recA Gene of *Escherichia coli* Complements Defects in DNA Repair and Mutagenesis in *Streptomyces fradiae* JS6 (mcr-6)

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We are interested in understanding the fundamental mechanisms of mutation, DNA repair, and recombination in *Streptomyces fradiae*, a filamentous actinomycete that produces the macrolide antibiotic tylosin (4, 5). To aid these studies, we have isolated mutants of *S. fradiae* M1 which were defective in repair of damage induced by mitomycin C (MC) and characterized their responses to treatment with a variety of mutagenic agents (2, 3, 6, 25). One mutant, JS6 (mcr-6), is defective in the repair of potentially lethal damage induced by a variety of agents, including MC, UV light, 4-nitroquinoline-1-oxide (NQO), methyl methanesulphonate (MMS), hydroxylamine (HA), and N-methyl-N-nitro-N-nitrosoguanidine (MNNG), and is less mutable by MNNG, MMS, NQO, UV light, HA, and ethyl methanesulphonate (EMS) than is the wild-type strain (2, 25). A spontaneous MC-resistant mutant of JS6 expresses normal levels of resistance to all of the agents and shows wild-type levels of induced mutagenesis, indicating that a single mutation causes the multiple defects in JS6 (25). The mcr* gene product thus appears to control an SOS response in *S. fradiae* and to be functionally analogous to the RecA protein in *Escherichia coli* (28–30) and the RecE protein in *Bacillus subtilis* (5). One difference in the error-prone DNA repair systems expressed in *S. fradiae* and in *E. coli* or *Salmonella typhimurium* is that *S. fradiae* JS6 (mcr-6) is 10- to 100-fold less mutable by EMS than is its parent strain M1 (2, 25), whereas *E. coli* and *S. typhimurium* recA mutants express normal levels of EMS-induced mutagenesis (8, 12, 24). The dependence of EMS mutagenesis on an error-prone repair system suggested that *S. fradiae* expresses error avoidance mechanisms more stringent than those observed in *E. coli* and similar to those expressed in the lower eucaryote *Saccharomyces cerevisiae* (25).

In *B. subtilis*, recE mutants display multiple defects in DNA repair, recombination, and mutagenesis similar to those observed in *E. coli* recA mutants (15, 16, 32). Like *S. fradiae* JS6 (mcr-6), *B. subtilis* recE mutants show a reduced level of induced mutagenesis by EMS (32). The multiple defects in a *B. subtilis* recE mutant have been complemented by a cloned recA gene from *E. coli* (16), indicating that the recA gene of *E. coli* and the recE gene of *B. subtilis* encode analogous proteins. Since the recA promoter from *E. coli* functions in *Streptomyces lividans* (7), we surmised that the cloned recA gene of *E. coli* might complement the mcr-6 mutation in *S. fradiae* if the mcr-6 mutation was located in an analogous gene. To explore this possibility, we cloned the recA gene of *E. coli* into a vector which was bifunctional for *E. coli* and *Streptomyces* spp. and transformed *S. fradiae* JS6 (mcr-6). Plasmid pJC859, a derivative of pBR322 containing the recA gene of *E. coli* inserted on a 3.3-kilobase-pair BamHI fragment originally cloned in pBEU14 (27), was isolated from *E. coli* JC14773 by the method of Kieser (13), cut with *Bam*HI, and ligated (17) with *Bam*HI-digested pOJ160, a bifunctional vector containing the pUC19 origin of replication for *E. coli* and the SCP2* origin of replication for *Streptomyces* spp. (20; R. Stanzak and B. E. Schoner, manuscript in preparation). The ligation mix was used to transform *E. coli* JM109 (31) to apramycin resistance (23). Plasmid pRHB300 containing the appropriately sized *Bam*HI fragment inserted in pOJ160 was transformed into *S. fradiae* PM73, a mutant defective in several restriction systems (19), by the method of Matsushima and Baltz (18). One transformant containing an appropriately sized plasmid was retained, and DNA was isolated and transformed into *E. coli* HB101 (recA13). pJC859 was also transformed into HB101, and transformants containing pRHB300 or pJC859 were tested for restoration of resistance to the lethal effects of UV light as previously described (17). Both transformants were resistant to UV light, whereas HB101 was sensitive, indicating that pRHB300 contained a functional recA gene. Plasmid pRHB300 isolated from PM73 was transformed into *S. fradiae* JS6, and several normally growing and slow-growing colonies were obtained. All of the normally growing colonies contained plasmids smaller than pRHB300, whereas the slow-growing colonies contained appropriately sized plasmid DNA. One transformant (PM74) that grew relatively slowly and contained pRHB300 was retained. (Presumably, the expression of the recA gene in PM74 caused the poor growth, but this theory has not been further studied.) Since JS6 is defective in the repair of damage induced by MC, UV light, and MMS (25), we determined the levels of resistance to these agents in PM74, JS6, and M1. PM74 was substantially more resistant to the potentially lethal effects of MMS than was JS6, but it was not as resistant as the wild-type strain M1 (Fig. 1). PM74 was completely resistant.
to 0.25 µg of MC per ml, whereas JS6 was reduced in viability to a surviving fraction of less than $10^{-5}$ at this dose. At a dose of 0.375 µg of MC per ml, PM74 grew poorly relative to M1, indicating that the cloned recA gene product was not as effective as the wild-type mer gene product at inducing excision repair or carrying out recombinational repair of potentially lethal damage induced by MC at high doses. PM74 was also much more resistant to the potentially lethal effects of UV light than was JS6 (Fig. 2). At 120 J of UV light per m², the viability of JS6 was reduced 100-fold, whereas the viability of PM74 was reduced only twofold. PM74 was almost as resistant to UV-induced lethal damage as was the wild-type M1. The vector (pOJ160) containing no insert was also transformed into JS6, and the transformants were as sensitive to inactivation by MC and UV light as was JS6 (data not shown). Also, PM74 was cured of PRHB300 by passaging cells without antibiotic selection, and the cured strain was as sensitive to inactivation by UV light and MC as was JS6. The combined results indicate that the cloned recA gene from E. coli can overcome the defects in repair of potentially lethal damage due to small lesions (MMS induced) and bulky lesions (UV light and MC induced). The E. coli RecA protein contains recombinase and protease activities (28, 29) and exerts its control of DNA repair pathways in E. coli first by becoming activated in response to treatment of cells with a mutagenic agent and then by participating in the proteolytic cleavage of LexA repressors. Thus, our data suggest that the genes encoding certain enzymatic pathways for repair of small and bulky lesions are present but not efficiently expressed in JS6 and that the RecA protein is able to induce them, presumably by participating in proteolytic cleavage of a protein repressor analogous to the LexA protein (28, 30).

Since EMS mutagenesis is mediated by an error-prone DNA repair pathway in S. fradiae (2, 25), and since efficient error-prone DNA repair in E. coli requires activated RecA protein to induce the umuCD system by cleavage of the LexA repressor and to participate directly in the process (9, 25, 28, 30), we also measured EMS-induced mutagenesis in PM74, JS6, and M1. It has been shown previously that comparable doses of EMS give 10- to 100-fold lower frequencies of spectinomycin-resistant mutants in JS6 than in M1 (2, 25). PM74 yielded a much higher frequency of induced mutants than did JS6, nearly equivalent to those obtained in M1 (Fig. 3).

The results of our study indicate that multiple defects in DNA repair and mutagenesis in S. fradiae JS6 (mer-6) can be complemented by the recA gene of E. coli and that the mcr-6 mutation, therefore, is located in a gene analogous to the recA gene of E. coli and the recE gene of B. subtilis. These results support the notion that the functions of the recA gene in regulation of DNA repair and induced mutagenesis (parts of the SOS response) may be highly conserved within procaryotes (10, 11, 14, 16, 21, 22, 28). Our data also imply that the E. coli RecA protein is activated by an inducing signal in S. fradiae and participates in the cleavage of an analog of the E. coli LexA protein (28, 29) to induce an SOS response in S. fradiae.
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LITERATURE CITED


