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Survival of Recombination-Deficient Mutants of *Escherichia coli* During Incubation with Nalidixic Acid

L. SCOTT McDANIEL, LYNETTE H. ROGERS,† AND WALTER E. HILL*
Department of Microbiology, University of Southwestern Louisiana, Lafayette, Louisiana 70504

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The ability of several *Escherichia coli* strains deficient in recombination (rec) to survive in the presence of nalidixic acid was determined. Genetic blocks of the RecBC or the RecF pathways resulted in increased sensitivity to nalidixic acid when compared with the wild-type strain. Mutants lacking functional *recA*, *recL*, or *recB recC recF* genes showed the most rapid decrease in colony-forming ability when incubated with nalidixic acid. However, the *uvrB* gene also plays a role in maintaining cell viability.

To increase the probability of their survival, cells have evolved the capacity to repair a variety of physical and chemical lesions in their DNA. Repair processes include photoreactivation (17), excision repair (2, 19), postreplicative repair (18), and reinitiation repair (3). With the exception of photoreactivation, most of the processes are thought to involve DNA synthesis (11). The *rec*, *lex*, and *uvr* genes participate in repair and have been linked to inducible functions.

When the growth of *Escherichia coli* cells is prevented by incubation with nalidixic acid (NAL), a decrease in colony-forming ability is observed (7). The drug is reported to be a specific inhibitor of DNA synthesis in proliferating cultures (1, 8), but it does not inhibit DNA polymerase I (2, 15), endonuclease I, or exonucleases I, II, and III from *E. coli* (15). In addition to inhibition of DNA synthesis, single-stranded breaks accumulate in the DNA (10), and some of the DNA is degraded to acid-soluble material (9).

NAL is also reported to inhibit DNA repair synthesis, as shown by the accumulation and persistence of small-molecular-weight DNA after extensive UV irradiation (6). However, experiments using toluenized cells have shown inhibition at the replication fork with no effect on repair synthesis (20). It has been suggested recently that the *nalA* gene is a component of DNA gyrase and that NAL blocks the nicking-closing activity of the enzyme, which may lead to DNA synthesis inhibition (21). Because of the possible role of DNA repair in sustaining cell viability, we have tested the ability of strains deficient in genetic recombination to survive during incubation with NAL.

† Present address: Department of Internal Medicine, University of Texas Health Science Center, Houston, TX 77025.

The bacterial strains employed in this study and their relevant genotypes are shown in Table 1. LB broth consists of the following (in grams per liter): tryptone, 12; yeast extract, 5; NaCl, 10; and glucose, 1. LB plates (LB broth plus 15 of agar [Difco] per liter) and LB top agar (LB broth plus 7 of agar [Difco] per liter) were used in the plating procedure. Exponentially growing cells were diluted fivefold into prewarmed LB broth containing 100 μg of NAL per ml. Samples (0.1 ml) were taken at 0.5-h intervals, diluted, and plated on LB plates by using soft agar overlays held at 48°C. Plates were incubated at 37°C for 24 to 36 h before counting. Each experiment was repeated at least twice, and the averages were plotted. To determine the efficiency of plating of the mutants, various dilutions of overnight cultures were spread on LB plates containing concentrations of NAL up to 100 μg/ml. The susceptibility of the mutants to NAL was determined by the disk diffusion technique (13).

When incubated with NAL, strains deficient in recombination showed increased rates of viability loss when compared with the wild type (Fig. 1). Therefore, genes involved in recombination are also important in minimizing (repairing?) damage which accumulates during incubation with NAL. There is only a slight difference, if any, in the loss of colony-forming ability when a strain carrying both *recB recC* and *recF* mutations (JC3881) is compared with the strain carrying only *recA* (JC2926) (Fig. 1). The rate of viability loss of the *recF* strain is initially similar to that of the wild type up to 1.5 h of treatment, after which *recF* viability continues to decline. This suggests that the wild-type strain possesses a survival mechanism which is defective in *recF* strains, even though the latter is not appreciably deficient in recombination.

TABLE 1. *Bacterial strains*

Strain	Relevant genotype
AB1157[a]	Wild type
JC2926	AB1157 *recA13*
JC5519	AB1157 *recB21 recC22*
JC9239	AB1157 *recF143*
JC8471	AB1157 *recL152*
JC8679	AB1157 *recB21 recC22 sbcA23*
JC7623	AB1157 *recB21 recC22 sbcB15*
JC3881	AB1157 *recB21 recC22 recF143*
JC3890[b]	*uvrB301*
JC3893	JC3890 *recF143*
JC3896	JC3890 *recB21 recC22*
JC3899	JC3890 *recB21 recC22 recF143*

[a] The complete genotype of AB1157 is: F⁻ *thr-1 leu-6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 argE3 str-31 tsx-33 supE44* λ⁻. All strains are also *his-4*, except for JC8679, which is *his-60*.

[b] JC3890 contains the genotype of AB1157 plus the following mutations: *uvrB301*, *bio*, *chlA*, *phr*, *plg*, *lamattλ*⁻?.

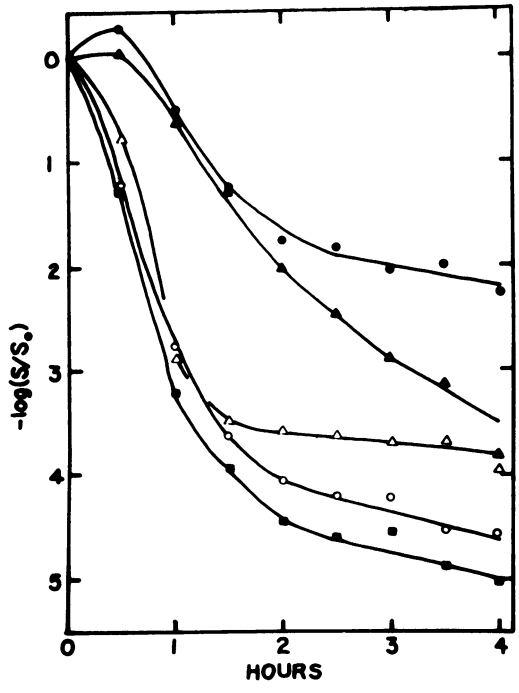

FIG. 1. *Effect of recA, recB recC, recB recC recF, and recF mutations on survival during treatment with NAL. Symbols: ●, AB1157 (wild type); ○, JC2926 (recA); ▲, JC9239 (recF); △, JC5519 (recB recC); ■, JC3881 (recB recC recF).*

To determine whether *sbcA* and *sbcB* mutations can restore wild-type levels of survival to *recB recC* strains, the *recB recC* strain (JC5519) was compared with *recB recC sbcA* (JC8679) and *recB recC sbcB* (JC7623) strains. Figure 2 shows that the *sbcA* and *sbcB* mutations cannot totally suppress the effects of the *recB recC* mutations.

The above experiments measure the ability of cells to form colonies after they have been removed from NAL. We tested the ability of mutant strains to form colonies on plates containing various concentrations of NAL, which measures the ability of cells to reproduce in the presence of NAL. At concentrations greater than 20 μg/ml, only occasional colonies were observed with all of the strains. Although the ability of the mutants to form colonies on plates containing NAL is reduced when compared with that of the wild type, the differences are not as pronounced as with those seen when the kinetics of viability loss are measured. Table 2 shows the estimated concentration of NAL required to reduce the plating efficiency to 1 and 0.01% of the number of colonies appearing on plates without NAL.

An effort was made to quantitate the relative sensitivity of the strains by measuring the diameter of the zone of inhibition produced in the susceptibility testing with paper disks impregnated with 30 μg of NAL (Difco). The results qualitatively agree with those obtained in the efficiency of plating assay (data not shown).

Recombination-deficient mutants of *E. coli*

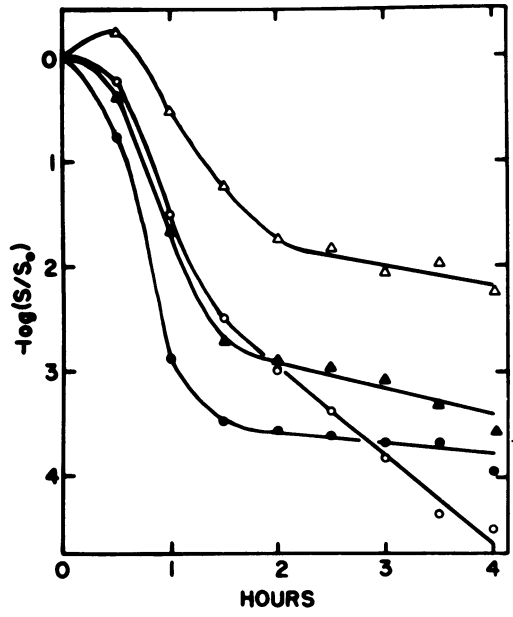

FIG. 2. *Effect of the sbcA and sbcB mutations on the survival of recB recC strains during treatment with NAL. Symbols: ●, JC5519 (recB recC); ○, JC8679 (recB recC sbcA); ▲, JC7623 (recB recC sbcB); △, AB1157 (wild type).*

show increased sensitivity to agents which are known to damage DNA (4) and probably lack some repair functions. The genetic blocks of the RecBC and RecF pathways result in comparable sensitivity to NAL and, hence, a comparable inability to repair the damage which accumulates during the inhibition of DNA synthesis by NAL.

In these experiments, the *sbcB* mutation failed to restore the wild-type level of survival to the *recB recC* strains (Fig. 2). However, the *sbcB* mutation seems to contribute more to the survival of the *recB recC* strains than does the *sbcA* mutation. This adds support to the possible functioning of the RecF pathway.

The fact that all cells in the cultures are not killed (see Fig. 1) indicates that there is a "resistant" fraction in the population. It has been shown that these cells are not genetically resistant to NAL but may be in a metabolic state that confers a transient physiological resistance (10).

Nishida and his colleagues (14) suggest that pyrimidine dimers may be involved in the bactericidal effect of NAL, with excision repair playing a key role. Our data (Fig. 3) suggest that a step in excision repair is important in protecting cells from the lethal effect of damage from NAL inhibition of DNA synthesis and support the findings of Nishida et. al. Both *recL* (16) and *uvrB* (12) are apparently involved in excision repair. The survival kinetics of the *recL* mutant show a more rapid decline in colony-forming ability than do those of the *uvrB* strain (Fig. 3), but the *recL* mutant is significantly more efficient at forming colonies in the presence of NAL than is the *uvrB* strain (Table 2). The significance of this rather curious difference in sensitivity to NAL is not known.

It has been reported recently that NAL alters the *nalA*$^+$ gene product, which may be a component of DNA gyrase (21). NAL prevents DNA gyrase from introducing superhelical turns into circular DNA, and this may result in the inhibition of DNA synthesis. However, the interaction of NAL with DNA gyrase cannot be solely responsible for the bactericidal effect. Because protein synthesis is required (5), the induction of the SOS repair system may also play a role.

Experiments to measure the rate of DNA molecule breakage during treatment with NAL and the ability of these strains to repair these breaks are being conducted.

TABLE 2. *Relative plating efficiency on NAL*[a]

Strain	1%	0.01%
AB1157	3.8	4.8
JC2926	1.8	2.4
JC5519	2.5	2.9
JC9239	3.4	4.5
JC8471	3.4	4.7
JC8679	2.5	3.5
JC7623	2.0	3.8
JC3881	2.1	2.8
JC3890	1.5	2.4
JC3893	1.6	2.6
JC3896	<0.5	0.5
JC3899	0.6	0.8

[a] The numbers reported are the concentrations of NAL in micrograms per milliliter required to reduce the plating efficiency to 1 and 0.01%.

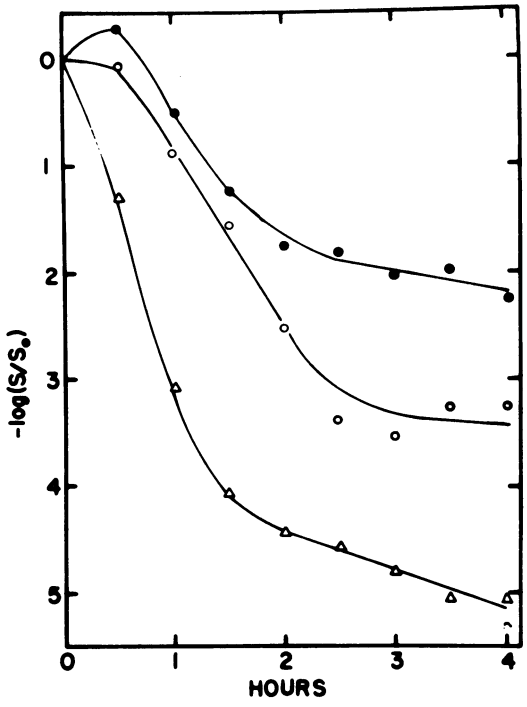

FIG. 3. *Effect of defects in excision repair on survival during treatment with NAL. Symbols:* ○, *JC3890 (uvrB);* △, *JC8471 (recL);* ●, *AB1157 (wild type).*

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