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NOTES

Isolation of *rel* Mutants of *Escherichia coli* B/r

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A method that relies on the biological effect of near-UV (340-nm) irradiation is described by which large numbers of independent *rel* mutants of *Escherichia coli* B/r may be rapidly isolated.

In *Escherichia coli* the control of ribosome synthesis by guanosine tetraphosphate (ppGpp) has generally been studied using mutants in which ppGpp metabolism is altered (2, 6, 8, 9, 12, 17, 19, 20). The *relA*-dependent accumulation of ppGpp after amino acid starvation has been well defined (1, 4, 5), but the reactions and effectors involved in a *relA*-independent pathway of ppGpp metabolism (16) have remained obscure. To study *relA*-independent ppGpp metabolism, it is necessary to develop methods by which mutants having deficiencies in the pathway may be isolated. One approach to identify mutants of this type would be to screen large numbers of *rel* mutants for altered levels of ppGpp during exponential growth.

To generate large numbers of independent *rel* mutants, attempts have been made to utilize the biological effects of near-UV (340-nm) irradiation (15, 18). At this wavelength of light, the 4-thiouridine residue in position 8 of some tRNA molecules forms an adduct with a cytidine residue at position 13 in the D-loop stem which inactivates the tRNA and results in amino acid starvation (14, 21). When the cells are removed from irradiation, they recover by de novo synthesis of active tRNA. In rel^+ strains a prolonged growth delay occurs since the bacteria have accumulated high levels of ppGpp that must first be drastically reduced before tRNA synthesis can resume (13, 14). Strains which are *rel* exhibit a much shorter growth delay since they accumulate much less ppGpp, and tRNA synthesis is rapidly resumed. Previous attempts to take advantage of this phenomenon in isolating large numbers of *rel* mutants were inefficient, however, due to the high incidence of tRNA modification mutants; among 105 mutants isolated, 102 were tRNA modification mutants (*nuvA*, *nuvC* [18]). These mutants are unable to introduce the 4-thiouridine modification into their tRNA and are therefore unaffected by near-UV irradiation. Here, we have modified the previously used method (18) to avoid the preferential selection of tRNA modification mutants.

The bacterial strains used in this study are listed in Table 1. For the isolation of near-UV-resistant mutants, ca. 500 bacteria from a culture of strain NC51 were spread onto LB plates (11), and the plates were incubated at 30°C for 6 h. The plates were then subjected to cycles of intermittent near-UV light (340 nm) for 24 h. Each irradiation cycle consisted of UV exposure (32 J/m^2 per s) for 1 h, followed by a 2-h recovery period. Thus, to avoid the selection of tRNA modification mutants, the previous method was modified by reducing the period of exposure to intermittent near-UV irradiation and the duration of each intermittent UV cycle. These measures were implemented to reduce the probability of a *rel* mutant colony being overgrown by a spontaneous mutant having a secondary tRNA modification mutation. Moreover, to avoid isolating tRNA modification mutants, which on average grow into large colonies, small colonies that formed on the selection plates were isolated. To test the implementation of these modifications to overcome the selection of tRNA modification mutants, 10 mutants each from the large (L mutants) and small (S mutants) colonies that formed were isolated and characterized by four criteria: (i) colony size after intermittent UV irradiation; (ii) auxotrophy for thiamine (iii) mass increase after amino acid starvation, and (iv) level of ppGpp after amino acid starvation.

The colony size after intermittent near-UV irradiation was determined by streaking the mutants onto LB plates which were then incubated and irradiated so that half of each streak received intermittent irradiation. All but one of the S mutants gave small colonies in the irradiated

TABLE 1. Bacterial strains

Strain	Genotype	Reference
NC51	*hsr lacZ valS*(Ts) *relA*$^+$ *relC*$^+$	14
NC52	*hsr lacZ valS*(Ts) *relA relC*$^+$	14
RL73	*metA rpoB* (Rif^r) *relA*$^+$ *relC*$^+$	This study
JF618	*thr-1 leuB6 proA2 pyrE60 his-4 cdd-6 pyrG51 argE3 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 rpsL (strA31) supE44*	3

area and large colonies in the unirradiated sectors, whereas the L mutants (exemplified by L1) gave colonies of the same size in both sectors (Table 2). The S mutant (S5) that exhibited the large-colony phenotype was not used for further analyses.

About 25% of the near-UV-resistant mutants isolated by the previous method (18) were found to be simultaneously deficient in 4-thiouridine modification and in the biosynthesis of thiazole, a precursor of thiamine (7); the mutations in these strains were found to map in *nuvC* (18). It was determined that all of the S mutants isolated here were Thi$^+$ (Table 2), whereas 4 of the 10 L mutants were Thi (the result for mutant L1 only is shown in Table 2).

After amino acid starvation, *rel* mutants exhibit a greater increase in cell mass, reflecting the continued accumulation of RNA, than do *rel*$^+$ strains (13). When the relative increase in cell mass of the mutants was measured after induction of amino acid starvation, the S mutants responded like the *rel* strain NC52, whereas the L mutants behaved like the *rel*$^+$ strain NC51 (Table 2).

The preliminary identification of the S mutants as *rel* was confirmed by measuring the levels of ppGpp in the mutants after amino acid starvation (Table 2). All of the S mutants exhibited low levels of ppGpp after amino acid starvation for 7.5 min, the time at which the ppGpp levels are maximal in the *rel*$^+$ strain NC51, whereas the L mutants had high levels of ppGpp. In some of the S mutants (e.g., S1), the concentration of ppGpp was somewhat elevated, although it was considerably lower than that in the *rel*$^+$ strain NC51. These differences may be due to the *rel* mutations in these strains being somewhat leaky but nonetheless sufficient to produce lower levels of ppGpp and hence a shorter growth delay than in *rel*$^+$ strains after UV irradiation. This is consistent with these strains forming very small colonies during intermittent UV irradiation (Table 2).

To determine whether the mutations in the S mutants were *relA* or *relC*, bacteriophage P1 lysates were prepared from each mutant and used to transduce strains JF618 and RL73 to Cyt$^+$ and Met$^+$ Rif^s, respectively. Such derivatives were then screened for a relaxed phenotype. It was found that none of the mutants were *relC*, whereas of the seven mutants tested, all were defective in *relA* function (Table 2).

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TABLE 2. Characterization of near-UV-resistant mutants

Strain	Colony size[a]	% Mass increase[b] after amino acid starvation	ppGpp[c]	Thi[d]	Mutation[e]
S1	Very small	43	258	+	*relA*
S2	Very small	43	218	+	*relA*
S3	Very small	62	150	+	*relA*
S4	Small	62	97	+	ND
S6	Small	69	50	+	*relA*
S7	Small	81	31	+	ND
S8	Small	68	9	+	*relA*
S9	Small	77	21	+	ND
S10	Small	81	19	+	*relA*
S11	Small	69	134	+	*relA*
L1	Large	28	1,780	−	ND
NC51	None	26	1,300	+	*relA*
NC52	Small	77	9	+	*relA*

[a] The size of the colonies was determined after 24 h of intermittent UV irradiation by comparing the colony size in the irradiated and unirradiated sectors of the plates.

[b] The percent mass increase was measured 1 h after a temperature shift from 30 to 42°C to induce amino acid starvation.

[c] Picomoles per optical density unit at 460 nm. The levels of ppGpp were determined, as previously described (10), 7.5 min after a temperature shift to 42°C to induce amino acid starvation.

[d] +, Prototrophs; −, auxotrophs.

[e] ND, Not determined.

The method described here is considerably simpler, faster, and more efficient than those previously used (15, 18) and is thus well suited for isolating large numbers of independent *rel* mutants. Moreover, the intermittent near-UV colony size screen used here to distinguish between *nuv* and *rel* mutants was found to be very reliable and considerably simpler and faster than autoradiographic methods (2, 15).

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