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Cloning of the *recA* Gene from a Free-Living Leptospire and Distribution of RecA-Like Protein among Spirochetes

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A recombinant plasmid carrying the *recA* gene of *Leptospira biflexa* serovar patoc was isolated from a cosmid library of genomic DNA by complementation of an *Escherichia coli recA* mutation. The cloned serovar patoc *recA* gene efficiently restored resistance to UV radiation and methyl methanesulfonate. Recombination proficiency was also restored, as measured by the formation of Lac$^+$ recombinants from duplicated mutant *lacZ* genes. Additionally, the cloned *recA* gene increased the spontaneous and mitomycin C-induced production of lambda phage in lysogens of an *E. coli recA* mutant. The product of the cloned *recA* gene was identified in maxicells as a polypeptide with an M_r of 43,000. Antibodies prepared against the *E. coli* RecA protein cross-reacted with the serovar patoc RecA protein, indicating structural conservation. Southern hybridization data showed that the serovar patoc *recA* gene has diverged from the *recA* gene of *L. interrogans*, *Leptonema illini*, and *E. coli*. With the exception of the RecA protein of *L. interrogans* serovar hardjo, the RecA protein of the *Leptospira* serovars and *L. illini* were synthesized at elevated levels following treatment of cells with nalidixic acid. The level of detectable RecA correlated with previous studies demonstrating that free-living cells of *L. biflexa* serovars and *L. illini* were considerably more resistant to DNA-damaging agents than were those of parasitic *L. interrogans* serovars. RecA protein was not detected in cells of virulent *Treponema pallidum* or *Borrelia burgdorferi*.

Organisms in the genus *Leptospira* are thin, tightly coiled, motile, aerobic spirochetes. Although genetic studies have indicated considerable heterogeneity within the genus, only two species, *Leptospira biflexa* and *L. interrogans*, are currently recognized (13). A major difference between these species is the ecological niche that they occupy. The primary habitat of *L. interrogans*, the etiologic agent of leptospirosis, is the mammalian kidney. When these organisms are shed in urine from infected hosts, they contaminate soil and water, creating transient foci of infection. In contrast, *L. biflexa* is a nonpathogenic, free-living spirochete that is indigenous to surface water and moist soil (11, 13). In this habitat, these organisms are likely to be exposed for prolonged periods to DNA-damaging agents. Stamm and Charon (28) have recently shown that *L. biflexa* cells are less sensitive to UV radiation and mitomycin C (MC) and are more efficient in photoreactivation repair of UV-damaged DNA than are *L. interrogans* cells. Dose-response curves for both organisms to UV radiation suggest the presence of an inducible DNA repair system.

In *Escherichia coli*, agents that damage DNA or inhibit DNA replication induce a process termed the SOS response. This response involves several genes that participate in DNA repair, mutagenesis, and coordination of cell division (26, 31). Expression of the SOS response and homologous recombination is dependent upon the product of the *recA* gene. Several reports have shown that the RecA protein is structurally and functionally conserved in a variety of procaryotes (3, 5, 7, 9, 12, 15–17, 19, 21–23, 25). However, many of these organisms represented related enteric species. Data for more phylogenetically diverse genera are usually not available (19).

We are interested in the molecular genetics and physiology of organisms in the genus *Leptospira*, as well as certain other spirochetal species. For the most part, information regarding genetic organization and gene expression in these organisms is lacking. With the exception of the study of Stamm and Charon (28), there is no information on DNA repair mechanisms in any members of the order *Spirochaetales*. In the hope of enhancing our basic knowledge of the biology and ecological fitness of free-living *Leptospira* organisms, we have made use of the technique of functional complementation of *E. coli recA* mutations to clone and characterize the *recA* gene from *L. biflexa* serovar patoc. We have demonstrated that the *L. biflexa* RecA protein shares antigenic similarity to its *E. coli* counterpart and that the *recA* gene is inducible following treatment of cells with nalidixic acid. Additionally, we have sought to determine whether cells of various other *Leptospira* serovars and *Leptonema illini*, as well as certain other pathogenic spirochetes, synthesize a RecA protein.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophage. The bacterial strains, plasmids, and bacteriophage used in this study are listed in Table 1.

Media. *E. coli* strains were grown in Luria (L) broth or on L agar plates containing 100 μg of ampicillin per ml unless otherwise indicated. Recipient cells for transduction were grown as described by Maniatis et al. (18). Strains of *L. biflexa*, *L. interrogans*, and *L. illini* were grown in bovine serum albumin-polysorbate 80 medium at 30°C (4). Growth was monitored with a Coleman photonephelometer by the method of Johnson and Harris (14).

Preparation of chromosomal and plasmid DNAs. High-molecular-weight chromosomal DNA from 2×10^{10} cells of *Leptospira* spp., *L. illini*, or *E. coli* was prepared by the method of Saito and Miura (24). Plasmid DNA was prepared

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TABLE 1. Bacteria, plasmids, and bacteriophage

Strain, plasmid, or bacteriophage	Genotype or phenotype	Source or reference
Strains		
E. coli		
HB101	F⁻ *hsdS20 recA13*	This study
SE5000	F⁻ *araD139 lacU169 relA thi recA56*	This study
JC14604	F⁻ *lacMS2860811 lacBK1 Δ(srl-recA) hsr*	22
C600	F⁻ *thr-1 leuB6 tonA21 lacY1 supE44 thi-1*	B. Bachmann[a]
L. biflexa serovar:		
patoc Patoc I		This study
andaman CH 11		CDC[b]
ranarum Iowa City Frog		CDC
L. interrogans serovar:		
pomona Wickard		CDC
hardjo hardjoprajitno		This study
L. illini 3055		This study
Plasmids		
pHC79	Amp^r Tet^r	18
pRecA35	Amp^r Tet^s; contains *E. coli* MLS *recA* region	22
Phage lambda	Wild type	P. Bassford, Jr.

[a] *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.
[b] CDC, Centers for Disease Control, Atlanta, Ga.

by the method of Birnboim and Doly (1). Plasmid pHC79 was obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Competent cells were prepared by the method of Hanahan (10).

Cosmid cloning and isolation of *recA*-complementing clones. *L. biflexa* serovar patoc DNA was partially digested with *Sau*3A. The resulting DNA fragments were ligated into the *Bam*HI site of pHC79, which had been dephosphorylated with calf intestine alkaline phosphatase (Boehringer Mannheim). Ligation reactions were in vitro packaged with GigaPack Gold extracts (Stratagene, La Jolla, Calif.) and transduced into cells of *E. coli* HB101, a *recA* mutant. Transductants were selected on L agar plates containing ampicillin. Master plates of randomly selected colonies were established, and the colonies were transferred to L agar containing 0.02% methyl methanesulfonate (MMS) (Sigma Chemical Co., St. Louis, Mo.). Putative *E. coli* clones expressing the RecA$^+$ phenotype (growth in the presence of MMS) were chosen for further study.

Southern blot analysis. Chromosomal and plasmid DNAs were digested to completion with the appropriate restriction enzymes, electrophoresed on 0.8% agarose gels, and transferred to GeneScreen Plus (New England Nuclear Research Products, Boston, Mass.) by the method of Southern (27). Hybridizations were done with probe DNA labeled with digoxigenin-dUTP and detected with the Genius nonradioactive DNA labeling and detection kit (Boehringer Mannheim).

Maxicell analysis of *recA* gene products. Labeling of plasmid-encoded proteins in cells of *E. coli* SE5000 with [^{35}S]methionine (10 μCi/μl; New England Nuclear) and immunoprecipitations were performed as previously described (29). Rabbit anti-*E. coli* RecA serum was supplied by G. Christiansen, University of Aarhus, Aarhus, Denmark. Radiolabeled, solubilized maxicell extracts and immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (29). Gels were stained with Coomassie blue, destained, and processed for fluorography (29). Molecular weights were determined on the basis of the positions of unlabeled known protein standards (Bio-Rad Laboratories, Richmond, Calif.).

UV sensitivity. Cells were grown in M9 medium (18) supplemented with 0.5% Casamino Acids (Difco Laboratories, Detroit, Mich.) and 0.4% glucose to an optical density of 0.3 at 600 nm (determined on a Spectronic 20 spectrophotometer; Bausch and Lomb, Rochester, N.Y.). Samples (5 ml) were placed in sterile plastic petri dishes and exposed to UV radiation from a 15-W G15T8 germicidal lamp (GTE Products Corp., Danvers, Mass.) for various time periods. The intensity of the lamp was measured with a Blak Ray UV dosimeter (model J-225; Ultraviolet Products, Inc., San Gabriel, Calif.). Following irradiation, samples were removed, diluted, and plated in duplicate on L agar. All manipulations were carried out under red light to prevent photoreactivation. Plates were covered with foil and incubated overnight at 37°C.

Recombination proficiency. Recombination proficiency was determined by the method of Keener et al. (15) with cells of *E. coli* JC14604. The appearance of Lac$^+$ papillae on colonies grown on MacConkey lactose agar (Difco) was scored after 72 h at 37°C.

Prophage induction. Lysogens of HB101 cells carrying the desired plasmid were selected as described by Owttrim and Coleman (21). To test for phage induction, we grew the lysogens in L broth to an optical density of 0.3 at 600 nm, washed them, suspended them in fresh L broth with or without MC (5 μg/ml) (Sigma), and incubated them in the dark for 2.5 h at 37°C with agitation. Cells were lysed by the addition of chloroform. Cell debris was removed by centrifugation, and the phage titers in the supernatant were determined with *E. coli* C600 as the host strain.

Induction of the *Leptospira* RecA protein. To test for the induction of the native RecA protein, we grew cultures of *Leptospira* serovars or *L. illini* at 30°C in bovine serum albumin-polysorbate 80 medium to 5×10^8 cells per ml. Each culture was divided into six 1-ml samples. Nalidixic acid (Sigma) was added to three of the samples at a final concentration of 120 μg/ml. The three remaining samples received buffer as a control. [^{35}S]methionine (70 μCi/ml) was added at 30, 60, and 90 min following the introduction of nalidixic acid. The samples were labeled for 60 min, and the cells were pelleted, washed, and solubilized. Immunoprecipitations with normal rabbit serum (NRS) and rabbit anti-*E. coli* RecA serum were performed as previously described, and the results were analyzed by SDS-PAGE and fluorography (20). Radioactivity in individual protein bands was quantitated with an AMBIS Radioanalytic Imaging System (Ambis Systems, San Diego, Calif.).

RESULTS

Identification of recombinant plasmids complementing an *E. coli recA* mutation. A library of *L. biflexa* serovar patoc

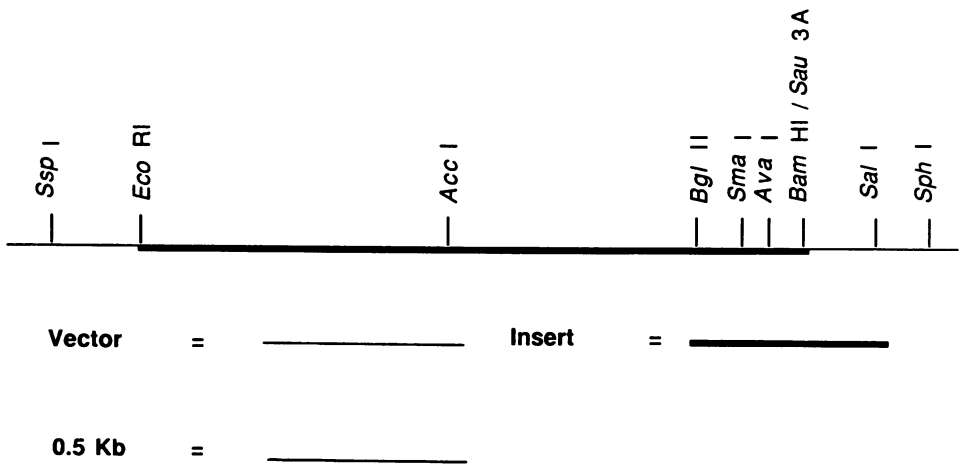

FIG. 1. Restriction map of plasmid pLS102. Plasmid pLS102 is the smallest subclone of the original plasmid (pLS100) that contains the *recA* gene of *L. biflexa* serovar patoc.

genomic DNA was constructed with the cosmid vector pHC79. Recombinant molecules were in vitro packaged and transduced into cells of *E. coli* HB101, a *recA* mutant. The titer of infective phage particles indicated that the library contained about 30,000 clones. Transductants exhibiting a RecA$^+$ phenotype were identified by growth on L agar containing MMS, an alkylating agent. Nine of the 190 clones initially screened were resistant to MMS. To ensure that *Leptospira* genetic information was responsible for the MMS resistant phenotype, we prepared plasmid DNA from seven MMS-resistant clones and reintroduced it into strain HB101 cells by transformation. For each plasmid, several transformants were tested for MMS resistance and, in addition, UV resistance by a qualitative UV cross-streak method (5). Virtually all of the randomly chosen transformants were resistant to both UV and MMS, whereas none of the transformants harboring the vector plasmid were resistant to these agents.

One recombinant plasmid of approximately 38 kb, designated pLS100, was selected for further study. Plasmid DNA was digested to completion with *Bam*HI, and intramolecular ligation of the fragments was performed. Using HB101 cells as the recipients for transformation, we isolated MMS-resistant subclones. *recA*-complementing activity was associated with the largest *Bam*HI fragment (10.7 kb) of pLS100. This fragment also contained the pHC79 vector, which allowed for continued replication and encoded ampicillin resistance. The new construct, pLS101, was further subcloned with *Eco*RI to produce a smaller *recA*-complementing plasmid (pLS102) that contained a 1.6-kb *Eco*RI-*Sau*3A fragment. A physical map of this fragment was constructed to facilitate further characterization (Fig. 1). Of the restriction enzymes tested, only four (*Acc*I, *Ava*I, *Bgl*II, and *Sma*I) cut within the insert DNA. There were no sites within the insert DNA for *Bam*HI, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Nde*I, *Pst*I, *Pvu*I, *Pvu*II, *Sal*I, or *Sph*I.

Southern blot analysis. Plasmid pLS102 was digested with *Eco*RI, labeled with digoxigenin-dUTP, and used as a probe against *Eco*RI digests of the following DNAs: *L. biflexa* serovars patoc, andaman, and ranarum, *L. interrogans* serovars pomona and hardjo, and *L. illini*. Homology to the *L. biflexa* serovar patoc *recA* gene was detected only in the saprophytic *L. biflexa* serovars (Fig. 2B, lanes 3 to 5). The *recA* gene of serovars patoc and andaman was present on a 2.0-kb fragment of *Eco*RI-digested genomic DNA, while that of serovar ranarum was present on a 2.2-kb *Eco*RI fragment. No homology was detectable with the *Eco*RI digest of the *L. interrogans* serovars or *L. illini* (lanes 6 to 8). We also tested an *Eco*RI digest of genomic DNA from a RecA$^+$ *E. coli* strain and did not observe any homology to the pLS102 probe (data not shown). As expected, the pLS102 probe showed homology to the vector plasmid (lane 1) as well as to the unlabeled *Eco*RI-*Sal*I double digest of itself (lane 2). When we used the *Eco*RI-cut and labeled vector plasmid as a probe, it hybridized only to common vector sequences present in pLS102 and itself. The pHC79 probe did not show any homology to the *Leptospira* or *L. illini* DNA digests tested (data not shown).

Identification of the *recA* gene product of *L. biflexa* serovar patoc in maxicells. Plasmids pHC79, pRecA35, pLS100, pLS101, and pLS102 were transformed into cells of *E. coli* SE5000. Maxicell analysis was performed as previously

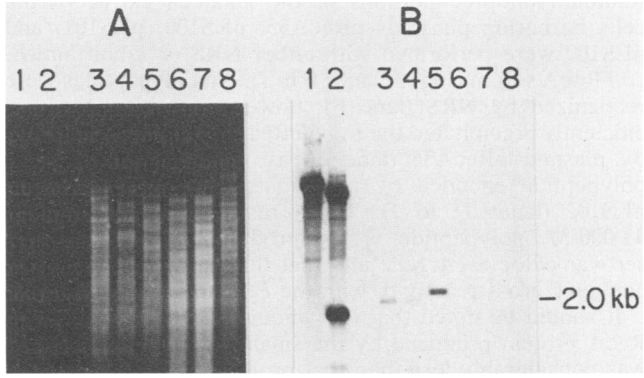

FIG. 2. Southern hybridization. (A) Ethidium bromide-stained plasmid and chromosomal DNAs. Lanes: 1, pHC79 digested with *Eco*RI; 2, pLS102 digested with *Eco*RI-*Sal*I; 3 to 7, *Eco*RI-digested genomic DNAs from *L. biflexa* serovars patoc, andaman, and ranarum and *L. interrogans* serovars pomona and hardjo, respectively; 8, *Eco*RI-digested genomic DNA from *L. illini*. (B) Corresponding hybridization patterns observed when DNAs were transferred to nitrocellulose and probed with *Eco*RI-digested and labeled pLS102.

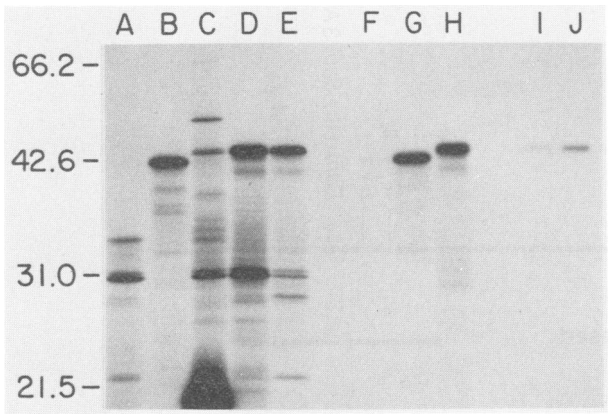

FIG. 3. Maxicell analysis of plasmid-encoded proteins and immunoprecipitations of the *L. biflexa* serovar patoc RecA protein. [^{35}S]methionine-labeled maxicell extracts were prepared and analyzed by SDS-PAGE and fluorography. Lane A, Proteins encoded by vector pHC79; lane B, pRecA35; lanes C to E, pLS100, pLS101, and pLS102, respectively. Immunoprecipitations with selected radiolabeled maxicell extracts were performed with NRS (control) or anti-RecA serum and analyzed by SDS-PAGE and fluorography. Lanes F and G, Immunoprecipitations of maxicells programmed with pRecA35 performed with NRS and anti-RecA serum, respectively; lanes H to J, immunoprecipitations of maxicells programmed with pLS101, pLS100, and pLS102, respectively, performed with anti-RecA serum. Numbers at left are kilodaltons.

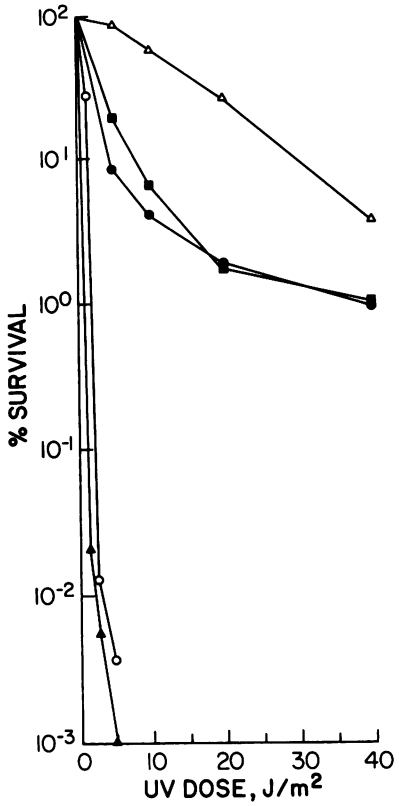

FIG. 4. UV sensitivity of *E. coli* HB101 harboring vector pHC79 or plasmids containing the cloned serovar patoc *recA* gene. Cells were grown in M9 medium to the appropriate density, irradiated for various times, diluted, plated on L agar, and incubated in the dark at 37°C. Survivors were determined after 1 to 2 days. Symbols: ▲, HB101(pHC79); △, HB101(pRecA35); ●, HB101(pLS100); ■, HB101(pLS101); ○, HB101(pLS102).

described (29), and the results were analyzed by SDS-PAGE and fluorography (Fig. 3, lanes A to E). Plasmid pHC79 encoded a polypeptide doublet representing the two forms of the β-lactamase enzyme (*bla* gene product) and an additional polypeptide representing the *tet* gene product (lane A). Plasmid pRecA35 encoded a major 42,400-M_r polypeptide previously identified by Resnick and Nelson (23) as the *E. coli* RecA protein (lane B). Plasmids pLS100, pLS101, and pLS102 encoded the *bla* gene product as well as several additional polypeptides not of vector origin (lanes C to E). A polypeptide with an M_r of 43,000 was the only prominent insert-encoded band that was clearly common to all of the maxicell extracts programmed by these three plasmids. Radioimmunoprecipitations of the maxicell extracts from cells harboring plasmids pRecA35, pLS100, pLS101, and pLS102 were performed with either NRS or rabbit anti-*E. coli* RecA serum (Fig. 3, lanes F to J). No polypeptides were recognized by NRS (lane F); however, anti-RecA serum efficiently precipitated the *E. coli* RecA polypeptide encoded by plasmid pRecA35 (lane G) as well as the 43,000-M_r polypeptide encoded by plasmids pLS100, pLS101, and pLS102 (lanes H to J). These results indicate that the 43,000-M_r polypeptide is the product of the *L. biflexa* serovar patoc *recA* gene and that this polypeptide exhibits antigenic cross-reactivity with the *E. coli* RecA protein.

It should be noted that the amount of the serovar patoc RecA protein produced by the smallest subclone, pLS102, was considerably less than that produced by pLS101. This fact is not readily apparent in Fig. 3, lane E, since a fivefold-greater amount of the maxicell extract of pLS102 was loaded onto the gel to facilitate visualization.

Complementation studies with the cloned *L. biflexa* serovar patoc *recA* gene. The ability of the cloned *recA* gene to complement a defective SOS response and recombination functions was investigated. Quantitative UV sensitivity experiments compared the abilities of *E. coli* HB101 cells harboring pHC79, pRecA35, pLS100, pLS101, and pLS102 to survive exposure to UV radiation (Fig. 4). Plasmid pRecA35 efficiently complemented the *recA* mutation in strain HB101 cells. In contrast, HB101 cells harboring pHC79 were extremely sensitive to UV radiation. Plasmids pLS100 and pLS101 complemented *recA* to the same degree, although this complementation was below the level conferred by pRecA35. Cells containing plasmid pLS102 were only slightly more resistant to UV radiation than were cells containing the vector plasmid.

We also tested the qualitative ability of the cloned serovar patoc recA gene to promote homologous recombination in cells of *E. coli* JC14604. This *srl-recA* deletion mutant contains two defective copies of the *lac* operon, making it Lac$^-$. Lac$^+$ papillae can arise from recombination upon introduction of a functional *recA* gene. Cells of strain JC14604 were transformed with plasmids pHC79, pRecA35, pLS100, pLS101, and pLS102. Ampicillin-resistant colonies were selected and transferred to MacConkey lactose agar. Colonies forming papillae were scored at 72 h. We found that cells containing plasmid pLS101 had a recombination proficiency of 100% as compared with cells containing plasmid pRecA35 (i.e., all colonies formed papillae). Although cells containing plasmids pLS100 and pLS102 did not produce papillae during the 72-h scoring period, papilla production was observed following an additional 72 h of incubation.

TABLE 2. Induction of lambda prophage in lysogens of *E. coli* containing plasmids with cloned *recA* genes

Lysogen	PFU/ml	
	Spontaneous	Induced[a]
HB101(pHC79)	3.9×10^2	2.4×10^2
HB101(pRecA35)	2.0×10^2	2.9×10^5
HB101(pLS100)	1.5×10^5	7.2×10^8
HB101(pLS101)	8.6×10^4	4.7×10^8
HB101(pLS102)	2.9×10^2	8.3×10^2

[a] MC was present at a final concentration of 5 μg/ml.

Plasmid pHC79 did not promote recombination during this time period.

Prophage induction. *E. coli recA* cells are defective in the ability to induce lysogenic prophages, either spontaneously or following exposure to DNA-damaging agents such as MC, unless a functional *recA* gene is introduced. Wild-type lambda lysogens of HB101 cells harboring plasmids pHC79, pRecA35, pLS100, pLS101, and pLS102 were constructed, and the levels of spontaneous and MC-induced phage production were determined. The presence of pHC79 did not enhance phage production after MC exposure (Table 2). In contrast, the presence of pRecA35 resulted in an approximate (1.5×10^3)-fold increase in MC-induced phage production. Plasmids pLS100 and pLS101 enhanced MC-induced phage production to an even greater extent, whereas plasmid pLS102 reproducibly enhanced MC-induced phage production by only about threefold.

Induction of the native *Leptospira* RecA protein. To determine whether the native RecA protein could be identified and whether its expression could be induced above a basal level, we exposed exponentially growing cells to buffer or nalidixic acid for various times before radiolabeling cells with [^{35}S]methionine. Immunoprecipitations of solubilized cellular extracts with anti-*E. coli* RecA serum indicated that a low basal level of the RecA protein was present in the cells of all of the *Leptospira* serovars and *L. illini* (Fig. 5, −Nal, A to E). Following the addition of nalidixic acid, the level of the RecA protein increased approximately five- to sixfold in the free-living serovars (patoc and ranarum) and fourfold in *L. illini* (Fig. 5, +Nal, A, B, and E, respectively). Induction of the RecA protein in the parasitic leptospires was either absent (serovar hardjo) or lower (about twofold in serovar pomona) (Fig. 5, +Nal, C and D, respectively).

We also observed that the RecA protein of the free-living *Leptospira* organisms and *L. illini* was slightly larger than those of the parasitic *Leptospira* organisms.

Other spirochetes. Since the RecA protein has been identified in many diverse bacterial species, we attempted to identify a corresponding homolog in certain other members of the order *Spirochaetales*. These experiments were performed with virulent *Treponema pallidum* (Nichols strain) and *Borrelia burgdorferi* in a manner similar to that used for the identification of the native *Leptospira* RecA protein. When immunoprecipitations of solubilized cellular extracts performed with anti-*E. coli* RecA serum were analyzed, we were unable to detect even a basal level of the RecA protein in cells of either of these spirochetes (data not shown).

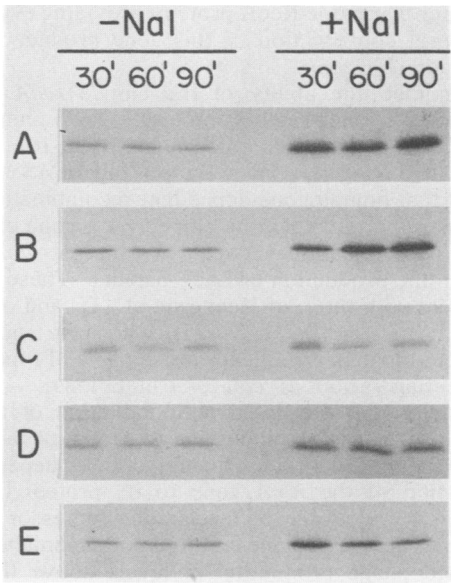

FIG. 5. Induction of the native RecA protein of *Leptospira* organisms. Nalidixic acid (+Nal) at 120 μg/ml or buffer (−Nal) was added to 5×10^8 cells of *L. biflexa*, *L. interrogans*, or *L. illini* per ml, and the cells were incubated at 30°C for 30, 60, or 90 min before the addition of [^{35}S]methionine (70 μCi/ml) for 60 min. Cells were washed and solubilized, and immunoprecipitations were performed with rabbit anti-RecA serum. Results were analyzed by SDS-PAGE and fluorography. Panels A to E show precipitates obtained with *L. biflexa* serovars patoc and ranarum, *L. interrogans* serovars hardjo and pomona, and *L. illini*, respectively.

DISCUSSION

We have cloned and partially characterized a genomic DNA fragment from *L. biflexa* serovar patoc that complements a range of *recA* mutations in *E. coli*, resulting in the restoration of MMS and UV resistance, recombination proficiency, and the ability to induce resident lambda prophage. Despite the functional conservation of the serovar patoc *recA* gene, Southern hybridization experiments indicated no detectable DNA homology between this gene and the *recA* gene of *E. coli*. Our data are in agreement with those of previous reports showing that DNA homology is frequently not detectable between the *E. coli recA* gene and cloned, heterologous *recA* genes (3, 9, 12, 15, 23, 25).

When chromosomal DNAs from two additional serovars of *L. biflexa*, three serovars of *L. interrogans*, and *L. illini* were digested with *Eco*RI and probed with the cloned serovar patoc *recA* gene, DNA homology was detected only among the *L. biflexa* serovars. DNA relatedness studies by Yasuda et al. (32) and others (2, 8) have placed serovars patoc and andaman in the same DNA relatedness group and serovar ranarum in a separate group. Our results appear to support this classification. The lack of DNA homology between the serovar patoc *recA* gene and the *recA* genes of the *L. interrogans* serovars and *L. illini* was not unexpected, since DNA hybridization studies have indicated the limited relatedness of these organisms to *L. biflexa* (32). It should be noted that, although *L. illini* strains are nonpathogenic and biochemically similar to *L. biflexa* strains, they are genetically distinct, exhibiting between 0 and 14% relatedness to other leptospires (32).

We have identified the product of the *recA* gene of serovar patoc as a polypeptide with an M_r of 43,000 by maxicell analysis. This M_r is similar to those reported for the RecA proteins of several other bacterial species (5, 9, 12, 15, 17, 19, 21–23, 25). Anti-*E. coli* RecA serum efficiently precip-

itated the serovar patoc RecA protein, providing evidence of the structural conservation of this gene product with the RecA protein of *E. coli*.

We examined the ability of the cloned *recA* gene of serovar patoc to functionally complement *recA* mutations in cells of *E. coli*. Plasmids pLS100 and pLS101 restored resistance to killing by UV radiation and MMS. We also concluded that homologous-dependent recombination proficiency was restored, since the cloned *recA* gene efficiently promoted recombination between two mutant *lacZ* genes, resulting in the production of Lac^+ papillae. These data are in agreement with those of Keener et al. (15) and others (3, 5, 7, 9, 12, 16, 17, 19, 21–23, 25), who have shown that cloned, heterologous *recA* genes are capable of functionally complementing various *E. coli recA* mutations.

A characteristic associated with induction of the SOS response in *E. coli* is the induction of prophage lambda by DNA-damaging agents (26). This induction is dependent on the activation of the *recA* gene to its proteolytic state, resulting in the cleavage of the lambda repressor protein. The serovar patoc *recA* gene restored both spontaneous and MC-induced phage production of levels above those observed in the $RecA^+$ control cells. While this result was not surprising, it should be noted that not all heterologous *recA* gene products are capable of binding to and inactivating the lambda repressor protein (3, 15, 19, 21).

The initial subclone of plasmid pLS100, designated pLS101, was most active in promoting complementation and prophage induction. Plasmid pLS101 was further subcloned, resulting in plasmid pLS102. Although maxicell analysis clearly demonstrated that the RecA protein was encoded on the 1.6-kb fragment of pLS102, this plasmid was considerably less efficient in complementation and prophage induction. A possible explanation is that the subcloned DNA fragment of pLS102 may not contain all of the genetic information required for full expression of the serovar patoc RecA protein in *E. coli*. A similar result was observed by Koomey and Falkow (17) during the subcloning of the *recA* gene of *Neisseria gonorrhoeae*.

Unlike the case with other bacterial species, such as *E. coli* or *N. gonorrhoeae*, in which genetic exchange mechanisms are reasonably well characterized, such mechanisms have not been documented in any spirochetes. Experiments involving inactivation of the cloned *recA* gene of serovar patoc and reintroduction of this gene into the native leptospire through allelic exchange would facilitate more definitive studies of the function and regulation of this gene and its product but cannot be performed at the present time. We did, however, test for the induction of the native *recA* gene product in both free-living (*L. biflexa*) and parasitic (*L. interrogans*) serovars as well as *L. illini*. Our results indicated that the expression of the RecA protein was inducible to several times the basal cellular level in the free-living leptospires and *L. illini* but that there was either no induction or a lower level of induction in the parasitic leptospires. These results correlate with those of Stamm and Charon (28) who showed that cells of *L. biflexa* serovars and *L. illini* were considerably more resistant to UV and MC than were those of *L. interrogans* serovars.

We also examined two other spirochetal species, virulent *T. pallidum* and *B. burgdorferi*, but could not detect even a basal level of the *recA* gene product in the cells of either organism. These results were surprising, since RecA homologs have been found in every species of procaryote examined thus far, indicating the widespread distribution and evolutionary conservation of the *recA* gene (19). Miller and Kokjohn (19) have postulated that the role of *recA* in DNA repair suggests that it contributes significantly to the ability of microbes to tolerate damage mediated by solar UV radiation and to the habitation of certain ecosystems. The absence of the RecA protein in *T. pallidum* and *B. burgdorferi* may relate to the ecological niches of these spirochetes. Whereas both free-living and parasitic *Leptospira* organisms survive in soil and water and are naturally exposed to DNA-damaging agents that may evoke an SOS response, *T. pallidum* and *B. burgdorferi* are fragile organisms that do not survive outside host tissues. Finally, there has been some concern regarding the potential for the acquisition of antibiotic resistance in these latter organisms, especially in view of the limited number of available antimicrobial agents with proven efficacy against them (30). The absence of homologous recombination mediated by the RecA protein may lessen the potential for such acquired resistance.

Our study is the first report of the presence of a *recA* gene in any bacterium of the order *Spirochaetales*. The approach that we have taken (i.e., cloning and complementation) has been previously used to explore basic questions concerning the metabolic capabilities of *L. biflexa* and *T. pallidum* (6, 33, 34). Additionally, this approach has been used by a number of investigators to clone and characterize *recA* genes from a variety of other procaryotes (3, 5, 7, 9, 12, 15–17, 19, 21–23, 25). Our complementation, induction, and immunochemical data indicate that, despite the absence of DNA homology, the *recA* gene of *L. biflexa* serovar patoc is structurally and functionally conserved and that it likely functions and is regulated in the spirochete as it is in *E. coli*.

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