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Salmonella typhimurium Mutants of RfaH⁻ Phenotype: Genetics and Antibiotic Sensitivities

By B. A. D. STOCKER, BONNIE M. MALES AND WAKO TAKANO*

Department of Medical Microbiology, Stanford University School of Medicine, Stanford, California 94305, *U.S.A.*

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Transductional mapping, with phage ES18 or ES18.*hl*, showed that several mutations causing the RfaH⁻ phenotype (defective formation of galactose I and also of more distal units of the lipopolysaccharide core) were located between *metE* and *pepQ* in the *Salmonella typhimurium* linkage map; the affected locus is designated *rfaH*. The mutation of one strain of RfaH⁻ phenotype was located elsewhere, at an unidentified *rfa* locus. Introduction of an F′ plasmid containing the *metE* segment of the *Escherichia coli* chromosome into several *rfaH* mutants restored the 'smooth' (Rfa⁺) phenotype. Several *rfaH* mutations, and that of the phenotypically similar *rfa* mutant, caused increased sensitivity to bacitracin, polymyxin, novobiocin, nafcillin and oxacillin, as expected if the mutations have no effect on the formation of the part of the lipopolysaccharide core proximal to the galactose units.

INTRODUCTION

Salmonella typhimurium mutants of class *galE* (lacking UDPgalactose epimerase activity) if grown in the absence of exogenous galactose cannot synthesize UDPgalactose and therefore make lipopolysaccharide (LPS) of type Rc, i.e. galactose-deficient LPS core – see Fig. 1 of the following paper (Lindberg & Hellerqvist, 1980). This LPS defect results in a characteristic pattern of susceptibility to 'rough-specific' phages, including sensitivity to phages C21 and Br2 (Wilkinson & Stocker, 1968; Wilkinson *et al.*, 1972). (C21-sensitive mutants of classes *rfaG* and *galU* differ from *galE* mutants in that they are resistant to phage Br2 and make LPS core lacking the glucose I unit, through lack of, respectively, the relevant glucosyltransferase or ability to synthesize UDPglucose.) Two rough mutants which, though able to make UDPgalactose, showed the same phage sensitivities as *galE* mutants were termed class *rfaH* (Wilkinson & Stocker, 1968). Osborn (1968) found that extracts of these two mutants did not catalyse the transfer of labelled galactose from UDPgalactose to C-3 of glucose I of type Rc (galactose-deficient) LPS, and that, correspondingly, their LPS would accept galactose from UDPgalactose when incubated with extracts of *rfa*⁺ bacteria. It was surmised (Wilkinson & Stocker, 1968; Osborn, 1968) that a gene affected in these two mutants, termed *rfaH*, specified an enzyme for the galactosyl-transfer reaction which forms the 'main-chain' galactose unit, galactose I, of the LPS core. Three additional mutants with the same phage sensitivity pattern and with similar *in vitro* galactosyl-transfer properties were assigned to class *rfaH* by Kuo & Stocker (1972); they showed that the affected genes of all five *rfaH* mutants were located elsewhere than the short *cysE–pyrE* segment of the linkage map, which includes nearly all other identified *rfa* genes. In the following paper, Lindberg & Hellerqvist (1980) describe chemical and serological analyses of the LPS of the original two *rfaH* mutants; the results indicate that each of the two mutations causes partial,

* Present address: Faculdade de Medicina de Fundação ABC, Santo André, Brazil.

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but not complete, failure to form the galactose I unit, but also causes partial defects in the reactions for formation of several core units distal to galactose I. We here report that the mutated genes of four of the five above-mentioned *rfaH* mutants are located between *metE* and *pepQ* and that the LPS core defect of several of these mutants is corrected by an F′ factor bearing the *metE–pepQ* segment from the *Escherichia coli* chromosome (cf. Creeger *et al.*, 1979). The affected gene (*rfa-658*) of strain SL3648, the fifth of the previously described *rfaH* mutants, is located elsewhere (and so should no longer be termed *rfaH*).

Defects of the deep part of the LPS core of *S. typhimurium* cause increased sensitivity to some antibiotics (Roantree *et al.*, 1977). We here report changes in antibiotic sensitivities caused by *rfaH* mutations (or by the mutation of a phenotypically similar strain mutated at a locus other than *rfaH*) and compare them with changes caused by *galE*, *galU* and *rfaG* mutations.

METHODS

Bacterial strains and phages. The strains used are listed in Table 1. Of the five mutants previously described as *rfaH* (Wilkinson & Stocker, 1968; Osborn, 1968; Kuo & Stocker, 1972) note that one, strain SL3648, appears from our transduction results (Table 2) to be mutated in a gene different from that affected in the others; its mutant gene is therefore termed *rfa-658*, instead of *rfaH658*. The phages used to distinguish smooth and different classes of rough mutant were those described by Wilkinson and his colleagues (1972), together with KB1, active on strains lysogenic for P22 (Boro & Brenchley, 1971) but not on strains lacking O antigen (B. A. D. Stocker, unpublished observations). The transducing phages used were: ES18, and its derivative ES18.*h1* which can be grown on LT2 sublines which retain Fels 2 lysogeny (Kuo & Stocker, 1970); and P22.HT105/1, and an *int* derivative of it (Schmieger, 1972). Phage P22 wild-type or P22.*sie* were used to lysogenize strains which were to be recipients in ES18 or ES18.*h1* transduction (Kuo & Stocker, 1970).

Phage and genetic methods. Phage pattern, i.e. sensitivity or otherwise to a variety of smooth-specific, rough-specific and other phages, was determined as described by Wilkinson *et al.* (1972). Hfr and F′ crosses were made by standard methods. For transduction we used the 'drop-on-lawn' procedure (Kuo & Stocker, 1970). Strains to be used as recipients in ES18 transduction were first made lysogenic for P22 or P22.*sie* (Kuo & Stocker, 1970). For selection of *metE*$^+$ transductants we used defined medium with purified agar (Difco Noble Agar), to minimize vitamin B12 contamination. Transductants acquiring *metE*::Tn10 transposon insertions were selected on nutrient agar with tetracycline (10 μg ml^{-1}). Smooth or RfaH$^-$ phenotype, and by inference *rfa* genotype, of purified transductants was determined by the phage pattern test, saline agglutinability or O agglutinability in a slide test, and by growth in broth, with uniform turbidity or with heavy deposit and little turbidity. For tests on large numbers of transductants we used only phages 9NA (active on smooth strains even if lysogenic for P22), C21 and a mixture of phages Br60 and Ffm (both active on rough strains). For *pmi* strains (deficient in phosphomannoisomerase and therefore unable to make O repeat units unless provided with mannose), the LPS phenotype was determined on mannose-supplemented medium.

Antibiotic sensitivity tests. Minimum inhibitory concentrations were determined by the method of Roantree *et al.* (1977).

RESULTS

Approximate mapping of rfaH487 by Hfr crosses

The *rfaH* strain SL1060 (or a mannose-fermenting, presumed *pmi*$^+$, revertant of it) was used as recipient in crosses with several smooth Hfr donor strains, with selection for donor Met$^+$ or Xyl$^+$ character and against donor auxotrophic character. [The first two *rfaH* mutants described, SL1060 and SL1033, were both derived, via a *pmi* mutant, from strain SL1027, which is smooth and requires methionine because it is *metA* (Wilkinson & Stocker, 1968). Some time after isolation of the *rfaH* mutants, strain SL1027 was found to have a leaky, cryptic *metE* mutation (Kuo & Stocker, 1970). However, we found the two *rfaH* mutants, SL1060 and SL1033 (and their *rfa*$^+$*pmi* parent, SL1030), to be phenotypically MetE$^+$ (Table 1, footnote*). Crosses involving these two mutants were therefore interpreted on the assumption that they were *metE*$^+$.] Several Hfr crosses in which selection was made for donor Met$^+$ character yielded recombinants, many of which were *rfa*$^+$, as indicated by their smooth phage sensitivity pattern and O agglutinability. The presence or absence of

Table 1. *Derivatives of Salmonella typhimurium* LT2 *used*

Strain no.	Description	Source/reference
Parent strains, rfaH mutants and transductional donors		
SL1027*	*metA22 trpD2 H1-b H2-e,n,x* 'cured of Fels 2' *flaA66 rpsL120 xyl-404 metE551*	Wilkinson *et al.* (1972)
SL1030*	As SL1027 but *metE⁺ pmi-404*	Wilkinson *et al.* (1972)
SL1033*	As SL1030 but *rfaH481*	Wilkinson & Stocker (1968)
SL1060*	As SL1030 but *rfaH487*	Wilkinson & Stocker (1968)
SL1741	As SL1060 but carries F′-*lac-ataA*-P22.HT105/1	From SL1060 by F′ transfer
SL3581	*his(rfb)519*$_\Delta$ Str^R *galK462* (F′8-*gal*)	Kuo & Stocker (1972)
SL3635	As SL3581 but *rfaH655*	Kuo & Stocker (1972)
SL3648†	As SL3581 but *rfa-658*	Kuo & Stocker (1972)
SL3625	*hisC527 xyl-416 flaA401*	Vary & Stocker (1973)
SL3657	As SL3625 but *rfaH659*	Kuo & Stocker (1972)
SL1772	*proB25*$_\Delta$ *pepP2 rfaH487*	From TN88 by transduction (Table 3, cross 4)
Strains used as transductional recipients		
metE47	*metE47*	
SL1723	*metE47*(P22)	
TA17	*hisR1223 hisD2473 metE338*	McHugh & Miller (1974)
SL1745	As TA17 but carries P22	
TN88	*proB25*$_\Delta$ *pepQ1 pepP2 metE1355*	McHugh & Miller (1974)
SL1763	As TN88 but carries P22	
SL1766	As TN88 but carries P22.HT105/1	
TN87	*proB25*$_\Delta$ *pepQ1 pepP1*	McHugh & Miller (1974)
SL1265	As TN87 but carries P22.*sie* and *metE862*::Tn10	
SL1266	As TN87 but carries P22.*sie* and *metE884*::Tn10	
F′ strains		
TR562	*hisG200 metE338 ilvC401 pur-847* (F′14)	Miller & Roth (1971)
TR563	As TR562 but *hisC117* instead of *hisG200*	Miller & Roth (1971)
TR564	As TR562 but *hisC342* instead of *hisG200*	Miller & Roth (1971)

* The 'leaky' *metE551* mutation of strain SL1027 causes a partial requirement for methionine or vitamin B12, evident when the effect of the *metA* mutation is reversed by provision of homocystine; its existence was discovered (Kuo & Stocker, 1970) after the isolation of *pmi* mutant SL1030 from SL1027. SL1030 and its *rfaH* mutants SL1033 and SL1060 are phenotypically MetE⁺, either by 'reversion' of *metE551* or because the *metE* mutation occurred after the isolation of SL1030.

† Strain SL3648, previously regarded as an *rfaH* mutant because of its phage sensitivity pattern and *in vitro* galactosyl-transfer character (Kuo & Stocker, 1972), appears from transduction (Table 2) to be mutated at a separate locus and is therefore now indicated as *rfa-658* instead of *rfaH658*.

rfa⁺ recombinants indicated that *rfaH* was transferred early by donors of types HfrK3, HfrK6, HfrK13, HfrK14, HfrK19, HfrA, HfrH10 and HfrH4, but not by HfrK10 (data not shown). From the origins of transfer of these donors (Sanderson *et al.*, 1972) we infer that *rfaH487* of SL1060 is between about unit 83 and unit 96 on the recalibrated *S. typhimurium* linkage map (Sanderson & Hartman, 1978).

Transduction by phage ES18 *or* P22 *grown in rfaH mutant* SL1060

Phage ES18 or ES18.*h1* grown on SL1060 (*rfaH487*) was used to evoke prototrophic transductants from strains with auxotrophic mutations mapping in the 83 to 96 unit segment of the linkage map. No *rfaH* clones were detected amongst transductants derived from *metB*, *metF*, *argH*, *argC*, *hemC* or *ilv* recipients (data not shown). When the recipient was *metE47* or *metE338*, cotransduction of the *rfaH487* allele of SL1060 with *metE⁺* was observed, usually at frequencies of about 25% (Table 2, crosses 1, 2 and 3). To test whether

Table 2. *Cotransduction of rfaH with metE+*

Cross no.	Donor: Strain no.	Donor: *rfa* allele	Recipient: Strain no.	Recipient: *metE* allele	Phage	*metE+rfaH*/ total *metE+* transductants
1	SL1060	*rfaH487*	*metE47*	*metE47*	ES18	5/44 = 11%
2	SL1060	*rfaH487*	SL1723	*metE47*	ES18	12/56 = 21%
3a*	SL1060	*rfaH487*	SL1745	*metE338*	ES18	13/49 = 27%
3b*	SL1060	*rfaH487*	SL1745	*metE338*	ES18	24/100 = 24%
4†	SL1741	*rfaH487*	TA17	*metE338*	P22.HT105/1	2/6 = 33%
5	SL1033	*rfaH481*	SL1723	*metE47*	ES18	4/14 = 29%
6	SL3635	*rfaH655*	SL1723	*metE47*	ES18.*h1*	3/12 = 25%
7	SL3648	*rfa-658*	SL1723	*metE47*	ES18.*h1*	0/27
8	SL3657	*rfaH659*	SL1723	*metE47*	ES18.*h1*	3/12 = 25%

* Cross 3a, 49 *metE+* transductants scored for Rfa phenotype after single-colony re-isolation; cross 3b, 100 additional transductants scored without purification.

† The transducing phage used was from spontaneous induction in *rfaH487* strain SL1060 made lysogenic by introduction of an F′(P22.HT105/1) plasmid (see text).

Table 3. *Distribution of unselected donor alleles in transduction of metE+ or pepQ+ from rfaH487 donors to metE pepQ recipients*

The recipient in cross 4 was TN88 (*proB pepP pepQ metE*) and in the other crosses its P22-lysogenic derivative, SL1763.

Cross no.	Donor	Phage	No. of transductants with donor alleles:			
Selection for metE+			*metE+*	*metE+rfaH*	*metE+rfaH pepQ+*	*metE+pepQ+*
1	SL1060	ES18	0	0	45	4
2	SL1060	ES18	26	13	11	0
3	SL1060	ES18	27	5	15	1
4	SL1060	ES18	14	3	2	1
5	SL1772	ES18.*h1*	18	5	3	1
Selection for pepQ+			*pepQ+*	*pepQ+rfaH*	*pepQ+rfaH metE+*	*pepQ+metE+*
6	SL1772	ES18.*h1*	1	4	6	0
7	SL1772	ES18.*h1*	6	10	11	0

phage P22, as well as phage ES18, could cotransduce *rfaH* with *metE* we used the supernatant of a broth culture of strain SL1741, which is strain SL1060 (*rfaH487*) made lysogenic for P22 by acquisition of an F′-*lac-ataA* with an integrated P22.HT105/1 prophage (B. A. D. Stocker, unpublished). The filtered supernatant contained only 9×10^6 plaque-forming units ml^{-1}, but its transducing activity allowed detection of cotransduction of *rfaH487* with *metE+* (Table 2, cross 4).

Cotransduction tests with three other rfaH mutants and with a phenotypically similar rfa mutant

Cotransduction of *rfaH* with *metE+* by phage ES18 or ES18.*h1*, at frequencies of about 25%, was also observed for three other *rfaH* alleles (Table 2, crosses 5, 6 and 8). [Strain SL3635 is an *rfaH* mutant of a strain with a deletion of the *rfb* gene cluster, so that one could not tell whether its *rfaH655* mutation prevented addition of O chains. The *metE+ rfaH655* transductants derived from the smooth recipient SL1723 (*metE*, P22 lysogenic) were unaffected by smooth-specific phages 9NA and KB1, which shows that *rfaH655* in a *rfb+* strain prevents addition of O chains.] All of 27 *metE+* transductants evoked by phage ES18.*h1* grown on SL3648 (*rfa-658* and of RfaH− phenotype) were smooth, like the recipient (Table 2, cross 7).

Mapping of rfaH in relation to pepQ

Selection for metE⁺, using SL1060 (*rfaH487*) *as donor.* Gene *pepQ*, specifying peptidase Q, is located on the 'late' side of *metE*, and cotransducible with it by phage P22 at frequencies of 8 to 16% (McHugh & Miller, 1974). To map *rfaH* in relation to *pepQ* we made use of strain TN88 (= LT2 *proB pepP pepQ metE*), a proline auxotroph which, because of lack of peptidases P and Q, cannot use leucylproline as a proline source (McHugh & Miller, 1974). Phage ES18 grown on SL1060 (*rfaH487*) was used to evoke *metE⁺* transductants from P22-lysogenic derivatives of TN88; purified transductants were scored for *rfaH* and *pepQ* character. In three of four experiments (Table 3, crosses 2, 3 and 4) in which the donor was SL1060, the frequency of cotransduction with *metE⁺* was 25 to 48% for *rfaH487* and 15 to 33% for *pepQ⁺*, and the frequency of the *metE⁺* class with donor *rfaH* but without donor *pepQ⁺*, compared with the rarity of those with donor *pepQ⁺* but without donor *rfaH*, indicated the order *metE–rfaH–pepQ*. One experiment (Table 3, cross 1) gave an anomalous result; all of 49 transductants selected as *metE⁺* had the donor *pepQ⁺*, and 45 of them also had the donor *rfaH487*.

Selection for pepQ⁺, using a constructed rfaH487 strain as donor. To look for cotransduction of *rfaH* and *metE⁺* with *pepQ⁺* as selected allele we used the same *proB pepP pepQ metE* recipient as before, and selected transductants able to grow with leucylproline (Cyclo Chemical Division, Tavenol Laboratories, Los Angeles, Calif. 90001, U.S.A.) (McHugh & Miller, 1974) as proline source. To avoid selecting *pro⁺* or *pepP⁺* transductants we used as donor not SL1060 but strain SL1772, a *metE⁺rfaH487 pepQ⁺* transductant derived from strain TN88 (Table 3, cross 4). In two experiments the frequency of cotransduction of *rfaH487* with *pepQ⁺* by phage ES18.*h1* was about 82% and that of *metE⁺* with *pepQ⁺* was about 45%, and the relative frequencies of the two classes with only one unselected donor marker clearly indicated the order *metE–rfaH–pepQ* (Table 3, crosses 6 and 7). Transductants with donor *metE⁺* were also selected in this experiment; the frequency of different transductant classes (Table 3, cross 5) was about the same as when the donor was SL1060 (Table 3, crosses 2, 3 and 4).

'*Phenotypic curing*' *of rfaH strains by* F'14

Construction of rfaH metE recipients. The recent report of Creeger and her colleagues (1979) that the LPS defect and lack of *in vitro* galactosyl-transferring ability of *rfaH* mutant SL1060 are 'cured' by introduction of a 'Carbon' plasmid containing an unidentified fragment of the *E. coli* chromosome prompted us to test whether an F' plasmid carrying the *metE* region of the K12 chromosome would have the same effect. For this we needed *metE rfaH* strains as recipients. Our transductants of this character had been discarded, and the previously used *proB pepP pepQ metE* starting strain had regained the ability to grow with leucylproline as protein source. Two strains of constitution *proB pepP pepQ* (P22.*sie*) *metE*::Tn10 were therefore derived from strain TN87 (Table 1) by lysogenization, followed by transducing in either of two *metE*::Tn10 transposon insertions (Kleckner *et al.*, 1977). ES18 or ES18.*h1* lysates of *rfaH* mutants SL1033, SL3635 and SL3657 and of *rfa-658* mutant SL3648 were applied to the newly constructed recipient strains, SL1265 and SL1266, and selection was made for the ability to grow on defined medium with methionine and leucylproline, i.e. for *pepQ⁺* or *pepP⁺* or *pro⁺* transductants. The *pepQ⁺* transductants thus obtained included one or more which were still Met⁻ but had acquired the RfaH⁻ characters of donors SL1033, SL3635 and SL3657, and were therefore suitable for the test of 'curing' by an F' factor. None of several *pepQ⁺* transductants (and none of 11 *pepQ⁺metE⁺* transductants obtained by selection on unsupplemented defined medium) evoked by phage grown on SL3648 (*rfa-658*) had its RfaH⁻ phenotype – which is further evidence that *rfa-658* is not between *pepQ* and *metE*.

Table 4. *Antibiotic sensitivities of* (i) *RfaH⁻ and Rfa⁺ strains and, for comparison,* (ii) *different classes of core-defective mutant*

(i) *RfaH⁻ and Rfa⁺ strains*

Strain no.	LPS genotype‡	(Relative) minimum inhibitory concentration* Erythromycin	Bacitracin	Polymyxin	Novobiocin	Nafcillin	Oxacillin
SL1030	*pmi rfa⁺*	(150)	(500)	(0·5)	(200)	(400)	(200)
SL1060	*pmi rfaH487*	0·7	0·3	0·8	< 0·5	0·4	0·5
SL1033	*pmi rfaH481*	1·0	0·3	0·6	< 0·5	0·4	0·5
SL3581	*rfb*$_\Delta$ *rfa⁺*	(50)	(150)	(0·3)	(500)	(50)	(< 25)
SL3635	*rfb*$_\Delta$ *rfaH655*	< 1·0	0·3	< 0·3	< 0·2	< 1·0	NT
SL3648	*rfb*$_\Delta$ *rfa-658*	< 1·0	0·7	< 0·3	< 0·2	< 1·0	NT
SL3625	*rfa⁺*	(100)	(500)	(0·7)	(500)	(400)	(500)
SL3657	*rfaH659*	1·0	0·3	0·3	< 0·2	0·25	< 0·2
(five)§	*rfa⁺*	(150)	(500)	(0·6)	(800)	(200)	(150)
(four)‖	*rfaH487*	1·0	0·4	0·7	< 0·13	0·75	0·7

(ii) *Core-defective mutant classes*

Mutant class	LPS chemotype	Relative minimum inhibitory concentration† Erythromycin	Bacitracin	Polymyxin	Novobiocin	Nafcillin	Oxacillin
rfaL	Ra	1·0	1·3	0·9	1·4	1·1	1·1
rfaJ	Rb2	1·0	1·1	0·6	1·4	1·2	1·1
rfa(R-res-2)	Rb3	1·0	0·5	0·1	0·05	1·0	0·9
galE	Rc	1·0	0·4	0·3	1·8	0·8	0·7
rfaG	Rd1	0·5	0·3	0·1	0·05	0·1	0·2
galU	Rd1	0·2	0·1	0·3	0·1, 1·0	0·2	0·2
rfaF	Rd2	0·1	0·2	0·1	< 0·05	0·2	0·2
rfaE	Re	0·03	< 0·01	0·3	0·01	0·02	< 0·05

* Results in parentheses indicate the minimum inhibitory concentration (m.i.c.) (μg ml^{-1}) for *rfa⁺* (control) strains; other results are the m.i.c. for the indicated *rfaH* or *rfa-658* strain relative to the m.i.c. of the corresponding *rfa⁺* strain (= 1·0). NT, Not tested.

† Summarized from the data of Roantree *et al.* (1977). Results show the mean m.i.c. of *rfa* strains relative to the m.i.c. of isogenic *rfa⁺* (control) strains (= 1·0); where two numbers are shown, these are the extreme values of the same parameter.

‡ See Table 1 for *pmi* and *his*(*rfb*)$_\Delta$ allele numbers.

§ Strain TN88, its P22-lysogenic derivative SL1763 and three *metE⁺* but *rfaH⁺* transductants evoked from SL1763 by ES18 lysate of SL1060.

‖ Four *metE⁺rfaH487* transductants evoked from SL1763 by ES18 lysate of SL1060.

Phenotype of transconjugants. pepQ⁺rfaH transductants derived from SL1265 or SL1266 and with the *rfaH* allele of SL1033, SL3635 or SL3657 were crossed by plate mating with LT2 *pur ilv metE his* strains carrying F'14 (Table 1); the donor strains had been reisolated on medium selective for Ilv⁺Met⁺ character to ensure the presence of the relevant chromosomal segment in the plasmid. Several purified Met⁺ transconjugants for each of the three *rfaH* alleles were grown up in liquid selective medium (to avoid accumulation of F⁻ segregants) and then used as inoculum for phage pattern tests. All gave smooth phage patterns and were also smooth in their type of growth in liquid medium and in O agglutinability.

Effect of RfaH⁻ character on antibiotic sensitivities

To test the effect of *rfaH* (or *rfa-658*) character on antibiotic sensitivities, we determined the minimal inhibitory concentrations (m.i.c.) of six antibiotics for several sets of nearly isogenic strains, each set comprising either *rfa⁺* parent and *rfa* mutant(s), or sister *metE⁺* transductants, *rfa⁺* like the recipient or with the mutant *rfaH* gene of the donor, SL1060.

The mutant alleles tested (Table 4) were the four *rfaH* shown to be cotransducible with *metE*⁺, and *rfa-658* from the phenotypically similar mutant SL3648. The *rfa* genes had little or no effect on sensitivity to erythromycin (m.i.c. of *rfa* strains $\geqslant$ 0·7 those of the relevant *rfa*⁺ control). All the *rfa* strains showed increased sensitivity to bacitracin (m.i.c. 0·3 to 0·7 of controls), polymyxin (m.i.c. < 0·3 to 0·8 of controls), novobiocin (m.i.c. < 0·13 to < 0·5 of controls), nafcillin (m.i.c. 0·25 to < 1·0 of controls) and oxacillin (m.i.c. < 0·2 to 0·7 of controls).

DISCUSSION

Two classes of mutant of RfaH⁻ phenotype. The assumption (Wilkinson & Stocker, 1968; Kuo & Stocker, 1972) that the five phenotypically similar mutants heretofore called *rfaH* were affected at a single locus is shown to be incorrect by our cotransduction data (Table 2 and text). The mutations of four strains, found to be cotransducible with *metE* and *pepQ* (and clearly between these two loci in the case of the mutation of SL1060; Table 3), may all be in one gene and we propose to retain the symbol *rfaH* for these mutants. The altered gene, *rfa-658*, of the remaining mutant (SL3648) is not between *metE* and *pepQ*, since none of 27 *metE*⁺, and none of 11 *metE*⁺*pepQ*⁺, transductants evoked by phage ES18.*h1* grown on SL3648 acquired its RfaH⁻ phenotype (and, as noted above, the *rfa* mutation of SL3648 is not between *cysE* and *pyrE*). No positive information is yet available as to the location of *rfa-658* and we consider it premature to assign it a letter symbol.

RfaH⁻ phenotype. We confirmed that the four *rfaH* mutants and the *rfa-658* mutant SL3648 showed the same pattern of phage sensitivity as *galE* mutants; but Lindberg & Hellerqvist (1980) show, in the following paper, that *rfaH* strains SL1060 and SL1033 adsorb phage C21 less rapidly than do *galE* mutants. Our data (Table 4) show that several *rfaH* mutations and mutation *rfa-658* have much the same effect on sensitivity to six antibiotics as those recorded (Roantree *et al.*, 1977) as resulting from mutation at *rfa*(R-res-2) or at *galE* (causing, respectively, failure to form the galactose I unit and failure to form either of the galactose units of the core), as would be expected if the *rfaH* or *rfa-658* mutations have no effect on the formation of core units proximal to galactose I. The mutations we tested resemble *rfa*(R-res-2) mutations by causing increased sensitivity to novobiocin, instead of the anomalous decreased sensitivity seen in one *galE* mutant (Roantree *et al.*, 1977). Osborn (1968) and Kuo & Stocker (1972) showed that the four *rfaH* mutants and the phenotypically similar mutant SL3648 are at least partly defective in galactose-transferring activity. All these phenotypic traits would be accounted for if their mutations are, as previously surmised, in the structural gene for the single polypeptide of the transferase which forms the 1,3-α-linked galactose I unit of the LPS core (Endo & Rothfield, 1969).

New evidence as to structure of LPS of mutants of RfaH⁻ phenotype: curing by E. coli genes. In the following paper, Lindberg & Hellerqvist (1980) describe methylation analysis and serological tests on the LPS of two *rfaH* mutants. Their results show that in the LPS of these mutants: (i) about 15% of the core chains lack both the galactose I and the galactose II unit; (ii) many chains which receive a galactose I unit are not continued to form 'complete core' chains, but terminate at intermediate points. Thus the function of gene *rfaH*⁺ is required for the efficient addition not only of the galactose I unit but also of several distal sugars of the core. Gene *rfaH*⁺ may be a positive regulatory gene, or may specify a protein which, though not a component of any glycosyltransferase, yet is required for the efficient action of several of the transferases for addition of core units distal to glucose I. In *E. coli* K12 the LPS core unit corresponding in position to galactose I of *S. typhimurium* LPS is a glucose, presumably added by a specific glucosyltransferase, specified by a corresponding gene. However, it was recently shown (Creeger *et al.*, 1979) that a hybrid ColE1 plasmid incorporating an unidentified fragment of the *E. coli* K12 chromosome when transferred to *rfaH* mutant SL1060 made it phenotypically Rfa⁺, as indicated by phage sensitivities and by restoration of *in vitro* galactosyltransferase activity, presumably because the plasmid

contains the wild-type form of an *E. coli* gene equivalent to gene *rfaH* of *S. typhimurium*. Correspondingly, we found that transfer of *E. coli* F'14, carrying the *metE* region of the K12 chromosome, to *pepQ*$^+$*metE* transductant strains with three other *rfaH* alleles restored the smooth phage sensitivity pattern and O agglutinability, i.e. Rfa$^+$ phenotype. The presence of such a gene in *E. coli* K12 would be explained if *rfaH* of *S. typhimurium* is a positive regulatory gene for several *rfa* loci, or if it specifies a polypeptide needed for the efficient action of several glycosyltransferases.

Function of wild-type allele of gene rfa-658, causing RfaH$^-$ phenotype of strain SL3648. As noted above, our transduction results show that the mutated gene *rfa-658* of strain SL3648 is not located in the *metE–pepQ* segment. Yet in its phage and antibiotic sensitivities, in its galactosyltransferase activity and ability of its LPS to accept galactose *in vitro* (Kuo & Stocker, 1972), this mutant is indistinguishable from the four *rfaH* mutants. The arguments, above, as to the function of gene *rfaH*$^+$ therefore hold also for the wild-type gene corresponding to *rfa-658* of strain SL3648, and it seems that mutation at either *rfaH* or at the locus affected by mutation *rfa-658* (elsewhere) can interfere with efficient addition of both galactose I and several distal units of the core. Perhaps the protein needed for RfaH$^+$ phenotype is made up of two kinds of polypeptide.

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REFERENCES

Boro, H. & Brenchley, J. E. (1971). A new generalized transducing phage for *Salmonella typhimurium* LT2. *Virology* **45**, 835–836.

Creeger, E. S., Chen, J. F. & Rothfield, L. T. (1979). Cloning of genes for bacterial glycosyltransferases. II. Selection of a hybrid plasmid carrying the gene for UDP-galactose:lipopolysaccharide α.3 galactosyltransferase. *Journal of Biological Chemistry* **254**, 811–815.

Endo, A. & Rothfield, L. T. (1969). Studies of a phospholipid-requiring bacterial enzyme. I. Purification and properties of uridine diphosphate galactose:lipopolysaccharide α-3-galactosyl transferase. *Biochemistry* **8**, 3500–3507.

Kleckner, N., Roth, J. & Botstein, D. (1977). Genetic engineering *in vivo* using translocatable drug-resistance elements. New methods in bacterial genetics. *Journal of Molecular Biology* **116**, 125–159.

Kuo, T.-T. & Stocker, B. A. D. (1970). ES18, a general transducing phage for smooth and nonsmooth *Salmonella typhimurium*. *Virology* **42**, 621–632.

Kuo, T.-T. & Stocker, B. A. D. (1972). Mapping of *rfa* genes in *Salmonella typhimurium* by ES18 and P22 transduction and by conjugation. *Journal of Bacteriology* **112**, 48–57.

Lindberg, A. A. & Hellerqvist, C. G. (1980). Rough mutants of *Salmonella typhimurium*: immunochemical and structural analysis of lipopolysaccharides from *rfaH* mutants. *Journal of General Microbiology* **116**, 25–32.

McHugh, G. L. & Miller, C. G. (1974). Isolation and characterization of proline peptidase mutants of *Salmonella typhimurium*. *Journal of Bacteriology* **120**, 346–371.

Miller, C. G. & Roth, J. R. (1971). Recessive-lethal nonsense suppressors in *Salmonella typhimurium*. *Journal of Molecular Biology* **59**, 63–75.

Osborn, M. J. (1968). Biochemical characterization of mutants of *Salmonella typhimurium* lacking glucosyl or galactosyl transferases. *Nature, London* **217**, 957–960.

Roantree, R. J., Kuo, T.-T. & MacPhee, D. G. (1977). The effect of defined lipopolysaccharide core defects upon antibiotic resistances of *Salmonella typhimurium*. *Journal of General Microbiology* **103**, 223–234.

Sanderson, K. E. & Hartman, P. E. (1978). Linkage map of *Salmonella typhimurium*, Edition V. *Microbiological Reviews* **42**, 471–519.

Sanderson, K. E., Ross, H., Ziegler, L. & Mäkelä, P. H. (1972). F$^+$, Hfr and F′ strains of *Salmonella typhimurium* and *Salmonella abony*. *Bacteriological Reviews* **36**, 608–637.

Schmieger, H. (1972). Phage P22-mutants with increased or decreased transduction abilities. *Molecular and General Genetics* **119**, 75–88.

Vary, P. S. & Stocker, B. A. D. (1973). Nonsense motility mutants in *Salmonella typhimurium*. *Genetics* **73**, 229–245.

Wilkinson, R. G. & Stocker, B. A. D. (1968). Genetics and cultural properties of mutants of *Salmonella typhimurium* lacking glucosyl or galactosyl lipopolysaccharide transferases. *Nature, London* **217**, 955–957.

Wilkinson, R. G., Gemski, P., jr & Stocker, B. A. D. (1972). Non-smooth mutants of *Salmonella typhimurium*: differentiation by phage sensitivity and genetic mapping. *Journal of General Microbiology* **70**, 527–554.