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Characterization of the *recA* Gene Regions of *Spiroplasma citri* and *Spiroplasma melliferum*

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In previous studies (A. Marais, J. M. Bové, and J. Renaudin, J. Bacteriol. 178:862–870, 1996), we have shown that the *recA* gene of *Spiroplasma citri* R8A2 was restricted to the first 390 nucleotides of the N-terminal part. PCR amplification and sequencing studies of five additional strains of *S. citri* have revealed that these strains had the same organization at the *recA* region as the R8A2 strain. In contrast to *S. citri*, *Spiroplasma melliferum* was found to contain a full-length *recA* gene. However, in all five *S. melliferum* strains tested, a TAA stop codon was found within the N-terminal region of the *recA* reading frame. Our results suggest that *S. melliferum*, as well as *S. citri*, is RecA deficient. In agreement with the *recA* mutant genotype of *S. citri* and *S. melliferum*, we have shown that these organisms are highly sensitive to UV irradiation.

Spiroplasmas are wall-less eubacteria with helical morphology and motility. They belong to the class *Mollicutes*, a group of organisms which are phylogenetically related to gram-positive bacteria (42, 44). Spiroplasmas occur primarily in arthropods, but some infect plants in which they cause diseases. *Spiroplasma citri*, the etiological agent of citrus stubborn disease, was the first mollicute of plant origin to be cultured (35) and, since then, has been extensively studied (1). A genomic map has been established (45, 46), and genes encoding spiroplasma-specific proteins such as spiralin and fibril protein have been characterized (3, 43). However, genetic analyses have been hampered by the lack of suitable gene transfer systems, and, consequently, genes involved in the basic properties of *S. citri* such as helicity, motility, transmissibility by the vector insect, and pathogenicity to plants are still unknown. Previous studies from our laboratory have centered on the development of cloning vectors that replicate in *S. citri*. We have first used the replicative form of *S. citri* virus SpV1 as a vector to introduce and express foreign genes, namely the *cat* gene and the G fragment of the P1 adhesin gene of *Mycoplasma pneumoniae*, in *S. citri* R8A2 (23, 37). More recently, artificial plasmids containing the origin of replication of *S. citri* (*oriC* plasmids) were constructed and could be used to clone and express the spiralin gene of *Spiroplasma phoeniceum* in *S. citri* ASP1 (32, 47). In contrast to *oriC* plasmids, in which inserted DNA was stably maintained, the recombinant SpV1 replicative form was found to be relatively unstable. Deletion formation was shown to proceed not only by illegitimate recombination, but also by homologous recombination between the viral replicative form and viral sequences present in the *S. citri* host chromosome (24).

The RecA protein is the enzyme primarily responsible for the homologous recombination of chromosomal DNA in bacteria (27) and is also essential for recombinational DNA repair (33). In mollicutes, *Mycoplasma genitalium* (14), *Mycoplasma pulmonis* (18), *Mycoplasma mycoides* subsp. *mycoides* (18), and *Acholeplasma laidlawii* (11) contain a functional *recA* gene. Because homologous recombination occurred in *S. citri* R8A2, it was assumed that this strain also had a functional *recA* gene. However, a search for the *recA* gene of *S. citri* R8A2 revealed that the gene was deleted over two-thirds from the C-terminal part, indicating that this particular strain was probably RecA deficient (24).

The aim of the present study was to determine whether the presence of a truncated *recA* gene in *S. citri* R8A2 was specific to this particular strain or was a property shared by other strains of *S. citri* and other *Spiroplasma* species. All five *S. citri* strains tested were found to have the same *recA* gene organization as the R8A2 strain. In contrast to *S. citri*, five strains of *Spiroplasma melliferum*, a spiroplasma species serologically related to *S. citri*, were found to carry a full-length *recA* gene. However, all *S. melliferum* strains tested had a stop codon in the *recA* reading frame, suggesting that strains of *S. melliferum* as well as strains of *S. citri* are *recA* mutants. The *recA* mutant genotype of these organisms was correlated with their high sensitivity to UV irradiation.

Characterization of the *recA* gene from various strains of *S. citri*. The *recA* gene regions of five different strains of *S. citri* were first characterized by PCR amplification of genomic DNA with oligonucleotide primers which were designed from the nucleotide sequence of the *recA* gene region of *S. citri* R8A2. Spiroplasmal genomic DNAs were isolated as previously described (43) and amplified with two different pairs of primers, pair SC1-SC3 and pair SC11-SC16. The positions of these primers are indicated in Fig. 1. Primer SC3 (nucleotides 590 to 607 of the published sequence [24]) is located within the *recA* gene, while primer SC1 (nucleotides 1913 to 1932) is located 1.4 kbp downstream, within open reading frame ORF 4. Primer SC11 (nucleotides 367 to 390) starts at the third nucleotide of the *recA* gene, and primer SC16 (nucleotides 807 to 824) is located 70 nucleotides downstream of the TAA stop codon of the *recA* gene.

The results of PCR amplifications with primer pair SC1-SC3 are shown in Fig. 2A. The expected fragment of 1.4 kbp was obtained with genomic DNA from *S. citri* R8A2 (lane 1) used as the positive control and with plasmids pRESC1 (lane 2) and pRT9 (lane 3), which contain the cloned *recA* gene of *S. citri*

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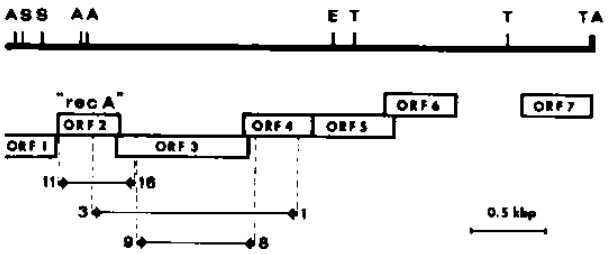

FIG. 1. Genomic organization of the *recA* region of *S. citri* R8A2 (24). The positions of primers (abbreviated in parentheses) SC1 (1), SC3 (3), SC8 (8), SC9 (9), SC11 (11), and SC16 (16) used for PCR amplifications are indicated by arrows. The nucleotide sequences of these primers are as follows: SC1, 5′-GC ACCAACTTTATCGTTACC-3′; SC3, 5′-GGGCCAGAATCATCAGGC-3′; SC8, 5′-CTCAAACATTACATGAGCC-3′; SC9, 5′-TTCACCGTATGATGGT CG-3′; SC11, 5′-GGAAGATAATAAACCAGTTCACCC-3′; and SC16, 5′-TC ATTGTCTATCTTGGGG-3′. ORFs are numbered from 1 to 7. ORF 2 corresponds to the *recA* sequences. A, *Alu*I; E, *Eco*RI; S, *Sau*3AI; T, *Taq*I.

R8A2 (24). A similar DNA fragment with a size of 1.4 kbp was obtained with the DNAs of *S. citri* strains Corse and R8A2B (lanes 8 and 9, respectively). Amplification of DNA from *S. citri* strains ASP1 (lane 7), Israël (lane 10), and Alcanar (lane 11) also yielded an amplified DNA fragment, but with a slightly smaller size. In contrast, no amplification signal could be detected with DNA from *S. phoeniceum* (lane 4), *Spiroplasma apis* (lane 5), *S. melliferum* (lane 6), and *M. pneumoniae* (lane 12). To confirm the specificity of the PCR, the amplified DNA fragments were transferred to a nylon membrane and hybridized with an internal probe under stringent conditions. The 760-bp probe was generated by PCR amplification of plasmid pRESC1 carrying the *recA* region of *S. citri* R8A2 with primer pair SC8-SC9. As indicated in Fig. 1, primer SC8 is located at the beginning of ORF 4 and primer SC9 is within ORF 3. The hybridization patterns presented in Fig. 2B show that the probe did hybridize with all amplified DNA fragments (lanes 1 to 3 and 7 to 11). As expected, no hybridization signal could be detected in the absence of amplified DNA (lanes 4 to 6 and 12).

To further compare the *recA* regions of the different strains of *S. citri*, genomic DNAs were amplified with primers SC11 and SC16, respectively, located at the start of the *recA* gene and within ORF 3 immediately downstream of the *recA* sequences (Fig. 1). Figure 2C shows that amplification yielded a unique 450-bp DNA fragment regardless of the *S. citri* strain tested, R8A2 (lane 2), ASP1 (lane 6), Corse (lane 7), R8A2B (lane 8), Israël (lane 9), or Alcanar (lane 10). The size of the amplified DNA agrees well with the value of 458 bp calculated from the nucleotide sequence of the *recA* region of *S. citri* R8A2 (24). Used as positive control, the PRT9 plasmid carrying the *recA* region yielded the same amplified DNA fragment (lane 11). In contrast, no amplification product could be detected with DNA from *S. melliferum* (lane 3), *S. phoeniceum* (lane 4), and *S. apis* (lane 5).

These results suggest that the *recA* regions of the six *S. citri* strains tested have the same gene organization. To confirm this hypothesis, the nucleotide sequence of the 450-bp PCR product of strain ASP1 was determined. This nucleotide sequence was found to be identical to that of the cloned chromosomal DNA fragment of strain R8A2. In particular, the *recA* coding sequence is followed by a TAA triplet located at the same position (nucleotide 755 of the published sequence of strain R8A2) in both strains. These results indicate that the *S. citri* strains tested so far (R8A2, R8A2B, Corse, ASP1, Israël, and Alcanar) contain a truncated *recA* gene. Only the first 390 nucleotides at the 5′ end are present. The results also suggest that these *S. citri* strains are *recA* mutants. In addition, the fact that the DNAs from *S. melliferum*, *S. apis*, and *S. phoeniceum* could not be amplified with *S. citri recA* specific primers (see Fig. 2) suggested that, in these organisms, the gene organization at the *recA* region could be different from that in *S. citri*. To further investigate the *recA* situation in other spiroplasmas, we have characterized the *recA* gene of *S. melliferum.*

Characterization of the *recA* gene from *S. melliferum*. To identify DNA fragments carrying *recA* sequences, genomic DNA of *S. melliferum* BC3 was restricted and probed under stringent conditions with the $recA_i$ probe. This probe was obtained by PCR amplification of *S. melliferum* DNA with primer pair SM1-SM2 as previously described (24). As shown in Fig. 3, restriction with *Taq*I (lane 1), which cleaves within the probe, yielded two fragments with sizes of 3.4 and 2.2 kbp hybridizing with the probe. Restriction with *Sau*3AI (lane 2) or *Eco*RV (lane 4) yielded one single fragment each with sizes of 1.8 and 2 kbp, respectively. As shown in lane 3, the combination of *Sau*3AI and *Hin*dIII (which has a recognition site within the probe) yielded three hybridizing fragments with sizes of 1.8,

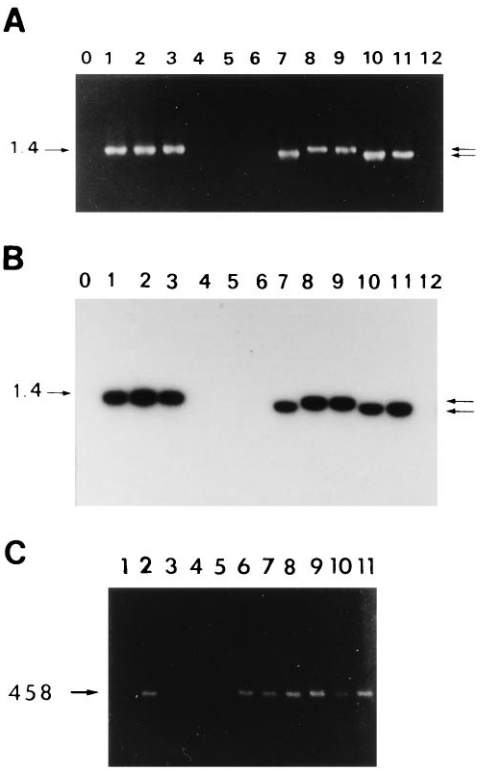

FIG. 2. (A) Ethidium bromide-stained 0.8% agarose gel of DNAs amplified with primers SC1 and SC3. Amplification with primer pair SC1-SC3 involved 30 cycles, each of 1 min at 92°C, 1 min at 57°C, and 2 min at 72°C. (B) Southern blot hybridization of PCR products with the PCR-generated probe SC8-SC9. Lanes: 0, no template DNA; 1, genomic DNA of *S. citri* R8A2; 2, plasmid pRESC1; 3, plasmid pRT9; 4 to 12, genomic DNAs of *S. phoeniceum* (lane 4), *S. apis* (lane 5), *S. melliferum* (lane 6), *S. citri* ASP1 (lane 7), *S. citri* Corse (lane 8), *S. citri* R8A2B (lane 9), *S. citri* Israël (lane 10), *S. citri* Alcanar (lane 11), and *M. pneumoniae* (lane 12). The sizes of fragments are indicated in kilobase pairs. (C) Ethidium bromide-stained 2% agarose gel of DNAs amplified with primers SC11 and SC16. Amplification involved 35 cycles, each of 1 min at 92°C, 1 min at 50°C, and 2 min at 72°C. Lanes: 1, no template DNA; 2 to 10, genomic DNA of *S. citri* R8A2 (lane 2), *S. melliferum* (lane 3), *S. phoeniceum* (lane 4), *S. apis* (lane 5), *S. citri* ASP1 (lane 6), *S. citri* Corse (lane 7), *S. citri* R8A2B (lane 8), *S. citri* Israël (lane 9), *S. citri* Alcanar (lane 10); 11, plasmid pRT9. The sizes of fragments are indicated in base pairs.

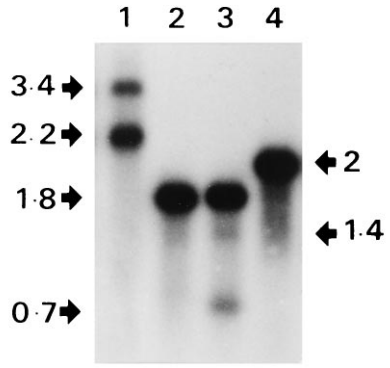

FIG. 3. Southern blot hybridization of restricted genomic DNA of *S. melliferum* with the ^{32}P-labeled $recA_i$ probe. DNA was restricted by *Taq*I (lane 1), *Sau*3AI (lane 2), *Sau*3AI-*Hin*dIII (lane 3), and *Eco*RV (lane 4). The sizes of restricted fragments are indicated in kilobase pairs.

1.4, and 0.7 kbp. Because the 0.7-kbp fragment's hybridization signal was stronger than that of the 1.4-kbp fragment it was thought that the 1.8-kbp fragment resulted from a *Hin*dIII partial digest of the DNA. The presence of two *Hin*dIII restriction sites within the 1.8-kbp *Sau*3AI fragment was later confirmed by analysis of the nucleotide sequence (Fig. 4).

In order to characterize the *recA* gene of *S. melliferum*, we first subcloned the 1.8-kbp *Sau*3AI fragment in *E. coli*. The genomic DNA of *S. melliferum* BC3 was restricted by *Sau*3AI, and restriction fragments were separated by agarose gel electrophoresis. Fragments with a size range of 1.6 to 2 kbp were ligated "in gel" with the *Bam*HI-linearized pBluescript SK$^+$ vector (Stratagene Cloning Systems, La Jolla, Calif.), and the ligation mixture was used to transform *E. coli* TG1 competent cells. The bacterial transformants were screened by in situ hybridization of colonies with the $recA_i$ probe under standard stringent conditions. Plasmid content of the hybridization-positive clones was further analyzed by Southern blot hybridization. One particular clone containing a recombinant plasmid with a 1.8-kbp insert hybridizing with the probe was selected. The recombinant plasmid was designated pSS1. The nucleotide sequence of the pSS1 insert is given in Fig. 4. It contains 1,793 nucleotides, of which 1,029 (nucleotides 128 to 1156) showed substantial homology (69% identity) with the *recA* gene from *M. mycoides* subsp. *mycoides* and with the *recA* sequences of *S. citri* (96% identity in a 390-bp overlap). Moreover, the amino acid sequence deduced from this nucleotide sequence showed striking homology (80% similarity, 63% identity) with the RecA protein of *M. mycoides* (Fig. 4). However, in *S. melliferum*, we found the reading frame of the *recA* gene to be interrupted by the presence of a TAA stop codon (at nucleotide position 359) instead of a GAA glutamic acid codon as in *M. mycoides* (Fig. 4). Interestingly, the *S. citri* R8A2 RecA-related polypeptide previously reported (24), like the *M. mycoides* RecA protein, contains a GAA-encoded glutamic acid at this position (Fig. 4). The same amino acid was also found in the RecA proteins of *Bacillus subtilis* and *A. laidlawii* (11, 38). This suggests that, in *S. melliferum*, a single base mutation, G→T at nucleotide position 359, has led to the replacement of a glutamic acid residue by a translational stop signal.

To ensure that the termination codon within the *recA* gene of *S. melliferum* did not result from a cloning artifact, the nucleotide sequence of this region was determined by direct sequencing of PCR products. Primers SM3 and SM9 (Fig. 4) were used for amplification of the *S. melliferum* genomic DNA, and the 257-bp PCR product was sequenced on both strands. The nucleotide sequence was found to be identical to that of the cloned DNA fragment of plasmid pSS1, showing that the *recA* gene of *S. melliferum* does contain a translation stop codon in the reading frame and that this codon was not introduced during cloning in *E. coli.*

Furthermore, we checked for the presence of this stop codon in the *recA* mRNA by reverse transcription PCR amplification and sequencing. Total RNAs from *S. melliferum* BC3 were extracted by the guanidinium-cesium chloride method (4) and were submitted to reverse transcription with the SM3 oligonucleotide as the primer. The resulting cDNA was then amplified by PCR with the SM3-SM9 primer pair. The results presented in Fig. 5A show that the expected 257-bp fragment was obtained, not only with the *S. melliferum* DNA used as the positive control (lane 1), but also with the cDNA (lane 2). In contrast, no amplification product could be detected with total RNAs purified from *S. melliferum* (lane 3), indicating that the amplification of the cDNA did not result from the presence of contaminating genomic DNA in the RNA preparation. The amplified DNA fragment was further characterized by restriction analysis with *Hae*III. As expected from the nucleotide sequence (Fig. 4), the PCR product of the genomic DNA (lane 4), as well as that of the cDNA (lane 5), yielded the same two *Hae*III fragments with sizes of 171 and 86 bp. The 257-bp fragment obtained by PCR amplification of the cDNA (Fig. 5A, lane 2) was inserted into the *Hin*cII-linearized pBS$^+$ vector (Stratagene Cloning Systems, La Jolla, Calif.) by cloning in *E. coli*, and its nucleotide sequence was determined (Fig. 5B). Sequence analysis showed the presence of a T at nucleotide position 359 (Fig. 4), indicating that the TAA stop codon found in the reading frame of the *recA* gene is also present in the *recA* mRNA.

To determine whether the *recA* genes of other strains of *S. melliferum* were similar to that of *S. melliferum* BC3, genomic DNAs from strains BW, B88, AS576, and G1 (Table 1) were amplified with primer pairs SM1-SM2 and SM3-SM9, and the PCR products were compared with those of strain BC3. All five strains of *S. melliferum* yielded identical PCR products. Sequencing of the 257-bp fragments obtained with primer pair SM3-SM9 revealed that they had identical nucleotide sequences. In particular, they all contained a TAA stop codon at the same position in the reading frame.

Organization of the *recA* gene region of *S. melliferum*. Sequence analysis of the 1.8-kbp *Sau*3AI fragment of *S. melliferum* (Fig. 3, lane 2) revealed the presence of three ORFs, including (i) ORF A, (ii) the *recA* gene, and (iii) ORF D, and a 0.5-kbp intergenic region between the *recA* gene and ORF D (Fig. 4). As previously mentioned, the coding region of the *recA* gene of *S. melliferum* was found to contain 1,029 nucleotides (including the TAA triplet within the reading frame), corresponding to a coding capacity of 343 amino acids. The ATG initiation codon is preceded by a putative ribosome binding site, AGAAAGGAG, characteristic of gram-positive eubacteria. An inverted repeat sequence (nucleotides 1173 to 1191 and 1197 to 1215), followed by a stretch of five uridine residues in the transcription product, characteristic of a rho-independent transcription terminator (16), was present immediately downstream of the *recA* gene.

Upstream of the *recA* gene, *S. melliferum* ORF A (Fig. 4) seems to be the counterpart of *S. citri* R8A2 ORF 1 (Fig. 1) (24). Indeed, comparison of the predicted amino acid sequences showed that 31 of the 34 C-terminal amino acids of ORF A were identical to those of *S. citri* R8A2 ORF 1. In previous studies (24), we had shown that the putative translation product of *S. citri* ORF 1 shared significant homology

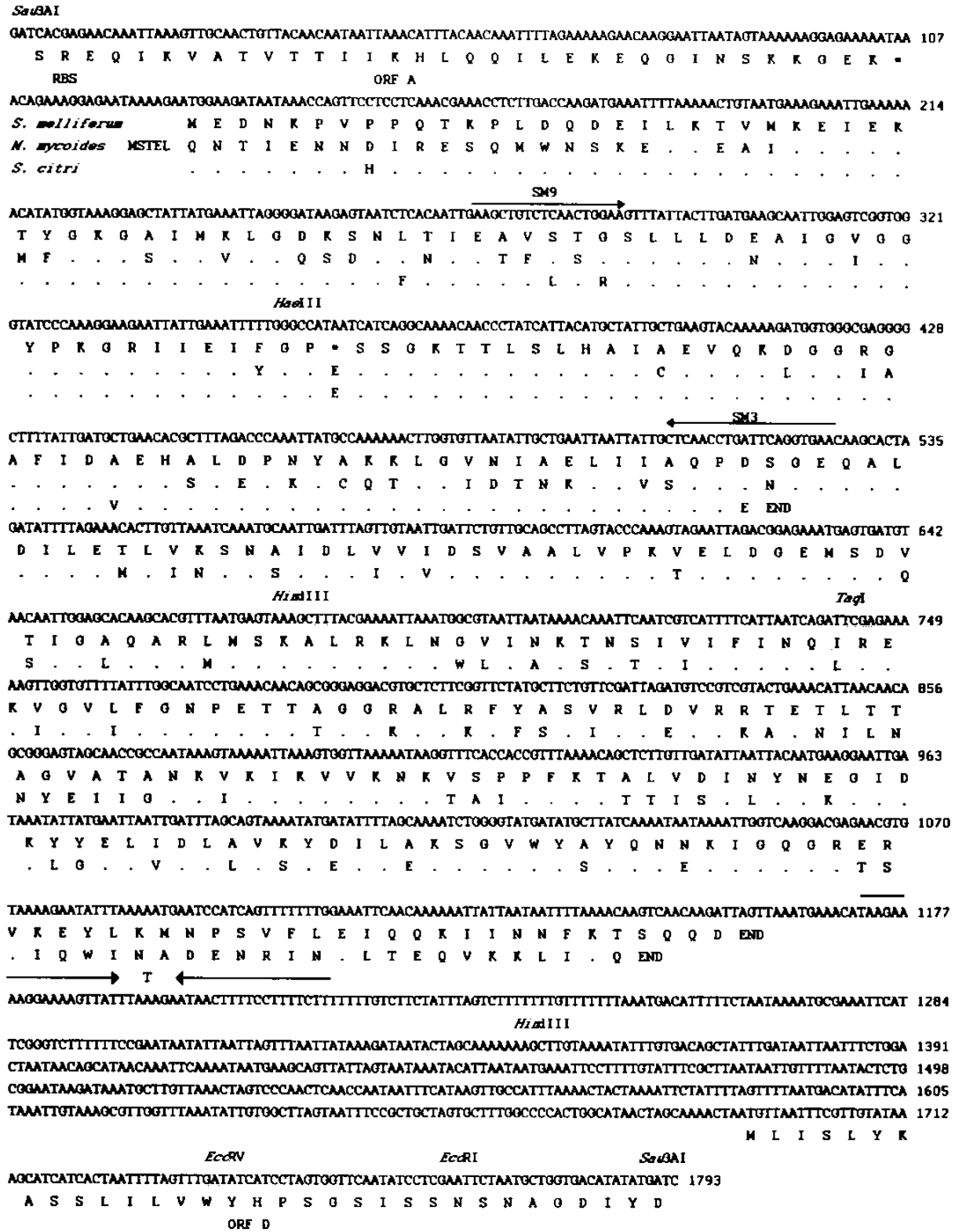

FIG. 4. Nucleotide sequence of the 1.8-kbp *Sau*3AI insert of *S. melliferum* and alignment of amino acid sequences of the putative RecA proteins of *S. melliferum*, *M. mycoides* subsp. *mycoides* (18), and *S. citri* (24). Primers SM3 and SM9 are indicated with arrows. The T between inverted arrows indicates a putative transcription terminator. RBS, putative ribosome binding site. Restriction sites *Eco*RI, *Eco*RV, *Hae*III, *Hin*dIII, *Sau*3AI, and *Taq*I are indicated. In the amino acid sequences of *S. citri* and *M. mycoides*, the dots (.) refer to amino acids identical to those of the *S. melliferum* sequence.

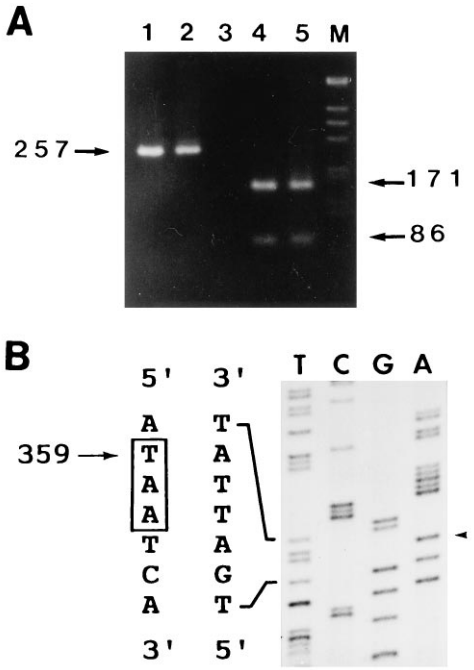

FIG. 5. (A) Ethidium bromide-stained 2% agarose gel of PCR products obtained with primer pair SM3-SM9. Lanes: 1, *S. melliferum* DNA; 2, *S. melliferum recA* cDNA obtained by reverse transcription of 0.15 ng of total RNAs; 3, *S. melliferum* total RNAs (1.3 ng); 4, PCR product from lane 1 restricted by *Hae*III; 5, PCR product from lane 2 restricted by *Hae*III; M, 1-kbp molecular weight ladder (GIBCO/BRL Life Technologies, Inc., Gaithersburg, Md.). Sizes of fragments are indicated in base pairs. (B) Sequencing reactions of the *recA* cDNA amplified with primers SM3 and SM9 and cloned in *E. coli*. Nucleotide 359 is indicated by an arrow, and the TAA stop codon is boxed.

(63% similar amino acids) with CinA from *Streptococcus pneumoniae*, a protein involved in the natural competence of this organism (25, 31).

Downstream of the *recA* gene, ORF D (nucleotides 1693 to 1792 in Fig. 4) encodes a putative polypeptide for which no homology was found with any of the protein sequences in the data banks.

UV sensitivity of *S. citri* and *S. melliferum*. In addition to its role in the homologous recombination process (19), the RecA protein is involved in two DNA repair systems: recombinational DNA repair (6) and induction of the SOS system (22). Accordingly, most of the *recA* mutants are more sensitive to DNA-damaging agents and, in particular, to UV irradiation (27). Therefore, it was of interest to determine the UV sensitivity of *S. citri* and *S. melliferum*, which were both found to be *recA* mutants, compared with that of a *recA*$^+$ organism. Because no spiroplasmas containing a functional *recA* gene have yet been found, we chose to compare the UV sensitivity of *S. citri* and *S. melliferum* with that of *A. laidlawii* JA1. *A. laidlawii*, like spiroplasmas, is a wall-less bacterium belonging to the class *Mollicutes*. In addition, *A. laidlawii* JA1 was shown to possess a functional *recA* gene (11, 12).

TABLE 1. Organisms used in this study

Species	Strain	Collection[a]	Reference
S. citri	Alcanar	Unpublished	
	ASP1	NCPPB 3095	39
	Corse		13
	Israël	NCPPB 2565	7
	R8A2	ATCC 27556	34
	R8A2B		30
S. melliferum	BC3	ATCC 33219	5
	BW		9
	B88		40
	G1		8
	AS576	ATCC 29416	10
S. phoeniceum	P40	ATCC 43115	36
S. apis	B31	ATCC 33834	28
M. pneumoniae	FH	ATCC 15531	2
A. laidlawii	JA1		21

[a] ATCC, American Type Culture Collection, Rockville, Md.; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, United Kingdom.

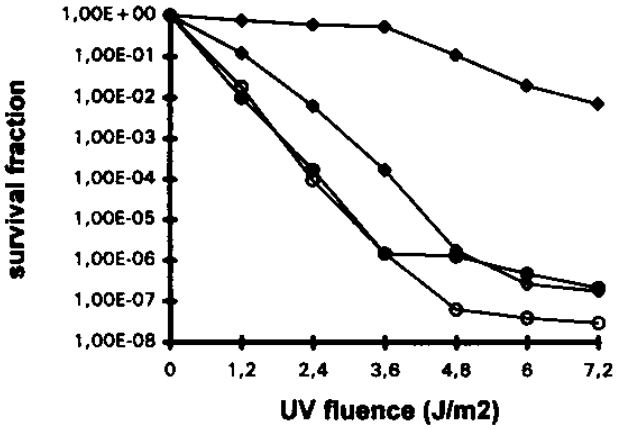

FIG. 6. UV sensitivity profiles of *S. citri* R8A2, *S. citri* ASP1, *S. melliferum* BC3, and *A. laidlawii* JA1. Cells were washed three times in PBS buffer (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4 [pH 7.4]) containing sucrose (7 g/liter) before being irradiated and then assayed for CFU. Each point of the curves represents an average of three independent experiments. ◆, *A. laidlawii* JA1; ◇, *S. melliferum* BC3; ●, *S. citri* R8A2; ○, *S. citri* ASP1.

The organisms were grown in SP4 medium up to early log phase, washed three times in PBS buffer containing 0.7% sucrose, and resuspended in the washing buffer to approximately 10^7 CFU/ml. Irradiation was performed with a UV lamp (model SVL 15 W; Bioblock Scientific, Strasbourg-Illkirch, France) at a fluence rate of 0.12 J/m^2/s. Samples were serially diluted and assayed for CFU in the dark. Curves in Fig. 6 represent the fractional survival of *S. citri* strains R8A2 and ASP1 and *S. melliferum* BC3 as a function of UV dose. The results show that *S. citri*, as well as *S. melliferum*, was dramatically more sensitive to UV irradiation than *A. laidlawii* JA1. The UV doses yielding 50% survival of *A. laidlawii* JA1, *S. melliferum* BC3, *S. citri* R8A2, and *S. citri* ASP1 were 1.7, 0.2, 0.15, and 0.08 J/m^2, respectively.

Conclusion. The data presented in this paper indicate that all of the *S. citri* strains tested (ASP1, Israël, Alcanar, Corse, and R8A2B) have the same organization at the *recA* gene region as that previously reported for the R8A2 type strain (24). In particular, the *recA* sequences in these spiroplasmas, like that in strain R8A2, represent approximately one-third (390 nucleotides at the 5′ end) of the *recA* gene sequences reported for *M. mycoides* (1,041 nucleotides) (18) or *B. subtilis* (1,044 nucleotides) (38). Thus, the *S. citri* strains tested contain a truncated *recA* gene. The presence of two *recA* genes has been reported in *Myxococcus xanthus* (29). However, in *S. citri*, hybridization experiments with two different probes (the *recA* sequences of *S. citri* and the full-length *recA* gene of *S. melliferum*) did not reveal any additional *recA* sequences, even under low-stringency conditions. In contrast to *S. citri*, *S. mel-*

liferum BC3 and all other strains tested (BW, B88, G1, and AS576) were found to possess a full-length *recA* gene. However, a TAA stop codon is present in the reading frame and leads to a partial translation product of 77 amino acids only, compared with a 343-amino-acid product for the full-length polypeptide which would be obtained in the absence of the stop codon. Because there is no frameshift in the *recA* reading frame and there is no hairpin structure sequence upstream of the internal stop codon, the possibility of a read-through process leading to a full-length RecA protein seems unlikely. The partial RecA polypeptide from *S. melliferum*, as well as the truncated *S. citri* RecA polypeptide, contains neither the nucleotide binding domain nor the domain involved in DNA binding. From these observations, and from the fact that no other *recA* sequences could be detected by hybridization experiments, it seems highly improbable that *S. citri* and *S. melliferum* produce functional RecA proteins. Our data indicate that most, if not all, *S. citri* and *S. melliferum* strains are *recA* mutants.

Labarère and Barroso (20) were the first to show the high sensitivity of *S. citri* to UV irradiation. Our findings that *S. citri* R8A2, *S. citri* ASP1, and *S. melliferum* BC3 were highly sensitive to UV irradiation, and thus have deficient DNA repair systems compared with that of *A. laidlawii*, are in good agreement with the fact that *A. laidlawii* JA1 has a functional *recA* gene while *S. citri* and *S. melliferum* strains do not. Indeed, it has been shown that strain 8195 of *A. laidlawii*, a restriction-deficient derivative of strain JA1 which has a nonsense mutation leading to a truncated RecA protein, was more sensitive to UV irradiation than the $recA^+$ strain JA1 (11). However, because so far no spiroplasma $recA^+$ strain has been available and, hence, could be tested, the conclusion that the *recA* mutant genotype of these organisms is responsible for their high sensitivity to UV irradiation is still in doubt. Nevertheless, it would be of interest to characterize the *recA* genes from spiroplasma species, such as *Spiroplasma floricola* present on plant surfaces, the ecosystem of which is expected to be more subject to DNA damage by exposure to solar UV radiation than that of *S. citri* and *S. melliferum* growing within phloem tissue (*S. citri*) or insect hemolymph (*S. citri* and *S. melliferum*) (15). Indeed, the role of *recA* in DNA repair suggests that this gene plays a major role in the ability of microorganisms to tolerate such damage and, hence, to exploit certain ecosystems (17, 27). However, the RecA protein is also thought to improve cell survival and persistence, even in environments which are not affected by solar UV radiation. For example, under nutrient stress, production of many bacteriocins, such as colicin E2 of *E. coli* and pyocin AP41 of *Pseudomonas aeruginosa*, is regulated by the RecA protein (26, 41). As a result, bacterial cells are more tolerant of these competitive stresses (27). However, in the case of spiroplasmas, the diversity of bacterial flora in the *S. citri* and *S. melliferum* ecosystems is relatively poor, and the competition for nutrients would be nonessential. Under these conditions, the RecA protein would not be critical for cell survival, and *S. citri* and *S. melliferum* would have dispensed with the *recA* gene during evolution.

According to Ye et al. (45), *S. citri* and *S. melliferum* have evolved from a common ancestor by inversion of a region of the genome. According to this hypothesis, the common ancestor would have contained a functional *recA* gene, suggesting that the *recA* mutant genotype in *S. citri* and *S. melliferum* might have originated recently. Whether the $recA^+$ phenotype of *S. citri* could be restored by complementation with a functional *recA* gene is under investigation.

Nucleotide sequence accession number. The sequence data reported here have been assigned GenBank accession numbers U43710 for the *recA* region of *S. melliferum* BC3 and U31909 for the *recA* region of *S. citri.*

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