Research Note

Evaluation of the Role of RecA Protein in Plant Virulence with *recA* Mutants of *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris* NRRL B1459 *recA* mutants were isolated by recombination with an interrupted *Rhizobium etli* *recA* gene and selection of double recombinants. The mutants were impaired in homologous genetic recombination and in DNA repair as judged by their sensitivity to methyl-methane-sulfonate and to UV irradiation; these defects are complemented in *trans* by the *R. etli* *recA* gene. Virulence of *X. campestris* pv. *campestris* NRRL B1459 to cabbage is considerably diminished by the *recA* mutation. The *recA* mutation is not correlated with the frequency of occurrence of a genetic rearrangement that affects chemotaxis, plant virulence, and xanthan gum production.

Additional keywords: *recA* independent.

Xanthomonas campestris pv. *campestris* strain NRRL B1459 (Jeanes et al. 1961) is a phytopathogenic bacterium commonly used for the production of the exopolysaccharide xanthan gum; this biopolymer has several commercial applications due to its high viscosity and pseudoplastic behavior under a wide range of pHs, temperatures, and salt concentrations (Galindo 1994). Under certain growth conditions morphological variants of this strain that are defective in xanthan gum production occurred at a high frequency (Cadmus et al. 1976; Kidby et al. 1977). *X. campestris* was thus thought to be genetically unstable (Kidby et al. 1977). In a previous paper, however, we reported that this bacterium is remarkably stable and that the morphological variants may not be true *Xanthomonas* (Martínez-Salazar et al. 1993).

Kamoun and Kado (1990) reported a genetic switch in *X. campestris* that affected chemotaxis, xanthan gum production, and plant virulence. We have previously shown that *X. campestris* pv. *campestris* NRRL B1459 presented this so-called “chemotactic switch” and that this bacterium was proficient in

homologous genetic recombination (Martínez-Salazar et al. 1993).

Programmed genetic rearrangements that affect the expression of specific genes, and are mediated by genetic recombination, have been described in a wide variety of microorganisms and are thought to be of importance for the adaptation of these organisms to different environmental changes (Dybvig 1993; Miller and Kokjohn 1990). The RecA protein is involved in several of these genetic rearrangements, as in *Vibrio cholera* (Goldberg and Mekalanos 1986), *Neisseria gonorrhoea* (Kooimey et al. 1987), and *Hemophilus influenza* (Hoiseth et al. 1986). In contrast to these cases, in *Enterobacteriaceae* different phenotypic switches have been described that are *recA* independent (Plasterk and van de Putte 1984). These inversions are catalyzed by a family of related invertases.

The sequence of the *recA* genes from bacteria belonging to the protobacteria is highly conserved (Lloyd and Sharp 1993; Karlin et al. 1995). Sixty-five percent of the amino acids of the *Rhizobium etli* (previously called *Rhizobium phaseoli*) RecA protein are identical to those of the corresponding *Escherichia coli* protein (Michiels et al. 1991). Based on this high degree of sequence conservation, we predicted that the *recA* genes from *X. campestris* pv. *campestris* and from *R. etli* could recombine, rendering a functional hybrid gene. Furthermore, we predicted that through recombination with an interrupted *R. etli* *recA* gene, a *X. campestris* *recA* mutant could be selected.

To obtain a *X. campestris* pv. *campestris* *recA* mutant we first selected for recombinants with an interrupted *R. etli* *recA* gene contained in plasmid pMS26 (Martínez-Salazar et al. 1991), which is derived from plasmid pSUP202. Double recombinants were later selected. Bacterial strains and plasmids used in this work are shown in Table 1. Matings were done by plating a 1:1 mixture of the donor and recipient strains on NYGB medium (Daniels et al. 1984) and incubating overnight at 30°C; the mixture was serially diluted and plated onto selective medium.

Plasmid pMS26 was introduced by conjugation from *E. coli* S17-1 to *X. campestris* IBT148, and rifampicin-, spectinomycin-, and kanamycin-resistant transconjugants were selected

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with a frequency of 1×10^{-6} . These transconjugants are expected to be single recombinants between plasmid pMS26 and the *X. campestris* chromosomal *recA* gene, and thus contain a functional hybrid *recA* gene and an interrupted copy of this gene. These transconjugants were presumed to be due to recombination between *recA* genes, since the plasmid vector pSUP202 does not replicate in *X. campestris* and does not integrate into the *X. campestris* chromosome at any observable frequency ($<5 \times 10^{-10}$).

Double recombinants, and presumably *recA* mutants, were selected as sucrose-resistant derivatives with a frequency of 4% and were later shown to be kanamycin-sensitive. Sucrose-resistant derivatives were isolated on NYGB medium supplemented with 5% sucrose. Two of these putative double recombinants, strains IBT R2 and IBT R3, were further characterized.

Strains IBT R2 and IBT R3 are extremely sensitive to methyl-methane-sulfonate (MMS). At a concentration of 0.02% MMS, both mutants exhibited a reduction in viability of three orders of magnitude (Fig. 1). Both putative *recA* mutants have an increased sensitivity to irradiation with UV light (Fig. 2).

To measure the frequency of homologous recombination, we used plasmid pBX404-7, which contains two nonoverlapping, truncated genes derived from a gene conferring kanamycin resistance. Homologous recombination between the two truncated genes can generate a functional copy. The frequency of kanamycin-resistant derivatives from a bacterium carrying plasmid pBX404-7 reflects its efficiency in recombining homologous DNA sequences. Both IBT R2 and IBT R3 have a reduction in homologous recombination frequency of at least three orders of magnitude. Strain IBT148 presented a fre-

quency of Km^r derivatives among the Tc^r colonies of 2×10^{-3} , while IBT R2 and IBT R3 presented frequencies of 9×10^{-7} and 3×10^{-6} , respectively.

To determine that the *recA* mutation was indeed due to the insertion of the gene coding for spectinomycin resistance, we measured the genetic linkage between the Spc^r phenotype of strains IBT R2 and IBT R3 and their sensitivity to MMS and UV irradiation. Plasmid pJB3JI (Table 1) was introduced to both *recA* mutants to mobilize their Spc^r phenotype to strain IBT150 (Table 1) and the MMS sensitivity of the corresponding transconjugants was determined. We found that 25 out of 27 transconjugants tested from the cross between IBT R2 and IBT150 were unable to grow in the presence of 0.03% MMS, and that 9 out of 11 transconjugants tested from the cross between IBT R3 and IBT150 also were sensitive to this mutagen. These data suggest that the *X. campestris recA* mutation is caused by a double recombination event with the interrupted *recA* gene present in plasmid pMS26. The UV sensitivity of one transconjugant of each cross was determined (strains IBT R20 and IBT R30; Fig. 2) and, as expected, both were found to be sensitive.

The *recA* mutants were analyzed by Southern blot hybridization with the following probes (Fig. 3): plasmid pSUP202, the vector in which *R. etli recA* gene was cloned; plasmid pMS26, which carries the interrupted *R. etli recA* gene; and plasmid pCR3, which carries the *E. coli recA* gene (Horii et al. 1980). DNA from strain IBT148 and the tested *recA* mutants contains a 4-kb fragment homologous to *R. etli* and *E. coli recA* genes (Fig. 3A and B). This result suggests that the hybridizing fragment contains only a fragment of the *X. campestris recA* gene, and that the part of the *recA* gene where the spectinomycin cassette was inserted, as the product of recom-

Table 1. Bacterial strains and plasmids used in this work^a

Strain or plasmid	Relevant characteristics	Reference
<i>Xanthomonas campestris</i>		
NRRL B1459	Strain selected for xanthan production	Jeanes et al. 1961
IBT148	Spontaneous Rif^r mutant derived from NRRL B1459	Martínez-Salazar et al. 1993
IBT150	Spontaneous Ery^r mutant derived from IBT148	This work
IBT R2	<i>recA</i> mutant derived from IBT148.	This work
IBT R3	<i>recA</i> mutant derived from IBT148.	This work
IBT R20	<i>recA</i> mutant derived from IBT150 by transfer of IBT R2 mutation	This work
IBT R30	<i>recA</i> mutant derived from IBT150 by transfer of IBT R3 mutation	This work
IBT148 <i>che</i>	Chemotactic derivative from IBT148	This work
IBT R2 <i>che</i>	Chemotactic derivative from IBT R2	This work
IBT R3 <i>che</i>	Chemotactic derivative from IBT R3	This work
<i>Escherichia coli</i>		
S17-1	Able to mobilize plasmids by means of an RP4 inserted in the chromosome	Simon et al. 1983
RR1	<i>SupE</i> 44 <i>hsdS</i> 20 (<i>hsdR hsdM</i>) <i>ara</i> -14 <i>leu proA</i> 2 <i>lacY</i> 1 <i>galK</i> 2 <i>rpsL</i> 20 <i>xyl</i> -5 <i>mtl</i> -1	Boyer and Roulland-Dussoix 1969
HB101	Same as RR1, but also <i>recA</i> 13	Boyer and Roulland-Dussoix, 1969
Plasmids		
pSUP202	ColE1 origin Ap^r , Cm^r , Tc^r , mob^+	Simon et al. 1983
pMS16	Plasmid pSUP202 with <i>Rhizobium etli recA</i> , Tc^r , Ap^r	Martínez-Salazar et al. 1991
pMS26	Plasmid pMS16 with the <i>R. etli recA</i> gene interrupted with Spc^r gene, and <i>sacRB</i> Km^r inserted in plasmid Tc^r	Martínez-Salazar et al. 1991
pBX404-7	Constructed to measure genetic recombination by the generation of a functional gene conferring Km^r from two truncated nonoverlapping genes, Tc^r	Xu et al. 1988.
pJB3JI	CMA, Ap^r , Tc^r	Brewin et al. 1980
pCR3	Carries <i>E. coli recA</i> gene	Horii et al. 1980

^a Abbreviations and antibiotic concentrations (μg/ml): Resistance to ampicillin (Ap^r , 200), chloramphenicol (Cm^r , 30) erythromycin (Ery^r , 100), kanamycin (Km^r , 60), rifampicin (Rif^r , 50), spectinomycin (Spc^r , 100) and tetracycline (Tc^r , 30). Ability to be mobilized by conjugation (mob^+). Chromosome mobilization ability (CMA).

bination, could not be detected by hybridization with *R. etli* and *E. coli* *recA* genes. The spectinomycin resistance cassette hybridizes with a 1.9-kb DNA fragment (Fig. 3A), the approximate size of the spectinomycin cassette itself. Therefore, the *recA* gene fragment where the spectinomycin cassette is inserted is likely so small that it is not detected with non-homologous *recA* genes as probes for hybridization. Thus, the most plausible explanation for the *RecA*⁻ phenotype of strains IBT R2, IBT R20, and IBT R30 is that the *recA* gene is interrupted by the spectinomycin cassette as a product of a double recombination event. Based on Southern blot analysis, however, we cannot rule out the possibility that another genetic rearrangement linked to the spectinomycin cassette is responsible for the inactivation of this gene.

The Southern blot analysis shows that strain IBT R3 contains a fragment of plasmid pSUP202 (Fig. 3C). Strain IBT R3 seems to be a product of a deletion that removed some of the plasmid sequence, since it is resistant to sucrose and sensitive to kanamycin. Strain IBT R30, which is the product of the genetic transfer of the spectinomycin resistance from strain IBT R3 to strain IBT150, does not contain sequences homologous to plasmid pSUP202 (Fig. 3C). The most plausible explanation for this is that the *recA* gene of strain IBT150 was substituted by the interrupted *recA* gene from strain IBT R3 and that the adjacent plasmid sequences were not co-transferred. The co-inheritance of the spectinomycin resistance with the *RecA*⁻ phenotype in strain IBT R30, without the transfer of other changes in sequence present in strain IBT R3, suggests that the *recA* mutation is caused by the substitu-

tion of the *X. campestris* *recA* gene by the interrupted *recA* gene present in plasmid pMS26, and not by some other genetic rearrangement.

To further confirm that the phenotype of the IBT R2 mutant was due to the inactivation of the *RecA* protein, its complementation by the *R. etli* *recA* gene was analyzed. This was achieved by selecting a co-integrate between plasmid pMS16, which carries a functional *R. etli* *recA* gene but is unable to replicate in *Xanthomonas*, and the self-transmissible plasmid pJB3JI. As shown in Figure 1, mutant IBT R2 is fully complemented by the co-integrated plasmids pMS16 and pJB3JI with respect to its resistance to MMS. As controls we included the *E. coli* strain RR1 and its *recA* mutant HB101 (Fig. 1). The UV sensitivity of IBT R2 also was complemented by the *R. etli* *recA* gene (data not shown). These results show that the IBT R2 mutant phenotype is due to a deficiency in *RecA* protein function.

In a previous paper (Martínez-Salazar et al. 1993), we studied the genetic stability of strain NRRL B1459 and reported that this strain underwent a genetic rearrangement that caused a change from a nonchemotactic, xanthan gum-producer bacteria to a chemotactic and nonmucoid derivative. This genetic rearrangement reverted with a frequency of 2×10^{-4} . Strain NRRL B1459 is proficient in homologous genetic recombination, and in the presence of 0.02% MMS the frequency of recombination is increased by one order of magnitude. This increment is due to the SOS response. We also demonstrated that the frequency of reversion of the "chemotactic switch" is not affected by exposure to 0.02% MMS. Here, however, we demonstrate that the "chemotactic switch" is clearly an *recA*-independent genetic rearrangement. Both mutant (IBT R2 and IBT R3) and wild-type strains gave rise to variants that were chemotactic and nonmucoid. Bacterial chemotaxis was determined as reported by Kamoun and Kado (1990), with medium M925 with 0.05% sucrose and 0.3% agar. The frequency of reversion of one chemotactic derivative from each *recA* mutant and that from the wild-type strain are of the same order of magnitude; the frequencies found are IBT148che 3.1×10^{-4} , IBT R2che 1.5×10^{-4} , and IBT R3che 3.3×10^{-4} . Xanthan gum-producing revertants from chemotactic variants were selected by visual screening of isolated colonies grown in Burk's medium (Newton et al. 1953), as described previously (Martínez-Salazar et al. 1993).

The nature of the genetic change involved in the "chemotactic switch" remains to be determined. Both the reversibility and the *recA* independence of this rearrangement are compatible with a "change of phase" type of change similar to the one presented in some *Enterobacteriaceae* (Plasterk and van de Putte 1984), but this model remains to be experimentally validated.

In order to determine whether the plant virulence of *X. campestris* pv. *campestris* was influenced by the *recA* mutation, 30 *Brassica oleracea* (cabbage) leaves were inoculated with each of the mutants isolated in this work. We used as a control the chemotactic derivatives of IBT148, IBT R2, and IBT R3, since the chemotactic derivatives of other *X. campestris* strains have been reported to be less virulent (Kamoun and Kado 1990). The virulence of different *X. campestris* strains was evaluated as described by Kamoun et al. (1992). Three leaves of each cabbage plant at the six-leaf stage (5 weeks old) were inoculated with a saturated suspension of

Fig. 1. Bacterial viability in the presence of methyl-methane-sulfonate (MMS). Survival curves correspond to the following strains: IBT148 (Δ), IBT R2 (■), IBT R2/pMS16::pJB3JI (○), IBT R3 (●), RR1 (□), and HB101 (▼). For all the experiments presented in this paper *Xanthomonas campestris* pv. *campestris* strains were grown on NYGB medium (Daniels et al. 1984), and *Escherichia coli* strains were cultured on LB medium (Miller 1972).

Fig. 2. Bacterial sensitivity to UV light irradiation. Part of the petri dish was irradiated for 30 s and the other part was protected with foil paper; the line shows the border between the two parts. The sensitivity to irradiation with UV light was tested as described by Miller (1972).

Fig. 3. Southern blot hybridization with, as probes, plasmid pMS26 (A), plasmid pCR3 (B), and plasmid pSUP202 (C). Lanes correspond to total DNA restricted with *Pst*I of the following strains: A and B, 1) IBT148; 2) IBT R2; 3) IBT R3. C, 1) IBT148; 2) IBT R2; 3) IBT R20; 4) IBT R3; 5) IBT R30. DNA manipulations, Southern blotting (high stringency), and nick translation procedures were carried out as described by Sambrook et al. (1989).

Table 2. Plant virulence of different *Xanthomonas campestris* derivatives

Strain	Relevant characteristics	Virulence ^a
Control		–
IBT148	Wild type, Rif ^r	++++
IBT148 <i>che</i>	IBT148 nonmucoid, chemotactic derivative	+
IBT R2	IBT148 <i>recA</i> mutant	++
IBT R2 <i>che</i>	IBT R2 nonmucoid, chemotactic derivative	+
IBT R3	IBT148 <i>recA</i> mutant	++
IBT R3 <i>che</i>	IBT R3 nonmucoid, chemotactic derivative	+
IBT150	IBT148 Ery ^r spontaneous mutant	+++
IBT R20	IBT150 <i>recA</i> mutant	+
IBT R30	IBT150 <i>recA</i> mutant	+

^a Rank of plant virulence given to each strain reflects both time of symptom development and degree of lesion produced. ++++ = symptoms were apparent after 1 week of infection and lesion affected almost the entire leaf after 4 weeks. Scores from +++ to + were given to strains that produced symptoms after 10 to 12 days of infection and the lesion produced after 4 weeks ranged from almost the entire leaf affected (+++) to only a small lesion (+). Each strain was inoculated to 30 cabbage leaves with three injections per leaf. Observed variability was very low, since all leaves injected showed symptoms at roughly the same time and of the same magnitude. Between one and five of the experimental leaves per treatment were lost due to senescence before the end of the 4-week observation period.

bacteria (approximately 3×10^9 CFU/ml) in sterile saline solution by injection of 1 µl of the suspension in the middle of the leaf (three injections per leaf). The control plant inoculated with sterile saline solution did not show any lesions. Typical lesions produced by strain IBT148 were seen after 1 week, and the leaves presented in Figure 4 were photographed after 2 weeks of inoculation. The results presented in Table 2 summarize the plant virulence assay, considering both the time of appearance of infection symptoms as well as the intensity of the lesion developed by each strain. All strains were pathogenic on cabbage, but their degree of virulence varied. The IBT R2 and IBT R3 *recA* mutants showed a considerable delay in plant infection, compared with strain IBT148, but were considerably more virulent than strain IBT148*che*. The “chemotactic switch” diminished the virulence of both *recA* mutants, showing that the effect of the *recA* mutation and the “chemotactic switch” on plant virulence are caused by independent mechanisms. On the other hand, the IBT R20 and IBT R30 *recA* mutants showed a reduction of virulence similar to that presented by the chemotactic derivatives. The differences observed between *recA* mutants might be due to differences in plant virulence of their parental strains. For example, strain IBT150 (IBT148 Ery^r spontaneous mutant) was found to be slightly less virulent than strain IBT148, developing symptoms of infections 3 to 5 days later (Table 2).

Plants are known to produce reactive oxygen intermediates (ROIs) as part of their defense response to pathogen infection

Fig. 4. Photograph of cabbage leaves with characteristic lesions caused by *Xanthomonas campestris* infection after 15 days of inoculation. The control plant was inoculated with sterile saline (A) and the virulence of the following strains was determined by inoculation of 10 different plants: IBT148 (B), IBT148*che* (C), IBT R2*che* (D), IBT R2 (E), IBT150 (F), IBT R20 (G), IBT R30 (H), IBT R3 (I), IBT R3*che* (J).

(Dixon et al. 1994; Baker et al. 1997), and ROIs are known to produce severe DNA damage in all living organisms (Halliwell and Gutteridge 1989). Thus, the reduced plant virulence of the *X. campestris* *recA* mutants described in this work might be due to their impairment in the repair of the DNA damage produced by the plant defense response. An alternative explanation is that the RecA effect in plant virulence is indirect, reflecting growth rate or another general metabolic characteristic of the *recA* mutants, and not the direct involvement of the RecA protein in plant pathogenesis.

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LITERATURE CITED

- Baker, B., Zambrinsky, P., Staskawicz, B., and Dinesh-Kumar, S. P. 1997. Signaling in plant-microbe interactions. *Science* 276:726-733.
- Boyer, H. B., and Roulland-Dussoix, D. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* 4:459-472.
- Brewin, N. J., Beringer, J. E., and Johnston, A. W. B. 1980. Plasmid-mediated transfer of host range specificity between two strains of *Rhizobium leguminosarum*. *J. Gen. Microbiol.* 120:413-420.
- Cadmus, M. C., Rogowin, S. P., Burton, K. A., Pittsley, J. E., Knutson, C. A., and Jeanes, A. 1976. Colonial variation in *Xanthomonas campestris* NRRL B1459 and characterization of the polysaccharide from a variant. *Can. J. Microbiol.* 22:942-948.
- Daniels, M. J., Barber, C. E., Turner, P. C., Clearly, W. G., and Sawczyc, M. K. 1984. Isolation of mutants of *Xanthomonas campestris* pv *campestris* showing altered pathogenicity. *J. Gen. Microbiol.* 130:2447-2450.
- Dixon, R. A., Harrison, M. J., and Lamb, C. J. 1994. Early events in the activation of plant defense responses. *Annu. Rev. Phytopathol.* 32:479-501.
- Dybvig, K. 1993. DNA rearrangements and phenotypic switching in prokaryotes. *Mol. Microbiol.* 10:465-471.
- Galindo, E. 1994. Aspects of the process of xanthan gum production. *Trans. Inst. Chem. Eng. (c)* 72:227-237.
- Goldberg, I., and Mekalanos, J. J. 1986. Effect of *recA* mutation on cholera toxin gene amplification and deletion events. *J. Bacteriol.* 165:723-731.
- Halliwell, B., and Gutteridge, J. M. C. 1989. *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford.
- Hoiseth, S. K., Moxon, E. R., and Silver, R. P. 1986. Genes involved in *Hemophilus influenza* type b capsule expression are part of an 18-kilobase tandem duplication. *Proc. Natl. Acad. Sci. USA* 83:1106-1110.
- Horii, T., Ogawa, T., and Ogawa, H. 1980. Organization of the *recA* gene of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 77:313-317.
- Jeanes, A., Pittsley, J. E., and Senti, F. R. 1961. Polysaccharide B-1459 a new hydrocolloid polyelectrolyte produced from glucose by bacterial fermentation. *J. Appl. Polym. Sci.* 5:519-526.
- Kamoun, S., and Kado, C. I. 1990. Phenotypic switching affecting chemotaxis, xanthan production and virulence in *Xanthomonas campestris*. *Appl. Environ. Microbiol.* 56:3855-3860.
- Kamoun, S., Kamdar, H. V., Tola, E., and Kado, C. I. 1992. Incompatible interactions between crucifers and *Xanthomonas campestris* involve a vascular hypersensitive response: Role of the *hrpX* locus. *Mol. Plant-Microbe Interact.* 5:22-33.
- Karlin, S., Weinstock, G. M., and Brendel, V. 1995. Bacterial classification derived from RecA protein sequence comparison. *J. Bacteriol.* 177:6881-6893.
- Kidby, D., Sanford, P., Herman, A., and Cadmus, M. 1977. Maintenance procedures for the curtailment of genetic instability: *Xanthomonas campestris* NRRL B1459. *Appl. Environ. Microbiol.* 33:840-845.
- Koomey, M., Gotschlich, E. M., Robbins, K., Bergström, S., and Swanson, J. 1987. Effects of *recA* mutations on pilus antigenic variation and phase transitions in *Neisseria gonorrhoea*. *Genetics* 117:391-398.
- Lloyd, A. T., and Sharp, P. M. 1993. Evolution of the *recA* gene and the molecular phylogeny of bacteria. *J. Mol. Evol.* 37:399-407.
- Martínez-Salazar, J. M., Palacios, A. N., Sánchez, R., Caro, A. D., and Soberón-Chávez, G. 1993. Genetic stability and xanthan gum production in *Xanthomonas campestris* pv *campestris* NRRL B1459. *Mol. Microbiol.* 8:1053-1061.
- Martínez-Salazar, J. M., Romero, D., Girard, M. L., and Dávila, G. 1991. Molecular cloning and characterization of the *recA* gene of *Rhizobium phaseoli* and construction of *recA* mutants. *J. Bacteriol.* 173:3035-3040.
- Michiels, J., Broek, A. V., and Vanderleyden, J. 1991. Molecular cloning and nucleotide sequence of the *Rhizobium phaseoli* *recA* gene. *Mol. Genet.* 228:486-490.
- Miller, J. H. 1972. *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Miller, R. V., and Kokjohn, T. A. 1990. General microbiology of *recA*, environmental and evolutionary significance. *Annu. Rev. Microbiol.* 44:365-394.
- Newton, J. W., Wilson, P. W., and Burris, R. H. 1953. Direct demonstration of ammonia as an intermediate in nitrogen fixation by *Azotobacter*. *J. Biol. Chem.* 204:445-451.
- Plasterk, R. H. A., and van de Putte, P. 1984. Genetic switches by DNA inversions in prokaryotes. *Biochem. Biophys. Acta* 782:111-119.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Simon, R., Priefer, U., and Pühler, A. 1983. A broad host range mobilization system for in vivo genetic engineering: Transposon mutagenesis in gram-negative bacteria. *Bio/Technology* 1:784-791.
- Xu, B., Patzy, C., and Lurquin, P. F. 1988. A plasmid based method to quantitate homologous recombination frequencies in gram-negative bacteria. *BioTech* 6:752-760.