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Genetic analysis of *sul* mutants of *Escherichia coli* B

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SUMMARY

The *sul* gene of B/rw (Witkin) and 26 independently isolated *sul* mutants were examined for their suppression of radiation sensitivity. The suppressed *lon* gene was demonstrated by transducing it into a K12 *proC* recipient. The *sul* gene of B/rw and all but one of the B/r mutants was found to be linked to a gene controlling azide resistance. Transduction data established the order of markers as *leu azi mutT sul*. One B/r mutant was found not transducible with azide resistance. *sul* mutations were found to suppress the capsular polysaccharide production of *lon* strains.

1. INTRODUCTION

In a previous paper it was demonstrated that *Escherichia coli* B/r contained a gene *sul* (suppressor of *lon*) which controlled the radiation resistance of that strain (Donch, Chung & Greenberg, 1969). *sul* was not an allele of *lon*, because *lon* could be transduced from strain B/r with *proC*$^+$ into a *proC* K12 strain. Transductants receiving the *lon* gene of B/r were sensitive to ultraviolet radiation (UV) and mucoid-like *lon* mutants of *E. coli* K12 (Howard-Flanders, Simson & Theriot, 1964; Donch & Greenberg, 1968*b*). *sul* was tentatively mapped between *tonA* and *lac*. In this paper it will be shown that *sul* is linked to the gene controlling azide resistance, the order of the markers in the region being *leu azi mutT sul lac* (Fig. 1).

2. METHODS

Bacterial strains. Bacterial strains used are shown in Table 1.

Phages. Phage P1*bvir* (Donch & Greenberg, 1968*a*), was used throughout these experiments, and shall be referred to as P1. P1 was grown on donor strains for at least two cycles by picking single plaques and harvesting as described in Adams (1959).

Media. The minimal (DM) and complete media (JN) used were described by Donch & Greenberg (1968*a*). Sodium azide was prepared as a 3 % aqueous solution, autoclaved for 10 min at 15 lb pressure and 121 °C, and added to melted JN agar such that the final concentration was 150 μg/ml.

Transductions. The method for transduction was described by Donch &

Greenberg (1968*a*) except with respect to the expression of the azide marker. Azide resistance is dominant (Hayes, 1957), but requires time for expression. The scoring of azide resistance was found to be enhanced when a mutant selected for azide resistance was also streptomycin-resistant. The reason for this is not clear. When selection was made for azide resistance, transducing mixtures were diluted in phosphate (0·1 M, pH 6·8) buffered saline and filtered (Millipore membrane filter

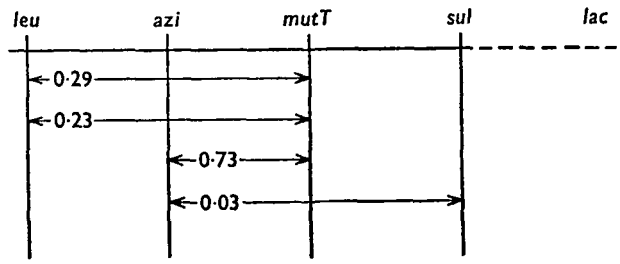

Fig. 1. Genetic map of the *leu–lac* region of the *Escherichia coli* chromosome (modified from Taylor & Trotter, 1967). The distances are given in terms of cotransduction frequencies determined with phage P1 using isogenic donor and recipient.

Table 1. *Characteristics of bacterial strains*

	Genotype (relevant markers only)	Source
B251	*lon*	W. Arber
PAM 2512	*leu lon str*^{r}	Derivative of B251
PAM 2513	*azi*^{r} *mutT lon str*^{r}	Transduction of PAM 2512 using P1 GW15
B/rw	*sul lon*	E. Witkin
GW15	*azi*^{r} *mutT sul lon*	GW derivative of B/rw
HB45	*thr leu sul mutT lon str*^{r}	H.B., derivative of B/rw
Bs8	*sul lon str*^{r}	R. Hill
χ478	*leu proC str*^{r}	R. Curtiss III
PAM 482	*azi*^{r} *proC*	Derivative of χ478
PAM 9951	*thr leu proA metA str*^{r}	Derivative of AB1899
AB1899	*thr leu proA str*^{r}	P. Howard-Flanders

Abbreviations conform where possible to the recommendations of Demerec *et al.* (1966). For azide and streptomycin, *azi*^{r} and *str*^{r} mean resistance to sodium azide and streptomycin respectively. *lon* means the gene responsible for UV sensitivity and UV-induced filamentation.

0·22 μ). Filters were transferred to JN agar containing 200 μg/ml of streptomycin. Plates were incubated for at least 3 h, at which time the membranes were transferred to JN agar plates containing 150 μg/ml of sodium azide and 200 μg/ml of streptomycin. All incubations were at 37 °C except as otherwise indicated.

Ultraviolet survival curves. Curves describing the survival of colony-forming ability following UV were derived as described by Donch & Greenberg (1968*a*). A rapid test for response to UV has been described (Greenberg, 1964). All colony counts were done with a prototype of the colony counting instrument described by Ingels, Daughters & Burzio (1968) and made by Varian Associates.

Isolation of radiation-resistant mutants. E. coli B251 was grown overnight in JN broth. The following day 0·1 ml amounts of appropriately diluted cells were spread on JN agar plates and exposed to 924 ergs/mm² of UV and incubated for 24 h. Survivors were picked into 2 ml of JN broth and incubated overnight. The following day samples were spotted on to JN plates with capillary pipettes, the plates irradiated with 924 ergs/mm² of UV and incubated overnight. Under these conditions B/r mutants gave a dense unbroken spot, while B or other UV-sensitive strains gave blank spots or highly discontinuous growth. After re-isolation survival curves were performed on the UV-resistant survivors. All the survival curves resembled that or B/rw. Like B/rw, none formed filaments after UV, and 26 such mutants were examined further and are the subject of this report.

Isolation of azide-resistant mutants. 0·1 ml of culture containing about 5×10^9 cells/ml was spread on JN agar containing 150 μg/ml sodium azide and the plates were incubated for 2 days. Surviving clones were restreaked on azide agar and incubated for 2 days.

Filamentation. The induction of filaments by UV was examined by the method of Donch & Greenberg (1968*a*).

Direct selection of UV resistance. Direct selection of UV resistance was performed by adding an homologous P1 preparation at an m.o.i. of 5 to early exponential phase cells ($1–3 \times 10^8$ cells/ml). After adsorption for 30 min cells were centrifuged and resuspended in sterile water, and 0·1 ml of appropriate dilutions were spread on supplemented DM glucose plates. Plates were incubated for 30–45 min, then UV-irradiated with 616–1078 ergs/mm² and incubated for at least 48 h before surviving colonies were counted.

3. RESULTS

Transductions of sul⁺ *to a B/r recipient.* In a previous communication (Donch, Chung & Greenberg, 1969) the *sul* gene was mapped by conjugation. It was tentatively placed between *lac* and *tonA*. Experiments, using P1 mediated transductions,

Table 2

Selected marker	No. examined	Frequency of unselected markers (%)			
		leu⁺	*azi*ʳ	*mutT*	*sul*⁺
leu⁺	823	—	29	23	0
*azi*ʳ	1526	22	—	73	2·9

P1 donor was PAM 2513 *azi*ʳ *mutT sul*⁺ and the recipient was HB45 *leu azi mutT*⁺ *sul*. Control plates consisted of recipient cells plated without phage and were either negative or contained no more than ten colonies.

were therefore designed to test whether the *sul* gene could be cotransduced with known genes in this region of the chromosome: *thr*⁺, *leu*⁺, *azi*ʳ, *mutT* and *proA*⁺.

To test for cotransducibility of *sul* with *azi*ʳ or *leu*⁺ P1 grown on the UV-sensitive (*sul*⁺) strain PAM 2513 (*azi*ʳ *mutT sul*⁺) was used to transduce the B/rw derivative HB45 (*leu azi*ˢ *mutT*⁺ *sul*) to *leu*⁺ or *azi*ʳ. The response to UV of 823 *leu*⁺ and

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1526 *azi*^{r} clones was tested by the rapid method. Table 2 shows that though *leu*$^+$, *azi*^{r} and *mutT* were cotransduced, sensitivity to UV was observed only among transductants selected for azide resistance. Fig. 2 shows the UV survival curve of one such transductant PAM 4535, together with that of each parent. All UV-sensitive transductants were found to form filaments following UV irradiation, as did the donor but not the recipient. The 44 (2·9 %) UV-sensitive (*sul*$^+$) transductants were found to fall into two classes. Sixty-six per cent were of the donor type (*mutT sul*$^+$) and 34 % were recombinant type formed by double crossing-over (*mutT*$^+$ *sul*$^+$). (Data confirming the position and reactivity of the *mutT* locus will be described in detail elsewhere by G. Warren.)

Position of the sul *gene.* Since *sul* was not cotransducible with *leu*$^+$ but was with *azi*^{r}; and *leu*$^+$, *azi*^{r} and *mutT* were cotransducible, and from the frequencies of the various classes involving *mutT* and *sul*, the order of markers deduced is *leu azi mutT sul* (Fig. 1).

The sul *locus of B/rw and independently isolated B/r mutants.* As described previously, B/rw contains the gene *lon*, which is cotransducible with *proC*$^+$ (Donch *et al.* 1969). Each of the independently isolated B/r mutants was also found to contain a *lon* gene cotransducible with *proC*$^+$ into χ478 at a frequency of 3 %. When introduced into χ478, the *lon* genes were expressed phenotypically by producing mucoid colonies characteristic of the *lon* mutants of K12 described by Howard-Flanders, Simson & Theriot (1964) and Donch & Greenberg (1968*a*, *c*), or *capR* mutants (Markovitz, 1964; Markovitz & Baker, 1967). The *lon* transductants were UV-sensitive and filamenting after irradiation. Fig. 3 shows the UV survival curve of one such *lon* transductant, PAM 4701, and those of strains χ478 and B/rw.

After demonstrating the existence of the suppressed *lon* gene in all 26 B/r mutants, experiments were performed to test whether with those mutants the gene, *sul*, which suppressed *lon* could be transduced by P1 with *azi*^{r} into a *sul*$^+$ recipient. In 25 of the mutants the *sul* gene was cotransducible with *azi*^{r} at frequencies varying from 0·4 % to 2·1 %. In one mutant, PAM 1209, *sul* was not cotransducible with *azi*^{r}.

PAM 1209 was subjected to further analysis. An attempt was made to transduce the *sul*$^+$ gene into it using PAM 2513 as a donor and selecting for azide resistance. Of 871 azide-resistant transductants examined, none were UV-sensitive.

The possibility existed that PAM 1209 was *sul*$^+$ at the azide-linked gene and its *sul* gene was located elsewhere on the chromosome. This was tested by using PAM 1209 as a donor in P1 transductions, and PAM 1202, another B/r mutant which has an *azi* linked *sul* gene, as a recipient. No UV-sensitive (*sul*$^+$) transductants were observed among the 1000 azide-resistant transductants selected. PAM 1209 is probably *sul* at the *azi*^{r}-linked locus, but the *sul* mutation must be displaced too far to the right to be cotransduced with *azi*^{r}. Whether PAM 1209 is related to the *fts* mutants of Van de Putte, Van Dillewijn & Rörsch (1964) and Hirota, Ryter & Jacob (1968) is not known at this time. This mutant is undergoing further study.

The sul *gene of strain Bs8.* Bs8, originally isolated from strain B by Hill & Feiner (1964), is a double mutant (Donch & Greenberg, 1968*b*). It carries *uvrB* and a second mutation (*sul*), a suppressor of the demonstrated *lon* gene of Bs8. Was this *sul* gene cotransducible with *azi*^{r}? When PAM 2513 (*sul*$^+$) was used as a donor in P1 transductions of azide resistance to strain Bs8 the *sul*$^+$ allele was introduced at a frequency of 1·9%. Fig. 4 shows the UV survival curves of Bs8 and one of the transductants, PAM 888, which had received the *sul*$^+$ gene. The transductant is more sensitive to UV than Bs8 and forms filaments following UV irradiation.

The sul$^+$ *gene of strain K12.* In a previous paper it was deduced that most K12 strains were *sul*$^+$ (Donch *et al.* 1969). This was based on the observation that the *lon* gene could be transduced into K12 recipients and its pleiotropic effects observed: UV sensitivity, mucoidy and UV-induced filamentation. If strain K12 were *sul* as is B/r, those results could not have been obtained, because, as will be shown below, the *sul* gene suppresses the production of excessive mucoid polysaccharide as well as UV sensitivity and filamentation in *lon* strains.

Table 3. *Transduction of* sul *recipient by P1·*sul$^+$ *K12 strain*

Selected	No. examined	Frequency of unselected markers (%)		
		leu$^+$	*azi*^{r}	UV^s
leu$^+$	312	—	29	0
azi^r	300	33	—	3

P1 donor was PAM 482 *azi*^{r} and the recipient was HB45 *leu*. UV^s means sensitivity to ultraviolet light. Control plates consisted of recipient cells plated without phage and were either negative or contained no more than ten colonies.

To test definitively whether strain K12 was *sul*$^+$, P1 transductions were performed, using P1 grown on the K12 strain PAM 482 *azi*^{r} to transduce HB45 (*leu*, *sul*) to *leu*$^+$ and *azi*^{r}. 312 *leu*$^+$ and 300 *azi*^{r} transductants were examined. Table 3 shows that when azide resistance was selected 3% of the transductants were sensitive to UV, but none of the *leu*$^+$ transductants were UV sensitive. (All the UV-sensitive clones were filamentous after exposure to UV.) The UV survival curve of one of the UV-sensitive transductants, PAM 4527, is shown in Fig. 2 together with the UV survival curves of parental strains HB45 and PAM 482. PAM 4527 has the typical biphasic curve of strain B. This is taken to mean that the K12 donor is *sul*$^+$.

Transduction of sul *to a mucoid* lon *K12 recipient.* The effect of *sul* on the production of excessive mucopolysaccharide associated with the *lon* gene was determined. In conjugal crosses between Hfr B/rw (HB33) and PAM 9951 *lon*, performed as described by Donch & Greenberg (1968*a*), it had been observed that non-mucoidy and UV resistance were linked. P1 grown on B/rw (*azi*^{r}) was used to transduce *azi*^{r} into PAM 9951 (K12 *lon*, mucoid). Nine hundred and twenty-one transductants were isolated and tested for their response to UV and for mucoid production. All (15) UV-resistant transductants were non-mucoid and were not

induced to form filaments by UV, and all UV-sensitive transductants were mucoid and were induced by UV to filament. A total of 156 non-mucoid transductants were ultimately isolated from several experiments. All were found to be UV-resistant. Thus, *sul* was a single mutation which suppressed both sensitivity to UV and the excess production of mucoid polysaccharide.

Dominance of sul. Using the method of direct selection of UV resistance previously described by Donch & Greenberg (1968*c*), P1*bvir* grown on B/rw was used to infect PAM 2513. Table 4 shows that of the 350 cells surviving the initial ex-

Table 4. *Direct selection of UV resistance*

			Phenotype*†			
P1 donor	Recipient	Survivors*	Mucoid UV^s (%)‡	Mucoid UV^r (%)	Non-mucoid UV^s (%)	Non-mucoid UV^r (%)
B/rw	PAM 2513	350	0	0	80	20
B/rw	PAM 9951	921	83	0	0	17
PAM 2513	PAM 2513	8	0	0	100	0
PAM 9951	PAM 9951	23	100	0	0	0

* Growth and expression occurred on DM glucose plus supplements after 48 h at 37 °C.
† Survivors were tested for UV resistance by the rapid method.
‡ All mucoid colonies were UV-sensitive.

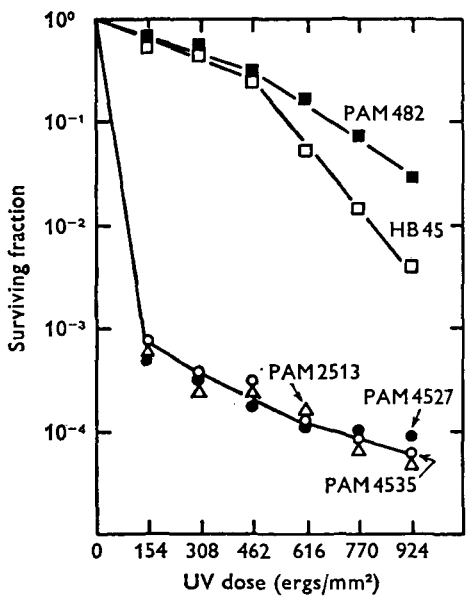

Fig. 2

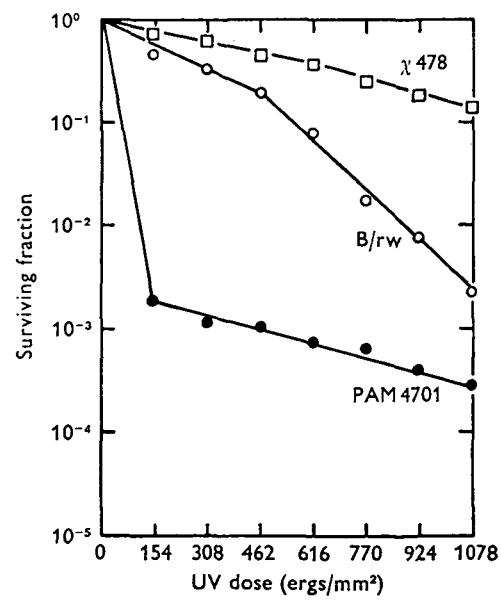

Fig. 3

Fig. 2. Ultraviolet survival curves of HB45, PAM 482 and its *sul*⁺ transductant PAM 4527, PAM 2513 and its *sul*⁺ transductant PAM 4535. Both transductants were obtained using HB45 as a recipient.

Fig. 3. Ultraviolet survival curves B/rw, and a mucoid *proC*⁺ transductant, PAM 4701, and the recipient, χ478.

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posure of UV to form colonies and reisolated into broth, 70 (20 %) were resistant to UV. These are considered to be stable recombinants. Two hundred and eighty of the surviving clones lost the property of UV resistance in one passage. Some of these are considered to be that small fraction of the population which chances to survive the dose of UV and the number can be estimated from number of survivors observed in the selfing experiment (i.e. about 8). The remainder (272) are considered to have been unstable heterozygotes, carrying the *sul* gene sufficiently long to permit them to survive the lethal effects of UV irradiation. If this interpretation is correct, then episomally carried *sul* acts as a dominant in strains carrying *sul*$^+$ on their chromosome.

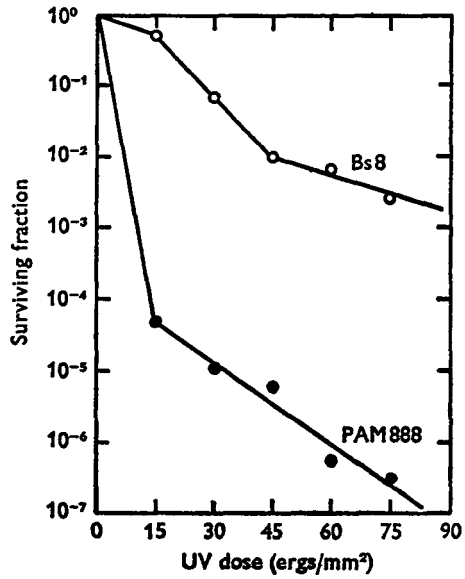

Fig. 4. Ultraviolet survival curve of Bs8 and a *sul*$^+$ transductant PAM 888. The recipient was Bs8.

This led to the question of what would happen to the mucoid properties of a K12 *lon* under the same experimental conditions. The effect of episomally located *sul* was therefore tested on the K12 *lon* mutant, PAM 9951 (mucoid, filamenting, UV-sensitive). Again selection was made directly for UV resistance. In the results shown in Table 4 17 % of the survivors were found to be UV-resistant and these were non-mucoid. No segregation to mucoidy was observed when these were restreaked on minimal medium. The remaining 83 % were mucoid and UV-sensitive. Again only a small fraction of these could be accounted for as survivors as in the selfing experiments.

These results differ from those reported earlier, transducing *lon*$^+$ into mucoid *lon* recipients (Donch & Greenberg, 1968*c*). In these earlier experiments the complementing particle established a relatively stable heterogenotic state, reflected in the observation that segregation from non-mucoidy to mucoidy persisted for several passages.

4. DISCUSSION

These results confirm and extend earlier observations that there is a gene, *sul*, which suppresses the phenotypic expression of *lon*. *sul* is now known to be co-transducible with a gene controlling resistance to azide, *azi*^{r}, and the order of markers in the region is *leu azi*^{r} *mutT sul tonA*. The *sul* mutation has been found to occur in each of 26 independently isolated radiation resistant (B/r) mutants of strain B, all of which are *lon*. The one B/r mutant in which *sul* could not be transduced with *azi*^{r} was, nevertheless, not *sul*$^+$. This observation makes it unlikely that it is mutant in a cistron other than *sul*.

The fact that all the B/r mutants thus far examined are *lon sul* leads to two conclusions. First, it is unlikely that the *lon* allele of strain B is a revertible point mutation. In this regard it is important to point out the following. Previous results have shown that radiation resistance can be transferred by conjugation from strain K12 to strain B. Resistant recombinants resulting from such crosses were phenotypically identical to strain B/r and very similar to wild-type strains of K12. Furthermore, it was possible to transfer from strain B to a wild-type (with regard to UV resistance) strain of K12 a gene which produced a phenotype similar to strain B. Mutants of strain K12 have been isolated which have all the characteristic responses to UV of strain B. These are mutant in the gene *lon*, which is cotransducible with *proC*, as is the *lon* gene of strain B. All of which leads to the conclusion that strain B differs from other laboratory strains of *E. coli* in lacking a *lon*$^+$ function. The evidence suggests that this lack is not the result of a point mutation, not the result of a missense or nonsense mutation, but likely the result of deletion. This would account for the failure to discover any *lon*$^+$ mutants of strain B, albeit in a limited series, nor any suppressor mutations other than *sul*. It is inferred that *sul* specifically suppresses *lon*, and involves the functional properties of *lon* rather than with restoring *lon* to *lon*$^+$. However, while it is not absolutely certain that *sul* is not a code-reading suppressor, though other evidence to be discussed suggest this strongly, it is certain that it is not an amber or ochre suppressor. None of the *sul* mutants including B/rw support the growth of amber or ochre mutants of phage T 4. Furthermore, nonsense mutants can be prepared in B/rw as shown by Bridges, Dennis & Munson (1967). If *sul* were a nonsense suppressor this would not have been possible. Furthermore, B. A. Bridges (personal communication) has tested a number of B/r strains and found no evidence of UGA suppressor activity. He was unable to grow either of two UGA mutants of T 4 on several B/r (*sul*) type strains B/r (Alper), WPS (Witkin), WU3610 (Witkin) or Hr/30-2 (Kondo).

The data in this report show also that strain K12 wild-type is *sul*$^+$ because a gene with the expected properties of *sul*$^+$ can be cotransduced with *azi*^{r} into strain B/r, producing a phenotype identical with that of strain B. Furthermore, the reciprocal is possible: *sul* can be cotransduced with *azi*^{r} into *lon* mutants of strain K12 and these transductants are then not phenotypically Lon but behave like wild-type. Furthermore, the data show that one additional phenotypic property of *lon*, the tendency to excrete excessive amounts of mucoid poly-

saccharide (Howard-Flanders, Simson & Theriot, 1964; Donch & Greenberg, 1968*a*, *c*) is suppressed by *sul*. Strain B, on the other hand, is not mucoid because of mutations elsewhere on the chromosome which suppress the synthesis of the capsular mucoid polysaccharide without significantly effecting the other expression of *lon* (Donch & Greenberg, in preparation).

It should be noted with regard to the transduction of *sul* to K12 *lon* mutants that this has been accomplished with two randomly chosen representatives of the two phenotypic classes of *lon* mutants which occur in strain K12 (Donch & Greenberg, 1968*c*). This observation increases the likelihood that *sul* directs some function which compensates for the conditionally (UV) lethal properties of *lon* mutants.

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