JOURNAL OF BACTERIOLOGY, July 1969, p. 238-241
Vol. 99, No. 1

Escherichia coli K-12 Mutants Resistant to Nalidixic Acid: Genetic Mapping and Dominance Studies[1]

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Received for publication 2 May 1969

Escherichia coli K-12 strains tested so far (approximately 20) can be separated into three groups on the basis of their abilities to form colonies on nutrient agar supplemented with nalidixic acid (NAL): (i) Nal^s or wild type (no growth at 1 to 2 μg/ml); (ii) NalA^r (growth at 40 μg/ml or higher); and (iii) NalB^r (growth at 4 μg/ml, but no growth at 10 μg/ml). The NalA^r group has a spectrum of sensitivity ranging from 60 to over 100 μg/ml. All Hfr strains of the NalA^r and NalB^r groups transfer NAL resistance to recipient cells at genetic loci which are at 42.5 $\pm$ 0.5 and 51 $\pm$ 1 min, respectively, on the Taylor-Trotter map. Some members of the NalA^r group also have the genetic locus for NalB^r. The *nalA*^{s} allele is completely dominant to *nalA*^{r} in a partial diploid configuration. In haploids, *nalA*^{r}-*nalB*^{r} is phenotypically NalA^r; *nalA*^{r}-*nalB*^{s} is NalA^r; and *nalA*^{s}-*nalB*^{r} is NalB^r. The map location of *nalA* and the easy differentiation between NalA^r and NalA^s allow this marker to be used as a counterselector in bacterial conjugation experiments.

Nalidixic acid (NAL) inhibits deoxyribonucleic acid (DNA) replication in many strains of *Escherichia coli* (5). This property has been useful in studying the dependence of chromosome transfer during conjugation on DNA synthesis (4, 6); more recently NAL-resistant strains of *E. coli* have been isolated and used in similar studies (1, 3).

In the present study two genes conferring different levels of resistance to NAL have been located on the *E. coli* genetic map and the dominance relationship between resistance and sensitivity has been determined.

MATERIALS AND METHODS

Strains. The *E. coli* K-12 stocks utilized and their pertinent genetic properties are listed in Table 1.

Media. BT broth contained: 0.3% Nutrient Broth (Difco), 0.5% tryptone (Difco), and 0.5% NaCl. Synthetic media were prepared in M-9 stock solution (Na_2HPO_4, 0.7%; KH_2PO_4, 0.3%; NaCl, 0.5%; NH_4Cl, 0.1%; $CaCl_2$, 0.002%; $MgSO_4$, 0.02%) properly supplemented as follows (concentrations in μg/ml): threonine, 40; leucine, 20; tryptophan, 10; proline, 20; arginine, 40; histidine, 20; tyrosine, 20; phenylalanine, 40; adenine, 20; thymine, 50; thiamine, 1; glucose, 2,000. Streptomycin (100 μg/ml) was generally used to eliminate donor cells. All media were solidified with 1.5% agar. Nalidixic acid was generously supplied by the Sterling-Winthrop Research Foundation, Rensselaer, New York.

Mating and assay. Male and female cells were grown at 37 C in broth to concentrations of about 2×10^8 cells/ml (exponential phase). For interrupted mating experiments, 10 ml of mating suspension (2×10^8 female cells per ml, 1×10^8 male cells per ml) was incubated for 5 min at 37 C, diluted gently (10-fold) into fresh broth in a prewarmed 250-ml flask and gently agitated during the course of the experiment. Samples (0.1 ml) were removed periodically, added to 3 ml of warm 0.75% agar-solution, exposed to severe agitation (blending) to break apart mating pairs (8), and plated on recombinant-selective synthetic media. Plate counts were made after 36 hr of incubation at 37 C. In entry curve experiments involving the transfer of NAL resistance, NAL was added to the assay plates (broth) at the proper concentrations (800 μg/plate for NalA and 100 μg/plate for NalB) in a layer of 0.75% agar after 2 hr of incubation.

RESULTS

Effects of NAL on cell survival and Nal^r mutants. The fraction of wild-type cells of *E. coli* K-12 that are able to form colonies on nutrient agar supplemented with NAL decreases sharply with increasing NAL concentrations (Fig. 1; strains

[1] The data presented in this paper are taken from a thesis submitted to the Committee on Molecular Biology, University of Pennsylvania, by M. W. Hane in partial fulfillment of the requirements for the Ph. D. degree.

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TABLE 1. *E. coli K-12 strains utilized*[a]

Strain	Mating type	Relevant genotype[b]	Source
AB444	F⁻	*arg aroC purF str*ʳ	B. Low
AT2092	F⁻	*his pheA arg purF str*ʳ	A. L. Taylor
EM1302	F⁻	*aroC purF recA str*ʳ	E. McFall
EM2001	F′	AB444 + F32 (*aroC*⁺ *purF*⁺ *nalA*ˢ)	E. McFall
KL16	Hfr	*str*ˢ	B. Low
KL98	Hfr	λ⁺ *str*ˢ	B. Low
KL164	Hfr	*thy nalB*ʳ *str*ˢ	B. Low
KL830	F⁻	*his trp pro thr leu str*ʳ	B. Low
PA309	F⁻	*his trp thr leu arg str*ʳ	H. Marcovich
MH4	Hfr	*nalB*ʳ *str*ˢ	KL16
MH5	Hfr	*nalA*ʳ *str*ˢ	KL16
MH6	Hfr	*nalB*ʳ *nalA*ʳ *thy str*ˢ	KL164
MH7	F⁻	*aroC purF recA nalA*ʳ *str*ʳ	EM1302
MH8	Hfr	λ⁺ *nalA*ʳ *str*ˢ	KL98
MH1003	F⁻	*his pheA purF nalA*ʳ *str*ʳ	AT2092
MH1004	F′	MH7 + F32 (*aroC*⁺ *purF*⁺ *nalA*ˢ)	EM2001 × MH7

[a] The genetic symbols are those used by Taylor and Trotter (10).

[b] Some mutations affecting resistance to phage and inability to utilize carbon sources have been omitted.

KL16 and EM1302); 1 to 2 μg of NAL per ml decreases cell survival to the 10% level. Nalʳ mutants were selected by incubating exponentially growing cells for several hours in broth containing various concentrations of NAL. Two general classes of mutants may be defined: (i) those resistant to 4 μg of NAL per ml but sensitive to 10 μg/ml (e.g., strains MH4 and KL164; Fig. 1); and (ii) those resistant to 40 μg of NAL per ml or more (Fig. 1; strains MH5, MH6, and MH7). These two resistant classes will be designated as NalBʳ and NalAʳ, respectively. Some strains of the second class are sensitive to 60 μg/ml (e.g., MH5 and MH7), whereas others are resistant to concentrations above 100 μg/ml (e.g., MH6). The more resistant class (NalAʳ) may be obtained in a single mutational step from the wild type (e.g., MH5) or by an additional mutational event from the NalBʳ class (e.g., MH6, a derivative of KL164). These mutants are quite stable and show the same resistance to NAL after more than 40 generations of growth.

Chromosomal positions of the Nalʳ mutations. Preliminary experiments utilizing conjugation [HfrH(NalAʳ, *str*ˢ) × PA309 (*str*ʳ)] indicated that Nalʳ was transferred at about 55 min and would thus be at about 45 min on the Taylor-Trotter map (10). Nalʳ derivatives of Hfr KL16 (origin, *thy*⁺, *his*⁺, *trp*⁺, . . . *str*ˢ) are particularly useful in investigating this chromosomal region; marker entry curves for a cross between MH5 (*nalA*ʳ, *str*ˢ) and KL830 (*thy*, *nalA*ˢ, *his*, *str*ˢ) are given in Fig. 2. *NalA*ʳ enters at about 12 min after *thy* and about 4 min before *his* and would therefore be at about 43 min on the Taylor-Trotter map. In a cross between MH4 (*thy*⁺, *nalB*ʳ, *his*⁺, . . . *str*ˢ) and KL830 (Fig. 3), *nalB*ʳ is found to enter approximately 5 min after *thy* and 12 min before *his*; it would be at 51 min on the circular map. In the NAL-resistant donor strains tested to date (approximately 10), all strains resistant to 40 μg of NAL per ml transfer Nalʳ at the 43-min map location (*nalA*ʳ), whereas those showing resistance to 4 μg/ml but sensitivity to 10 μg/ml of NAL transfer Nalʳ at the 51-min map location (NalB). The high-resistance donor strain MH6 is of special interest because it was isolated from a low-resistance NAL strain (KL164); the results of a cross between it and AB444 (*arocC*, *purF*, *arg*, *str*ʳ) are given in Fig. 4. Since *aroC* and *purF* are both located at 44 min, *nalA*ʳ and *nalB*ʳ are located at 42 and 52 min, respectively, in this experiment. The weighted averages of all the matings performed locate *nalA*ʳ at 42.5 ± 0.5 min and *nalB*ʳ at 51 ± 1 min.

Dominance studies for NalA. Dominance was examined by preparing a partial diploid strain carrying both *nalA*ʳ and *nalA*ˢ. The episome F32 (9) comprises the *E. coli* map region between approximately 39 and 45 min and thereby carries the genetic loci *purF*, *aroC*, and probably *nalA*. The F′ strain EM2001 [AB444(*aroC*, *purF*, *arg*, *str*ʳ) + F32 (*aroC*⁺, *purF*⁺, *nalA*ˢ)] was found to transfer its episomal markers at about 60 times the efficiency of transfer of its chromosomal ones; a cross between this strain and F⁻ MH7 (*recA*, *aroC*, *purF*, *nalA*ʳ) produced AroC⁺-PurF⁺-Arg⁺ colonies; if the selection plates were overlaid with NAL (400 μg) either immediately upon plating or after a 2-hr lag, no colonies were produced. This result is consistent with the hypothesis that *nalA*ˢ is transferred on the F32 episome along with *aroC*⁺ and *purF*⁺ and that a partial diploid configuration is produced in the *recA*⁻ recipient (7) in which *nalA*ˢ is dominant to *nalA*ʳ.

To test this hypothesis several of these AroC⁺-PurF⁺-Arg⁺-Na1Aˢ cells were isolated (e.g., MH1004) and tested further. The following points confirm this interpretation. (i) MH1004 is still sensitive to ultraviolet (i.e., it is still RecA⁻);

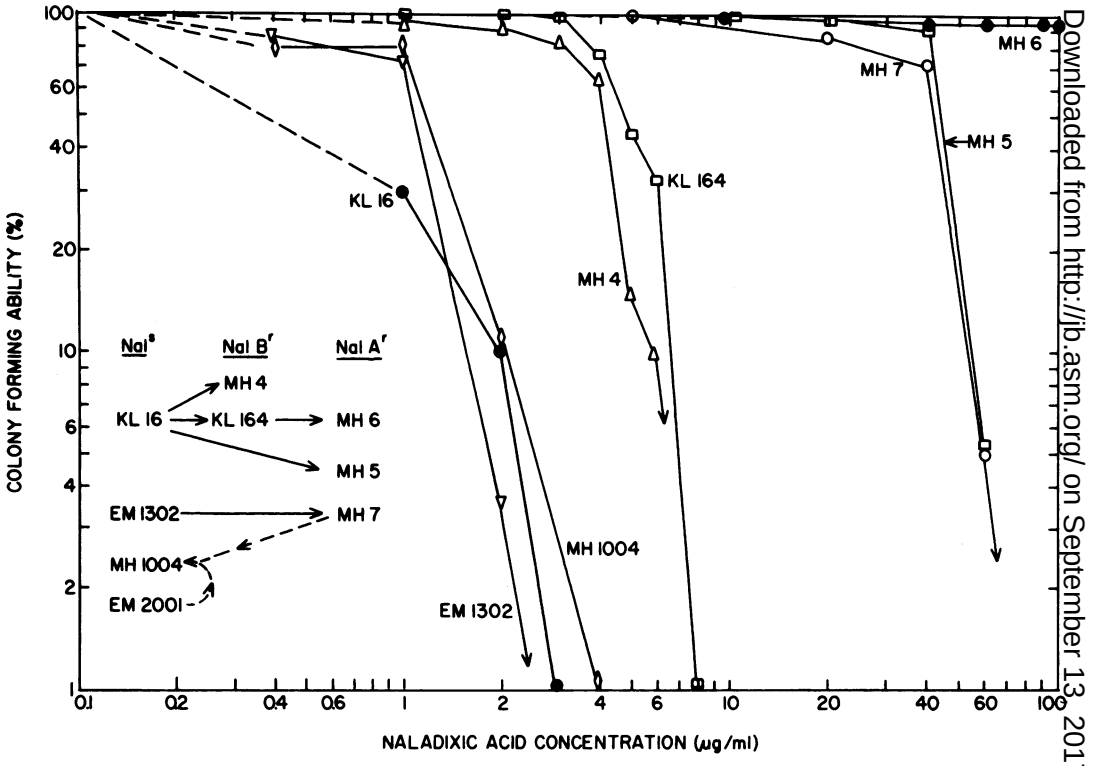

FIG. 1. *Colony-forming abilities of E. coli K-12 strains on nutrient plates supplemented with various concentrations of naladixic acid. Schematic in the lower left portion of the figure gives the origins of the various strains. MH1004 was formed by episomal transfer of nalA^s from EM2001 into MH7.*

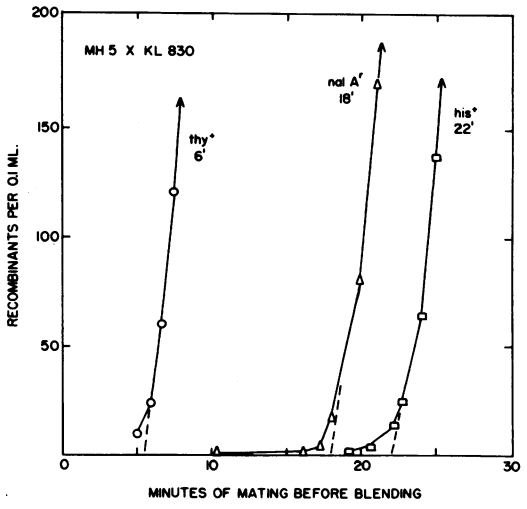

FIG. 2. *Marker entry curves for a mating between Hfr MH5 and F⁻ KL830. The donor was counter-selected with streptomycin.*

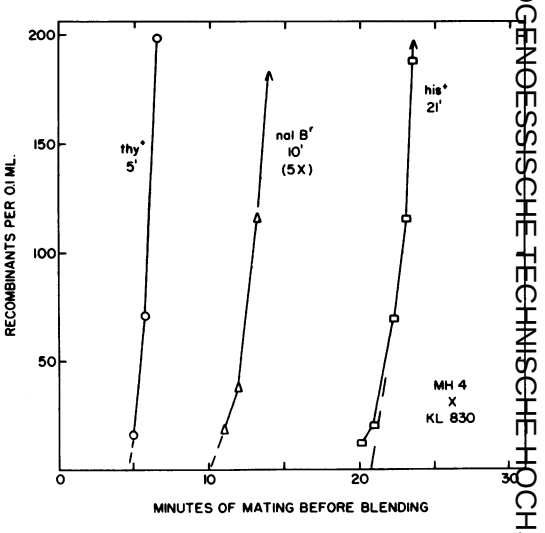

FIG. 3. *Marker entry curves for a mating between Hfr MH4 and F⁻ KL830. The donor was counter-selected with streptomycin. The recombinant numbers for NalB^r are five times those found experimentally, it is difficult to obtain a uniform concentration of NAL (2 to 4 μg/ml) on the assay plates and often a large fraction of the NalB^r recombinants are killed.*

(ii) it is sensitive to the male phage f1 (i.e., it carries at least a part of the F32 episome); (iii) it spontaneously produces haploid segregants having the phenotype AroC⁻-PurF⁻-NalA^r at a

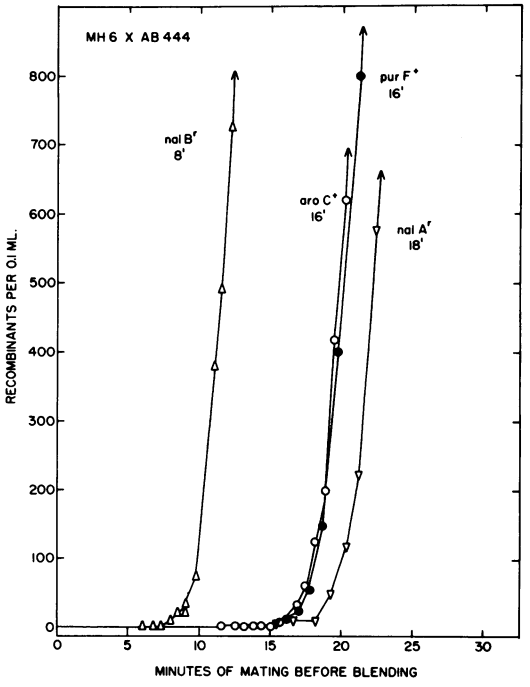

FIG. 4. *Marker entry curves for a mating between Hfr MH6 and F⁻ AB444. The donor was counter-selected with streptomycin.*

frequency of about 10^{-3} per generation; (iv) the phenotype of over 90% of the population changes from AroC⁺-PurF⁺ to AroC⁻-PurF⁻ on incubation with acridine orange [20 μg/ml for 5 hr at *p*H 7.85 (2)]; (v) MH1004 transfers the F32 episome into proper recipient cells with a relatively high efficiency [the analysis of 202 Ade⁺ colonies produced by a cross between MH1004 and F⁻ MH1003 (*pheA*, *purF*, *nalA*ʳ, *his*) showed that 17% had the phenotype Phe⁻NalA^{s}His⁻ which would be expected from a transfer of the modified F32 episome into MH1003]; and (vi) MH1004 has the same sensitivity to NAL as wild-type (Nal^s) strains (Fig. 1). We conclude therefore that MH1004 is a partial diploid carrying *nalA*^{s}/*nalA*ʳ and that sensitivity to NAL is completely dominant to resistance.

DISCUSSION

The *E. coli* strains tested so far (Fig. 1) can be separated into three groups on the basis of their abilities to form clones on nutrient agar supplemented with NAL: (i) Nal^s (no growth at 1 to 2 μg/ml), (ii) NalAʳ (growth at 40 μg/ml or higher), and (iii) NalBʳ (growth at 4 μg/ml, but no growth at 10 μg/ml). The NalAʳ group has a spectrum of sensitivity ranging from 60 to over 100 μg/ml.

All Hfr strains of the NalAʳ and NalBʳ groups transfer NAL resistance to recipient cells at genetic loci which are at 42.5 ± 0.5 and 51 ± 1 minutes, respectively, on the Taylor-Trotter map. Some members of the NalAʳ group also have the genetic locus for NalBʳ (e.g., MH6; Fig. 4). *NalA*^{s} is completely dominant to *nalA*ʳ in a partial diploid configuration (MH1004). In haploids, *nalA*ʳ-*nalB*ʳ is phenotypically NalAʳ (MH6); *nalA*ʳ-*nalB*^{s} is NalAʳ (MH1, MH5, MH7, and MH10); and *nalA*^{s}-*nalB*ʳ is NalBʳ (KL164 and MH4). Dominance relationships for *nalB*ʳ/*nalB*^{s} have not been studied.

The map location of *nalA* and the easy differentiation between *nalA*ʳ and *nalA*^{s} allow this marker to be used as a counterselector in bacterial conjugation experiments.

ACKNOWLEDGMENTS

We are most grateful to Brooks Low for many valuable suggestions made during the course of this study and for several bacterial strains. We acknowledge with pleasure the technical assistance of S. Randolph and E. Ambush. M. W. Hane is grateful for support from Molecular Biology training grant 5T01 GM-0069408; the work reported herein was supported by U.S. Public Health Service grant RH00501 from the National Center for Radiological Health, Consumer Protection and Environmental Health Service.

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