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P22-Mediated Transduction Analysis of the Rough A (*rfa*) Region of the Chromosome of *Salmonella typhimurium*

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Seven temperature-sensitive rough mutants of *Salmonella typhimurium* were found to be sensitive to smooth-specific phages at low temperature (25 C, 30 C) and resistant or partially resistant to rough-specific phages, whereas at high temperatures (37 C, 45 C) they were resistant or partially resistant to smooth-specific phages but sensitive to rough-specific phages. These data indicate that at low temperature each strain makes lipopolysaccharide which is relatively normal, but at high temperatures O-specific side chains are not added to the lipopolysaccharide. At 45 C, these strains have the R-res-1 or R-res-2 phage sensitivity phenotype, and their genetic lesions map by P22-mediated transduction in the *rfa* gene cluster between *cysE-pyrE*, suggesting a mutation in genes with transferase functions. P22-mediated joint transduction with temperature-sensitive *rfa* mutants, leaky *rfa* mutants, and *rfa* P22 lysogens have shown the following order of genes in the *S. typhimurium* linkage map: *xyl-mtlA-mtlB-cysE-rfaF-rfaG-pyrE*. An *rfaE* allele was not jointly transduced in the *cysE-pyrE* segment.

The synthesis in *Salmonella typhimurium* of the lipopolysaccharide (LPS) component of the cell wall, often referred to as the O (somatic) antigen or the endotoxin, is determined by many genes. There are two main clusters of genes: one, termed *rfb* (rough B) and closely linked to *his*, responsible for the synthesis of several special sugar nucleotides and for assembly of the O-repeating unit (7, 10), and the other termed *rfa* (rough A) containing genes concerned with core region synthesis, some known and others postulated to determine glycosyl transferases, located in the *str-xyl-metA* region (15, 16). A number of other smaller clusters or single genes affecting LPS synthesis (*gal*, *rfc*) are at other map locations. The location of these genes is shown on the linkage map (Fig. 1).

Detailed genetic analysis of the *rfa* group of genes is difficult, because the *rfa* mutants are normally resistant to P22, the only general transducing phage for *Salmonella* known at the time these studies were begun. Several methods of analysis of the fine structure of this group have been considered, and some have been used. (i) The phage ES18, able to multiply on rough mutants, was found to be a generalized transducing phage by Kuo and Stocker (4). (ii) A class of mutants with properties intermediate between rough and smooth, termed class D (9), and suspected to be leaky *rfa* mutants, were found by Gemski and Stocker (3) to be partially sensitive to P22 and able to act as transduction recipients. (iii) We isolated temperature-sensitive *rfa* mutants which are smooth at low temperature (30 C) but rough at high temperature (42 C), and used these strains as donors in P22-mediated transduction. (iv) We isolated *rfa* mutants from smooth parents which were lysogenic for P22, and induced them to release phage after ultraviolet irradiation; we then used the phage in transduction. (v) During attempts to isolate Hfr strains with point of origin near *rfa*, we isolated several Hfr strains, one of which simultaneously became Rfa in phenotype. Subsequent analysis has revealed a frequent class of Hfr strains in which the F factor has been inserted among the *rfa* genes. Such Hfr strains have been used in genetic analysis of the *rfa*

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group (14).

The structure of the LPS core region is shown in Fig. 2 (8, 16). The gene symbols for rough A mutants (*rfaE*, *rfaF*, etc.) indicate genes whose mutations affect formation of the core unit; these may be, but are not necessarily, glycosyl transferase genes.

The rough phenotype, defined originally on colony morphology, is now defined by chemotype, based on content of sugars in the LPS (6). However, this chemotype also determines sensitivity to many phages, and thus phage sensitivity can be used as a convenient method in determining the rough phenotype. For example, phage Felix O (FO) requires for attachment to the cell the *N*-acetyl glucosamine unit on the core of the LPS (16).

This paper describes the temperature-sensitive rough mutants and gives transduction data on the *rfa* cluster and nearby genes, based on crosses with the temperature-sensitive mutants, leaky mutants, and P22 lysogens as

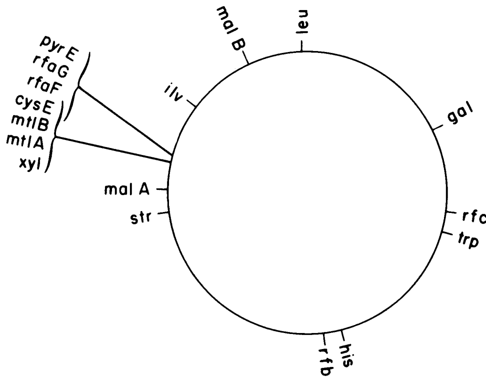

FIG. 1. *Partial linkage map of Salmonella typhimurium, not drawn to scale. For a more complete map, see Sanderson (11). The genes shown within brackets are jointly transduced by P22 phage. Joint transduction data (Fig. 3 and in reference 5) indicate that genes in the pyrE-xyl region are arranged on the chromosome in the order shown.*

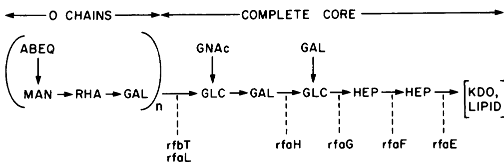

FIG. 2. *Structure of the LPS of Salmonella of group B, and symbols for genes concerned with formation of indicated linkages. Genes rfaG and rfaH determine the synthesis of transferase enzymes. Mutants termed rfaE and rfaF have defects in the addition of, respectively, the proximal and distal heptose units, but it is not known whether the affected genes specify heptosyl transferases (8, 16).*

donors. The group of Hfr strains in which F is inserted into the *rfa* cluster is described and used in analysis of the *rfa* genes in an accompanying paper (14). Another accompanying paper by Kuo and Stocker (5) gives transduction data obtained with ES18 and P22 phages, as well as conjugation data.

MATERIALS AND METHODS

Strains. The bacterial strains reported in this study came primarily from the Salmonella Genetic Stock Centre (SGSC), maintained at the University of Calgary, or from the collection of B. A. D. Stocker, originally at the Lister Institute, London, England, and later at Stanford University (Table 1). The bacteriophages used in isolation and characterization of the rough mutants were also from Dr. Stocker (see 16).

Media. Difco nutrient broth was used for routine growth of cells. Conjugation was carried out in Difco Penassay broth (Antibiotic Medium No. 3). Phage sensitivity tests were done on antibiotic agar (NA; Penassay broth plus 1.5% Difco agar) according to the methods of Wilkinson *et al.* (16). Davis minimal medium (MM; 12) with 0.2% glucose as carbon source was used as a selective medium; 20 μg of L-amino acids/ml or 2 μg of vitamins/ml was added as a supplement when needed. In some tests, other sugars, such as xylose, were added as sole carbon sources at 0.2%. Bile salts sensitivity was tested with 0.3% (w/v) Difco sodium deoxycholate in NA. Single enriched medium, used in transduction, is MM to which one-eightieth volume Difco nutrient broth was added.

Transduction. P22-mediated transduction was as described by Sanderson and Hall (13).

Induction of P22 lysogens. Overnight broth cultures were diluted in broth and grown with aeration to a titer of approximately 10^8 cells/ml, centrifuged, resuspended in 10 ml of saline, and treated with ultraviolet light (10-sec exposure at 50 cm from two 25-w General Electric germicidal lamps; i.e., an exposure of 5 ergs per cm^2 per sec). The cells were then centrifuged, resuspended in 50 ml of broth, and grown with aeration for 3 to 5 hr at 37 C. The cells were removed by centrifugation, and the culture filtrate was sterilized with chloroform. A titer of 10^8 to 10^9 plaque-forming units (PFU) of P22 per ml was usually obtained. The phage was further concentrated by centrifugation at 25,000 $\times$ *g* for 1 hr, yielding titers up to 10^{10} PFU/ml, adequate for transduction.

Isolation of temperature-sensitive rough mutants. Cells from a broth culture of a smooth strain of *S. typhimurium* were spread on NA, and phage (FO) was spotted over the cells. After overnight growth at 45 C, cells from the FO-resistant colonies appearing in areas of confluent lysis were suspended in broth and spotted on two NA plates; phages FO, P22.c2, and Ffm were spotted onto each strain; then one plate was incubated at 30 C and one at 45 C. All of the mutants thus isolated were resistant to FO at 45 C, but about 10% were sensitive to P22 and FO,

and resistant to Ffm, at low temperature (smooth phenotype), but resistant to FO and P22, and sensitive to Ffm, at high temperature (rough phenotype). These temperature-sensitive mutants were streaked on NA and single colonies were isolated; these were then retested at four temperatures, 25, 30, 37, and 45 C, with several phages (Table 2).

Soft-agar layer method for propagating phage. Into 2.5 ml of melted (45 C) half-strength Difco nutrient agar (which therefore contains 0.75% agar) was added 10^{6} PFU of P22 phage and 0.2 ml of nutrient broth culture of the bacterial strain to be tested. The contents of the tube were flooded onto a plate of NA and incubated at 37 C overnight. Then the soft agar layer was collected and centrifuged at low speed (10,000 × *g* for 30 min); the supernatant fluid was kept.

Abbreviations. The genetic nomenclature is as recommended in Demerec et al. (2). The basis for the *rfa* gene designations is given by Mäkelä and Stocker (8): *cys*, cysteine requirement; *gal*, galactose non-utilization; *his*, histidine requirement; *ilv*, isoleucine-valine requirement; *met*, methionine requirement; *mtl*, mannitol non-utilization; *pur*, purine requirement; *rfaE*, lipopolysaccharide core defect, proximal heptose deficient; *rfaF*, lipopolysac-

TABLE 1. *Strains of Salmonella typhimurium used*

No.	Source	Summary	Reference
SA624	N. Kredich	*cysE1709 pyrE125 xyl-3* F⁻	
SL1165	SGSC[a] (SA 26)	*proA26 rfaE629* (P22)⁺ HfrB2	
SL1166	SGSC (SA 18)	*proA26 rfaF624* (P22)⁺ HfrB2	
SL3600	T. Kuo and B. Stocker	*metA22 trpE2 H1-b H2-e,n,x flaA66 strA120 xyl-404 metE551 rfa-657* F⁻ (Fels 2)⁻	5
DB76	D. Berkowitz	*purC7 proA46 ilv-405 rha-461 iM10 fla-56 fim mtlA116 xyl* F⁻	1
DB89	D. Berkowitz	*purC7 proA46 ilv-405 rha-461 iM10 fla-56 fim mtlB121 xyl* F⁻	1
SA1437	SGSC	*cysE396* F⁻	
SA1438	SGSC	*pyrE125* F⁻	
SL1027	B. Stocker	*metA22 trpE2 H1-b H2-e,n,x flaA66 strA120 xyl-404 metE551* F⁻ (Fels 2)⁻	16

[a] The Salmonella Genetic Stock Centre (SGSC) is maintained at the University of Calgary.

TABLE 2. *Reaction of strains of S. typhimurium to phages*[a]

Strain[b]	Genotype (for the rough genes)	FO[c]				P22.*c2*[c]				Br60[c]				Ffm[c]				C21[c]				6SR[d]		Br2[d]		P221.*c2*[d]	
		25[e]	30	37	45	25	30	37	45	25	30	37	45	25	30	37	45	25	30	37	45	30	45	30	45	30	45
SL1027	Wild type	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−						
SA116	*rfa-3003*	+	+	+	−	+	+	±	±	−	−	+	+	−	−	−	±	−	−	−	−	−	−	−	±	+	−
SA117	*rfa-3004*	+	+	±	±	+	+	+	±	−	−	+	+	−	−	−	±	−	−	−	−	−	−	−	±	±	−
SA118	*rfa-3005*	+	+	−	−	±	±	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	±	±	−
SA120	*rfa-3006*	+	±	−	−	+	+	−	−	−	±	+	+	−	±	+	+	−	−	−	−	−	−	−	±	±	−
SA122	*rfa-3008*	+			−	+			−	±			+	±			+	−			−	−	−	−	±	±	−
SA126	*rfa-3012*	+			−	+			−	±			+	±			+	−			−	−	−	−	±	±	−
SA134	*rfa-3020*	+			−	+			−	−			+	−			+	−			−	−	−	−	−	±	−

[a] In addition, the strains were tested for galactose fermentation on Difco EMB agar plus 2% galactose; all strains were Gal⁺ at all temperatures. None of the strains was bile salts-sensitive, for all strains grew on 0.3% sodium deoxycholate in NA at 30 and 45 C.

[b] SL1027 is a smooth strain, described in Table 2. All other strains were derived from SL1027 by selection for resistance to FO phage at 45 C.

[c] These phages were tested at the University of Calgary, according to the following methods. An overnight broth culture was grown at 37 C, flooded on NA, and dried; suspensions of the phage (10^{8} PFU/ml) were spotted on top. Immediately after inoculation, duplicate plates were placed at the indicated temperatures; + indicates lysis, − indicates no lysis, ± is intermediate.

[d] Sensitivity to these phages, at 30 and 45 C, was tested by B. A. D. Stocker at Stanford University, with the methods and media stated in reference 16. Dr. Stocker also tested sensitivity to the other phages, with results which essentially agreed with those obtained at the University of Calgary.

[e] Temperature (C).

charide core defect, distal heptose deficient; *rfaG*, lipopolysaccharide core defect, glucose I transferase; *rfaJ*, lipopolysaccharide core defect, glucose II deficient; *rfaK*, lipopolysaccharide core defect, acetylglucosamine deficient; *rfaL*, lipopolysaccharide defect, O-ligase; *ser*, serine or glycine requirement; *thr*, threonine requirement; *trp*, tryptophan requirement; *str*, streptomycin resistance; *xyl*, xylose non-utilization.

RESULTS

Isolation of temperature-sensitive rough mutants. About 10% of the mutants isolated from SL1027 (*S. typhimurium* LT2) proved to be temperature-sensitive with respect to their rough phenotype. SL1027 is a smooth strain, and seven temperature-sensitive mutants were derived from it (Table 2). Six mutants (SA116 to SA126) at 45 C were resistant to phage FO and resistant (or partially resistant) to the smooth-specific phage P22.*c2*, but sensitive to three rough-specific phages, Br60, Ffm, and Br2. They were resistant, both at 45 C and at lower temperatures, to phage C21, which attaches to mutants unable to form the proximal glucose or the proximal galactose unit of the LPS core. In addition, they grew, both at 45 C and at lower temperatures, on nutrient medium containing 0.3% sodium deoxycholate, a supplement which inhibits growth of mutants with defects in synthesis of the heptose-containing part of the LPS. Thus, at 45 C their phenotype approximates mutants of class *rfa* (R-res-1), described by Wilkinson et al. (16). SA134 is Br2-resistant at 45 C as well as at 30 C, characteristic of the R-res-2 phenotype (16). At lower temperatures, the seven mutants regained sensitivity to phages FO and P22, and (to a large extent) lost their sensitivity to the rough-specific phages Br60, Ffm, and Br2. A proportion of the rough mutants isolated over a period of several years in B. A. D. Stocker's laboratory can propagate P22 at 37 C, even though they may not show visible lysis (3). All of the temperature-sensitive mutants listed in Table 2, as well as others isolated from SL1027 and from other smooth strains of *S. typhimurium*, were tested for their ability to support propagation of P22 by the soft-agar layer method at 25 C; all gave titers resembling those obtained with the control smooth strains, 5×10^{10} to 1×10^{11} PFU/ml.

Determining the map location of rfa mutants. Since Subbaiah and Stocker (15) and Wilkinson and Stocker (17) had shown *rfa* genes to be linked to *xyl* in conjugation crosses, we tested for P22-mediated joint transduction of *rfa* genes of temperature-sensitive Rfa mutants with wild-type alleles of auxotrophs for genes in the region of *xyl*. (See reference 11 for a detailed map of this region.) P22 phage was propagated at 25 C on the seven temperature-sensitive mutants which are described in Table 2, and these preparations were used as donors; smooth recipients carrying alleles of *cysE* (*cysE8*, *cysE30*, or *cysE396*) or of *pyrE* (*pyrE125*) were used as recipients. CysE$^+$ and PyrE$^+$ transductants were selected and were tested for their phage sensitivity, as an indicator of the rough phenotype, at 45 C. All of the seven temperature-sensitive *rfa* alleles tested are linked to *cysE* with 27 to 45% joint transduction, and to *pyrE* with 23 to 43% joint transduction [Table 3(A)]. There was 1.2 to 2.6% joint transduction of *cysE* and *pyrE*, depending on which strain was used as donor and which as recipient, indicating the gene order *cysE-rfa-pyrE* [Table 3(B)]. Thus, all the seven temperature-sensitive mutations to FO resistance, all determining the "R-res" phenotype at 45 C, map in the cluster of Rfa genes located between *cysE* and *pyrE* at ca. 110 min on the linkage map of *S. typhimurium* (11). The *rfa* genes for several functions necessary for LPS core synthesis, *rfaE, F, G, H*, and *L*, and probably also *rfaJ* and *K*, map by conjugation in the region of *xyl* on the linkage map (8, 16). Mutant alleles identified by chemotype and by serotype as members of each of these genes have subsequently been analyzed by transduction by a variety of methods by Kuo and Stocker (5) or by ourselves. Since the chemical analysis of new mutants is time-consuming, the chemotype of the temperature-sensitive mutants in Table 2 was not determined, and other methods have been used to obtain material to analyze further the genetics of the *rfa* mutants, as described below.

In Table 3(C) a group of *rfa* mutants for which the chemotype and hence the gene locus is known or suspected are analyzed. SL1165 (= SA26) *rfa-629* and SL1166 (= SA18) *rfa-624* are rough mutants (not temperature-sensitive) selected as FO-resistant mutants of an LT2 *proA26* (P22)$^+$ HfrB2 *tre*$^+$ *clb*$^+$ parent, chosen for investigation because they show the deoxycholate sensitivity (and phage-resistance pattern) typical of *rfa* mutants deficient in addition of the proximal heptose unit (class *rfaE*) or of the distal heptose unit (class *rfaF*) of the LPS core (16; Fig. 2). Chemical and serological tests on LPS extracted from these two mutants (O. Lüderitz and E. Ruschmann, *personal communication*) strongly suggest that the LPS of SL1165 lacks the proximal heptose

TABLE 3. *Frequency of P22-mediated joint transduction*[a]

Cross			Gene (*xyl* *mtlA* *mtlB* *cysE* *rfaF* *rfa* *pyrE*)
Donor: Stock no.	Donor: Unselected donor gene	Recipient	
(A) SA116	*rfa-3003*		← 22/82 (27) 16/55 (29) →
SA117	*rfa-3004*		← 22/79 (28) 124/426 (28) →
SA118	*rfa-3005*		← 40/90 (42) 33/132 (25) →
SA120	*rfa-3006*		← 60/135 (45) 64/274 (23) →
SA122	*rfa-3008*		← 9/30 (30) 47/110 (43) →
SA126	*rfa-3012*		← 12/32 (38) ——→
SA134	*rfa-3020*		← 10/29 (34) 10/29 (34) →
(B)	*cysE8*	*pyrE125*	6/251 (2.4) →
	cysE396	*pyrE125*	11/425 (2.6) →
	pyrE125	*cysE396*	← 7/585 (1.2)
(C) SL1166	*rfaF624*		← 15/50 (30) 5/62 (8) →
SL3600	*rfa-657*		← 33/88 (37)
SL1165	*rfaE629*		← 0/70 (0) 0/75 (0) →
(D) DB76	*mtlA*, *pyrE*+	SA624 *cysE1709*	15/293 (5.1) → ← 12/293 (4.1)
	mtlA	*pyrE125*	0/250 (0) →
DB76	*mtlA*	*cysE396*	16/982 (1.6) →
	xyl	*cysE396*	2/982 (0.2) →
DB89	*mtlB*, *pyrE*+	SA624 *cysE1709*	39/243 (16) → ← 13/243 (5.3)
	mtlB	*pyrE125*	0/220 (0) →
DB89	*mtlB*	*cysE396*	98/1,182 (8.4) →
	cysE396, *xyl*+	DB76 *mtlA*	45/360 (12.5) → ← 12/360 (3.3)

[a] The gene at the head of the arrow was the selected donor gene; the gene at the tail end was unselected. The proportion (shown as a fraction) and the percentage (in parentheses) indicate the frequency with which the selected transductants obtain the unselected donor allele. The recipient parents, if not specified, were *cysE396* and *pyrE125*.

unit and that the LPS of SL1166 contains the proximal but not the distal heptose unit; SL1165 is thus by definition an *rfaE* mutant and SL1166 is an *rfaF* mutant. These strains were induced with ultraviolet light, and the P22 phage which was released was used to transduce *cysE* and *pyrE* recipients.

SL3600 (*rfa-657*) is a "leaky" *rfa* mutant, received from Kuo and Stocker (5), which supports propagation of phage P22. Mutant SL3600 (*rfa-657*) shows the bile salt sensitivity of mutants with defects in the formation of the heptose-containing part of the core, but the exact nature of its LPS defect is not known (see discussion in reference 5).

The known *rfaF* allele of SL1166 gave 30% joint transduction with *cysE* but only 8% with *pyrE*. This suggests the map order *cysE-rfaF-pyrE*. The unidentified *rfa* allele of SL3600 showed 37% joint transduction with *cysE*, a value compatible with the mutation being at *rfaF*. The *rfaE* allele of SL1165 was not linked by transduction to either *pyrE* or *cysE*.

The transductional linkage of *mtlB* (mannitol permease) and *mtlA* (mannitol dehydrogenase) to *xyl*, *cysE*, and *pyrE* was tested with strains DB76 and DB89, which are mannitol-nonutilizing strains obtained from Berkowitz [1; Table 3(D)]. Joint transduction of *mtlA*-*cysE* and of *mtlB*-*cysE* was 1.6 to 5.1% and 8.4

to 16%, respectively, indicating the map order *mtlA-mtlB-cysE*. Joint transduction of 12.5% was detected between *xyl-mtlA*; linkage was barely detectable between *xyl* and *cysE* (2 joint transductants in 982 recombinants); and linkage was not detectable between *mtlA* or *mtlB* and *pyrE* or between *xyl* and *pyrE*. The total data are consistent with the map order *xyl-mtlA-mtlB-cysE-rfaF-pyrE*.

DISCUSSION

The genes for biosynthesis of the LPS are clustered into two main regions, *rfa* and *rfb*, as well as in other areas, *gal* and *rfc* (8; Fig. 1). The genetic locations and the order of most of the genes of the *rfb* cluster have been published (7, 10), and the data which reveal the location and the structure of some of the genes of the *rfa* cluster are published here and in the accompanying papers (5, 14), though the conclusions were presented earlier (8, 11).

Seven temperature-sensitive *rfa* mutants were isolated from SL1027, a smooth strain. Each of these strains is sensitive to smooth-specific phages at low temperatures (25 C, 30 C), but resistant or partially resistant to rough-specific phages. At high temperatures (37 C, 45 C), these strains shift to sensitivity to the rough-specific phages, and resistance or partial resistance to the smooth-specific phages. These data indicate that at low temperatures these strains attach O-specific side chains to their LPS, whereas at high temperatures these side chains are not added. Since all of these mutants map in the *rfa* cluster, and since all the genes so far identified in this cluster determine enzymes for transferase functions in the core region of the LPS (8, 16), it is likely that some or all of these mutants code for temperature-sensitive transferase enzymes.

This report reveals the map order *xyl-mtlA-mtlB-cysE-rfaF-pyrE*, based on P22-mediated joint transduction crosses. An *rfaE* allele in SL1165 is not jointly transduced with *pyrE* or *cysE*. These data confirm the more extensive data on P22- and ES18-mediated transduction for the *rfa* cluster reported by Kuo and Stocker (5).

The rates of joint transduction of the *rfa* mutations of the temperature-sensitive *rfa* mutants with *cysE* and with *pyrE* suggest that the loci affected in these mutants are about midway between *cysE* and *pyrE*, as expected if the mutations are at *rfa*(R-res-1) or *rfa*(R-res-2) of Kuo and Stocker (5), inferred by them to be between *rfaF* and *rfaG*.

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LITERATURE CITED

1. Berkowitz, D. 1971. D-Mannitol utilization in *Salmonella typhimurium*. J. Bacteriol. **105**:232–240.
2. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61–76.
3. Gemski, P., Jr., and B. A. D. Stocker. 1967. Transduction by bacteriophage P22 in nonsmooth mutants of *Salmonella typhimurium*. J. Bacteriol. **93**:1588–1597.
4. Kuo, T.-T., and B. A. D. Stocker. 1970. ES18, a general transducing phage for smooth and nonsmooth *Salmonella typhimurium*. Virology **42**:621–632.
5. Kuo, T.-T., and B. A. D. Stocker. 1972. Mapping of *rfa* genes in *Salmonella typhimurium* by ES18 and P22 transduction and by conjugation. J. Bacteriol. **112**:48–57.
6. Lüderitz, O., A. M. Staub, and O. Westphal. 1966. Immunochemistry of O and R antigens of *Salmonella* and related Enterobacteriaceae. Bacteriol. Rev. **30**:192–255.
7. Mäkelä, P. H. 1966. Genetic determination of the O antigens of Salmonella groups B (4, 5, 12) and C1 (6, 7). J. Bacteriol. **91**:1115–1125.
8. Mäkelä, P. H., and B. A. D. Stocker. 1969. Genetics of polysaccharide biosynthesis. Annu. Rev. Genet. **3**:291–322.
9. Naide, Y., H. Nikaido, P. H. Mäkelä, R. G. Wilkinson, and B. A. D. Stocker. 1965. Semi-rough strains of *Salmonella*. Proc. Nat. Acad. Sci. U.S.A. **53**:147–153.
10. Nikaido, H., M. Levinthal, K. Nikaido, and K. Nakane. 1967. Extended deletions in the histidine-rough B region of the *Salmonella* chromosome. Proc. Nat. Acad. Sci. U.S.A. **57**:1825–1832.
11. Sanderson, K. E. 1970. Current linkage map of *Salmonella typhimurium*. Bacteriol. Rev. **34**:176–193.
12. Sanderson, K. E. and M. Demerec. 1965. The linkage map of *Salmonella typhimurium*. Genetics **51**:897–913.
13. Sanderson, K. E. and C. A. Hall. 1970. F-prime factors of *Salmonella typhimurium* and an inversion between *S. typhimurium* and *Escherichia coli*. Genetics **64**:215–228.
14. Sanderson, K. E., and H. Saeed. 1972. Insertion of the F factor into the cluster of *rfa* (rough A) genes of *Salmonella typhimurium*. J. Bacteriol. **112**:64–73.
15. Subbaiah, T. V., and B. A. D. Stocker. 1964. Rough mutants of *Salmonella typhimurium*. I. Genetics. Nature (London) **201**:1298–1299.
16. Wilkinson, R. G., P. Gemski, Jr., and B. A. D. Stocker. 1972. Non-smooth mutants of *Salmonella typhimurium*: differentiation by phage sensitivity and genetic mapping. J. Gen. Microbiol. **70**:527–544.
17. Wilkinson, R. G., and B. A. D. Stocker. 1968. Genetics and cultural properties of mutants of *Salmonella typhimurium* lacking glucosyl or galactosyl lipopolysaccharide transferases. Nature (London) **217**:255–257.