site lesions after 24 h. By 48 h, three of six mice in this group had died, and by day five only one of six survived. All had developed ulcerated abdominal lesions. The lesion in the surviving animal was resolving at day 14.

DISCUSSION

The degenerate oligonucleotide primers designed to amplify a conserved region in gram-positive bacteria (9) are functional in *P. gingivalis*. Further, two conserved ATP binding motifs were observed in the deduced *P. gingivalis* RecA protein, which agrees with the evolutionary conservation of this motif across bacterial species (16). In addition, the deduced *P. gingivalis* RecA protein shows strong similarity to RecA proteins from other gram-negative anaerobes (14). Similar to the RecA pro-

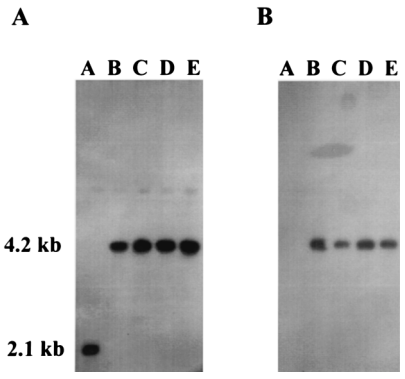

FIG. 4. Southern blot analysis of allelic exchange mutants of *P. gingivalis*. Total cellular DNA from *P. gingivalis* was cleaved with *Bam*HI, electrophoresed through 0.7% agarose, and bidirectionally transferred to nitrocellulose. (A) Lane A, W83; lanes B to E, allelic exchange mutants. The probe used was ^{32}P-labeled pFLL23, which carries the *P. gingivalis recA* homolog. (B) Lanes are as described for panel A. The probe used was ^{32}P-labeled pVA2198, which carries the *ermF-ermAM* cassette. A 4.2-kb fragment was detected in all strains which contain the *recA* homolog inactivated with *ermF-ermAM*.

teins from these gram-negative anaerobes, no consensus LexA binding sequence ($CTGTN_9CAG$) was present in the predicted promoter region of *recA*, which is in contrast to many SOS-regulated *recA* genes (39). This suggests that the predicted LexA homolog may bind a different sequence in the promoter region or that the *P. gingivalis recA* gene may not be regulated by a LexA-like protein. The *recA* genes from *B. fragilis* and *Thiobacillus ferrooxidans*, which also lack an SOS box, are not induced by DNA damage (13, 28), in contrast to *E. coli*, in which that gene, as part of the SOS regulon, is induced when the cell contains DNA damage (39). Alternatively, it is possible that the *P. gingivalis recA* gene is not part of an *E. coli*-like SOS regulon. There are other cloned *recA* genes (14, 28) that lack identifiable LexA binding sites whose regulation is unknown, and they may represent a class of non-SOS-regulated *recA* genes.

The RecA protein is functionally conserved in bacteria (16). The ability of the *recA* homolog from *P. gingivalis* to complement the *recA* mutation in *E. coli* suggests functional conservation of the RecA protein from *P. gingivalis*. Further, expression of the *recA* gene was independent of any *E. coli* promoters. Insertional inactivation of the *recA* gene with the *ermF-ermAM* cassette significantly reduced the ability for DNA repair in both *P. gingivalis* and *E. coli.*

Strain FLL33 showed a virulence profile similar to the wild-type, W83, which is consistent with a previous report (29). Strain FLL33 confirms that the *recA* gene in *P. gingivalis* W83 plays an important role in DNA repair; however, inactivation of this gene did not affect the virulence potential of *P. gingivalis* in our mouse model. A possible explanation for this phenomenon would be an ability of *P. gingivalis* to affect the normal function of polymorphonuclear leukocytes (PMNs), thus limiting or inhibiting oxidative stress. Consistent with this hypothesis, it has been demonstrated that *P. gingivalis* W83 can inhibit the induction of superoxide production in human PMNs (27). Further, the LPS from *P. gingivalis* can prevent the production of inflammatory mediators in the mouse model (30) and inhibit E-selectin expression and neutrophil adhesion, two components necessary for the recruitment of leukocytes in response to infection (7). It is possible, therefore, that *recA* may be important for the survival of *P. gingivalis* in an oral environment, with its occasional exposure to air, or in a mixed infection, where the normal function of PMNs could be induced by the presence of other bacteria. It is believed that the persistent colonization of bacteria on the tooth root surface is responsible for the inflammatory response characteristic of periodontal disease. As a late colonizer of the tooth root surface (18), *P. gingivalis* could be exposed to oxidative stress due to the host response to the accumulation of the early colonizers; thus, the *recA* gene also may be important for DNA repair in vivo. We are investigating this further.

We have constructed the first *recA*-defective strain of *P. gingivalis* by allelic exchange mutagenesis. This represents an important step that will facilitate genetic studies (e.g., complementation analysis) for elucidating the precise role of several virulence factors in the pathogenicity of *P. gingivalis*. It is also possible that the *recA* mutation may assist in the construction of whole-cell vaccine or replacement therapy strains or in the production of subunit vaccinogens with *P. gingivalis.*

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