Genetic Homologies between Flagellar Antigens of *Escherichia coli* and *Salmonella abony*

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SUMMARY

Genes determining the flagellar antigens were introduced from *Hfr Salmonella abony* into *F⁻Escherichia coli* by sexual recombination. The gene *H1* for the phase-1 flagellar antigen of *Salmonella* was found to be allelic to *H*, the gene for the only flagellar antigen of *E. coli*. The phase-2 antigen of *Salmonella* has no counterpart in *E. coli*; if the corresponding salmonella gene *H2* is introduced in *E. coli*, diphasic strains are produced whose phase-2 antigen is that of the *Salmonella* parent; the phase-1 antigen can be that of either parent. In each case the diphasic strains undergo a true phase variation, with either the phase-1 or the phase-2 antigen being expressed at one time. The rates of this variation are comparable to those in the *Salmonella* parent.

INTRODUCTION

Bacteria of the family Enterobacteriaceae show remarkable variation of their surface antigens, flagellar as well as cell wall. Immunological cross-reactions exist between various species (or serotypes) of a genus, and also between different genera. This holds true especially of the somatic (polysaccharide) antigens of *Escherichia*, *Salmonella*, *Arizona*, and *Citrobacter* (Westphal, Kauffmann, Lüderitz & Stierlin, 1960), but some cross-reactions are known even between the flagellar (protein) antigens of these genera (summarized by Kauffman, 1954). These similarities are so extensive that, together with similarities in morphology and cultural behaviour, they suggest a close relationship between these bacteria.

As hybridization between different genera of enteric bacteria has recently become practicable, it is now possible to make further tests of the suspected genetic homologies (Baron, Carey & Spilman, 1959; Miyake & Demerec, 1959; Zinder, 1960; Miyake, 1962; Mäkelä, Lederberg & Lederberg, 1962). It has become clear that many genes of *Escherichia coli* associated with synthesis of amino acids or the fermentation of sugars can function when transferred into *Salmonella*. Many of them map in corresponding places in the chromosomes of the two organisms (Zinder, 1960; Demerec *et al.* 1960; Mäkelä, 1963).

Most *Salmonella* species differ from *Escherichia coli* in having two possible flagellar antigens and exhibiting phase variation between them, whereas *E. coli* shows no phase variation, having only one flagellar antigen in any one strain. Genetical analysis by transduction (Lederberg & Edwards, 1953) shows that the phase-1 antigens of various *Salmonella* species are determined by a series of alleles

at a locus termed *H1*, and the various phase-2 antigens by a second series at a different locus, *H2*. Lederberg & Iino (1956), in a genetical analysis of phase variation, showed that the phase of a bacterium, that is whether the *H1* or the *H2* gene is expressed, is determined by the state of a 'factor' identical with or closely linked to the *H2* gene: in one state the *H2* gene is expressed and the *H1* gene unexpressed; in the other state the *H1* gene is expressed and the *H2* gene unexpressed. It therefore seemed of interest to test (1) whether the gene for the single flagellar antigen in *E. coli* is allelic to one of the Salmonella flagellar antigen genes *H1* and *H2*, and if so, to which; (2) whether *E. coli* can be made diphasic by introducing the factor which controls phase variation along with the gene *H2* which determines the phase-2 antigen from Salmonella; (3) whether, if this can be done, the *E. coli* flagellar antigen gene will respond to the control of the phase-variation factor from Salmonella.

METHODS

The bacterial strains used are listed in Table 1. The SW and W stocks come from the collection of Dr J. Lederberg, SH and EH from the author's collection in Helsinki.

Table 1. *Bacterial strains*

Strain number	O antigens	H antigens	Other characters	Source and/or references
<i>Escherichia coli</i>				
w 3703	025	h16coli*	<i>Hfr leu⁻ try⁻ lac⁻ str-r fla⁻†</i>	Ørskov & Ørskov (1962)
EH 1	0100	h2coli*	<i>F⁻ his⁻ str-r</i>	From w 3462 (Ørskov & Ørskov, 1962)
EH 23	0100	h2coli*	<i>F⁻ his⁻ arg⁻ str-r</i>	<i>arg⁻</i> mutant of EH 1
<i>Salmonella abony</i>				
sw 803	4, 5, 12	b ↔ e, n, x	<i>F⁻ str-s</i>	Edwards & Bruner (1942)
sw 1444	4, 5, 12	b ↔ e, n, x	<i>Hfr met⁻ aro⁻ str-r</i>	Same as sw 1391 (Mäkelä, 1963)
sw 1446	4, 5, 12	b ↔ e, n, x	<i>Hfr str-s</i>	Mäkelä (1963)
sw 1462	4, 5, 12	b ↔ e, n, x	<i>Hfr str-s</i>	Mäkelä (1963)
sh 67	1, 4, 5, 12	i ↔ 1, 2	<i>F⁻ his⁻ str-r</i>	From sw 803
sh 538	1, 4, 5, 12	i ↔ 1, 2	<i>F⁻ his⁻ arg⁻ str-r</i>	<i>arg⁻</i> mutant of sh 67

* To avoid confusion, H antigens of *E. coli* are in this paper designated thus: h16coli instead of H 16, and h2coli instead of H 2. The genes for the flagellar antigens are italicized: *H* in *E. coli*, *H1* and *H2* in *S. abony*.

† This *fla⁻* mutation is not linked to *H* or *his*; all recombinants obtained were motile.

For gene symbols, etc., see Methods.

The following symbols are used to denote genes, with + standing for the wild-type synthetic or fermentative ability, — for mutant inability: *arg* for arginine, *aro* for phenylalanine and tyrosine, *fla* for flagellar, *his* for histidine, *leu* for leucine, *met* for methionine, *try* for tryptophan synthesis; *lac* for lactose fermentation; *str-s* for streptomycin sensitivity, *str-r* for streptomycin resistance.

The genes for flagellar antigen determinants are denoted by *H* for the *Escherichia coli* gene, *H1* for the phase-1 and *H2* for the phase-2 genes of Salmonella. The flagellar antigens of the *E. coli* strains used are designated as H 16 and H 2 in the serological nomenclature of Escherichia. Now the use of the symbol H 2 for the specificity of an Escherichia antigen, and *H2* for the gene of phase-2 antigenic determinants of Salmonella is likely to cause confusion, and therefore we shall in the

present paper designate *E. coli* flagellar antigens in an unorthodox way by a small roman letter h plus the figure indicating specificity and the word 'coli' to emphasize the origin of these antigens: e.g. h2coli instead of H 2. The flagellar antigens of *Salmonella abony* used in the experiments are b and e, n, x, and the corresponding genes hence *H1-b* and *H2-e, n, x*. In some *S. abony* strains the genes *H1-i* and *H2-1,2* were introduced by transduction to replace the original *H1* and *H2* and to give an organism with the flagellar antigens $i \leftrightarrow 1, 2$.

F^- are female or recipient bacteria, *Hfr* are high frequency donors, as described for *Salmonella abony* by Mäkelä (1963).

The media used were mainly standard ones described by Lederberg (1950). Bacterial crosses between *Hfr* donors and F^- recipients were made as described by Mäkelä (1963). Recombinants were purified on nutrient agar plates by single colony isolation. To test for flagellar antigens the bacteria were first passed through a tube of 'motility agar' (semi-solid nutrient agar with gelatin; Stocker, Zinder & Lederberg, 1953); then grown overnight at 37° in Difco Antibiotic medium 3, and tested by tube agglutination at 37° with rabbit antisera. Anti-h2coli serum was kindly given by Drs F. and I. Ørskov. An alternate phase was always sought by passage through motility agar containing an appropriate concentration of the corresponding anti-H serum. Rates of phase variation were determined by the procedure of Stocker (1949).

RESULTS

Crosses involving the *his* region

In *Escherichia coli* the gene H which determines the specificity of the flagellar antigen is closely linked to the locus for histidine synthesis (*his*) (Ørskov & Ørskov, 1962). In *Salmonella abony*, *H1*, the locus for the phase-1 antigen, is similarly linked to *his* (Mäkelä, 1962). Furthermore, in *S. typhimurium* all known *his* mutants map together (Hartman, Loper & Šerman, 1960), and the *his* loci of *S. typhimurium* and *E. coli* map in corresponding regions of the linkage maps of the two organisms (Demerec *et al.* 1960; Mäkelä, 1962).

Crosses were therefore made selecting recombinants which had received the donor allele *his*⁺.

The results are recorded in Table 2. Both *Escherichia coli* and *Salmonella abony* *Hfr* donors were mated to *his*⁻ *E. coli* and *S. abony* recipients. In such crosses the

Table 2. Flagellar antigens of *his*⁺ recombinants from crosses between a *his*⁺ *Hfr* donor and a *his*⁻ F^- recipient

<i>Hfr</i> donors		F^- recipients		Recombinants analysed* (all motile)	
Strain	H antigens	Strain	H antigens	Number	H or H1 of donor
<i>E. coli</i> w 3703	h16coli	<i>E. coli</i> EH 1	h2coli	41	87 % (h16coli)
<i>S. abony</i> sw 1444	b ↔ e, n, x	<i>E. coli</i> EH 1	h2coli	28	57 % (b)
sw 1462	b ↔ e, n, x	EH 1	h2coli	105	52 % (b)
<i>S. abony</i> sw 1462	b ↔ e, n, x	<i>S. abony</i> SH 67	i ↔ 1, 2	104	67 % (b)

* All recombinants were selected on minimal glucose streptomycin medium.

donor allele will be present in a high proportion of recombinants for all loci closely linked to *his*, the allele selected from the donor parent. In all these crosses 52–87 % of the *his*⁺ recombinants manifested a flagellar antigen derived from the *Hfr* donor, i.e. the H antigen h16coli of the *E. coli* donor or the phase-1 antigen b of the *S. abony* donor. This suggests that the *H* locus for the flagellar antigen of *E. coli* is homologous with the *H1* locus for phase-1 flagellar antigen of *Salmonella*. Some of the *S. abony* *Hfr* → *E. coli* *F*[−] recombinants were agglutinated by anti-b serum, some by anti-h2coli serum, but none by both sera. Selection in motility agar with appropriate antisera did not reveal any latent antigens in the b or h2coli hybrids. A few of the b clones yielded swarms not reacting with anti-h2coli serum and not reacting with anti-e, n, x serum. These clones were presumed to be z33 variants which are known to emerge rather often from b bacteria (Edwards & Moran, 1946). Two of the swarms were tested with anti-z33 serum and were agglutinated as expected.

Thus the *Salmonella* → *Escherichia* hybrids had either antigen h2coli or antigen b; in the latter class the *Escherichia* gene which determines the flagellar antigen h2coli had very probably been replaced by the *Salmonella* gene *H1-b* which determines the phase-1 antigen b. Therefore, *H* of this *E. coli* and *H1* of *S. abony* may be considered to be allelic.

Crosses involving H2, the gene for phage-2 antigen of Salmonella

None of the *his*⁺ *Salmonella abony* → *Escherichia coli* recombinants described above showed a second flagellar antigenic phase. In *Salmonella* the gene *H2* for the phase-2 antigen is situated between *his* and *str*, and is linked to *arg-2*, a locus for arginine synthesis, which also lies between *his* and *str* (Mäkelä & Ziegler, to be published).

Two different *Salmonella abony* *Hfr* donors were crossed to *Escherichia coli* strain EH 23 and, as a control, to *S. abony* strain SH 583, which are *arg*[−] mutants of the *F*[−] recipient strains used in the previous crosses. In both cases the mutant *arg* loci lie between *his* and *str*. Either *his*⁺, *arg*⁺, or both *his*⁺ and *arg*⁺ were selected from donor, and *str-r* from the recipient. The results are recorded in Table 3. In some crosses with the *E. coli* recipient a minority of the recombinants were non-motile or too sluggish to allow determination of their flagellar antigens, as were a proportion of the bacteria of the *E. coli* recipient itself. This could be prevented by carefully selecting an actively motile clone of the recipient to be used in the cross; in several crosses this was done. The results were not appreciably different from those without pre-selection for motility, and the results of all these crosses are therefore presented together. In these crosses some recombinants, especially those obtained by selection of both *his*⁺ and *arg*⁺ from the *S. abony* donor, showed the phase-2 antigen e, n, x of the donor. The *S. abony* → *E. coli* hybrids with the phase-2 antigen e, n, x also possessed a phase-1 antigen (either h2coli of the *E. coli* recipient parent or b of the *S. abony* donor) and underwent phase variation just like diphasic *Salmonella*. The relative frequencies of hybrid recombinants with both *H1* and *H2* genes from the donor or with *H2* only from donor are comparable to the corresponding frequencies in the intraspecies *Salmonella* crosses.

The diphasic hybrids are inferred to be still mainly *Escherichia coli* for the following reasons. (1) The selected genes *his* and *arg* are rather near the point of origin of

the *Hfr* strains used, and since with these *Hfr* strains spontaneous chromosome breaks are frequent, little more than the selected region probably entered these recombinants. (2) Gene *str-r* was a recipient gene appearing in all the recombinants by selection. (3) All the recombinants were also *lac*⁺, like the recipient. (4) All the recombinants selected for *arg*⁺ only from the donor were *his*⁻, like the recipient.

Table 3. *Flagellar antigenic constitution of recombinants in crosses with Salmonella abony Hfr donors and his and arg as selective markers*

<i>Hfr str-s*</i> donors <i>S. abony</i>	<i>F⁻ str-r</i> recipients <i>his⁻ arg⁻</i>	Selected donor alleles	Motile/total recombinants	Proportion of total recom- binants re- ceiving the donor allele (%)		Numbers of recombinants with the anti- genic con- stitution of	
				<i>H1</i> (b)	<i>H2</i> (e, n, x)	b ↔ e, n, x	h2coli ↔ e, n, x or i ↔ e, n, x
sw 1462 (b ↔ e, n, x)	<i>E. coli</i> EH 23 (h2coli)	<i>his</i> ⁺	105/105	52	0	0	0
		<i>his</i> ⁺ <i>arg</i> ⁺	25/42	52	10	3	1
		<i>arg</i> ⁺	4/4	0	25	0	1
sw 1462 (b ↔ e, n, x)	<i>S. abony</i> SH 538 (i ↔ 1, 2)	<i>his</i> ⁺	104/104	67	7	5	2
		<i>his</i> ⁺ <i>arg</i> ⁺	34/34	68	65	14	8
		<i>arg</i> ⁺	87/87	30	45	16	23
sw 1446 (b ↔ e, n, x)	<i>E. coli</i> EH 23 (h2coli)	<i>his</i> ⁺	145/158	56	9.5	12	3
		<i>his</i> ⁺ <i>arg</i> ⁺	30/44	61	61	27	0
		<i>arg</i> ⁺	103/114	0	11	0	13
sw 1446 (b ↔ e, n, x)	<i>S. abony</i> SH 538 (i ↔ 1, 2)	<i>his</i> ⁺	54/54	56	24	11	2
		<i>his</i> ⁺ <i>arg</i> ⁺	9/9	67	67	5	1
		<i>arg</i> ⁺	41/41	0	54	0	22

* All crosses use streptomycin selection against the donor.

If one wants to analyse further the outcome of the crosses summarized in Table 3, the crosses with the two donors must be considered separately. These two *Hfr*-strains differ in the location of their origin and in the direction in which they inject the chromosome (Fig. 1). For sw 1462, *his* is near the origin and is transmitted at high frequency: it is a proximal gene. By contrast, *arg* is a relatively distal gene transmitted at a much lower frequency. If we select for *arg*⁺, a distal gene, from the donor, we can be sure that all proximal genes will have entered these recombinants, though they may have been eliminated from some of them by subsequent cross-overs. With sw 1462 as the donor, the frequencies of *H1* and *H2* of donor origin among *arg*⁺ recombinants reflect their relative distances from *arg*. The same is true of the relative frequencies of *H1* and *H2* of *his*⁺ recombinants when sw 1446 is the donor. The linkage of *H1* and *H2* to the distal genes is somewhat lower in the *Salmonella* → *Escherichia coli* crosses than in the corresponding *Salmonella* → *Salmonella* crosses; the more so, the further the unselected gene from the selected one. This probably means that in an interspecies combination usually rather short pieces of the foreign material are incorporated.

Rate of phase variation in Salmonella → Escherichia coli hybrids

The rate of phase variation was determined in some of the hybrids to see whether it was of the same order of magnitude as in the *Salmonella* donor species. The growth

of the cultures took place at 25° in broth; under these conditions the generation time was very nearly 1 hr for both the *Salmonella abony* donor strain and the hybrid strains. The results of the determinations are in Table 4.

Two hybrids, 1 and 3 ($b \leftrightarrow e, n, x \text{ arg}^+ \text{ his}^+$; $h2\text{coli} \leftrightarrow e, n, x \text{ arg}^+ \text{ his}^-$) gave rates of variation from the non-specific (e, n, x) to the specific phase (b or $h2\text{coli}$) of 5.4 and 3.5×10^{-4} , which is very similar to the rate 5.1×10^{-4} in the *S. abony* control. Two other hybrids gave lower rates, namely, 8 and 2×10^{-5} . In each strain the rate

Fig. 1. Chromosomal transfer by *Hfr* strains of *Salmonella abony*. The arrows indicate the leading locus of each *Hfr* strain and the direction of chromosome transfer. The probability of a given donor gene being transferred decreases, the further it is situated from the leading locus.

Table 4. Rates of phase variation in *Salmonella abony* and some of its hybrids with *Escherichia coli* determined by the method of Stocker (1949)

Strain	Change observed	Time of growth (hr)	Changed cells (%) (1-5 parallel cultures)	Changed cells/bacterium/hr = rate of phase variation
<i>S. abony</i> sw 803	$e, n, x \rightarrow b$	392	16, 19, 20, 20, 25	5.1×10^{-4}
Hybrid 1*	$e, n, x \rightarrow b$	168	6.2, 6.9, 9.7, 10, 12.8	5.4×10^{-4}
Hybrid 2	$e, n, x \rightarrow b$	240	0, 1.5, 1.6, 2.1, 2.7	8×10^{-5}
Hybrid 3	$e, n, x \rightarrow h2\text{coli}$	260	6.1, 6.5, 14.5	3.5×10^{-4}
Hybrid 4	$e, n, x \rightarrow h2\text{coli}$	216	0.4	2×10^{-5}
<i>S. abony</i> sw 803	$b \rightarrow e, n, x$	482	0.9, 1.6, 1.7, 2.1, 4.3	4.4×10^{-5}
Hybrid 1	$b \rightarrow e, n, x$	480	1.8, 1.9	4×10^{-5}
Hybrid 2	$b \rightarrow e, n, x$	984	0, 2.0	10^{-5}
Hybrid 4	$h2\text{coli} \rightarrow e, n, x$	984	0.5, 1.0	8×10^{-6}

* Hybrid 1 is a $his^+ \text{ arg}^+ \text{ str-r}$ recombinant from cross $\text{sw 1446} \rightarrow \text{EH 23}$; hybrid 2 is a $his^+ \text{ arg}^- \text{ str-r}$ recombinant from cross $\text{sw 1446} \rightarrow \text{EH 23}$; hybrid 3 is a $his^- \text{ arg}^+ \text{ str-r}$ recombinant from cross $\text{sw 1446} \rightarrow \text{EH 23}$; hybrid 4 is a $his^+ \text{ arg}^- \text{ str-r}$ recombinant from cross $\text{sw 1446} \rightarrow \text{EH 23}$.

of variation from specific to non-specific phase is about one tenth of the reverse, both in the control *S. abony* and in the hybrid recombinants. One can probably conclude that the control of phase variation (exerted by *H2* locus of *Salmonella*) operates in both *Salmonella* → *E. coli* hybrids and in *Salmonella* in a similar way.

DISCUSSION

Hybrids of *Salmonella abony* and *Escherichia coli* combining antigenic characteristics of both parents have been obtained. In this case the antigens, each determined by distinct structural genes, retain their original identity. Only flagellar antigens have so far been studied, and these strains would not be suitable for studies of other antigens, because for example, the *S. abony Hfr* strains were rough and not agglutinated by anti-O sera.

Diphase *Escherichia coli* was obtained by the introduction of a relatively short segment (including the genes *arg-2* and *H2*) of *Salmonella* chromosome. This agrees with the results of Lederberg & Iino (1956), namely, that phase variation is controlled by a factor, closely associated or identical with the locus *H2*, which determines the specificity of the phase-2 antigen. It seems significant that both the gene for *E. coli* flagellar antigen and the gene for *Salmonella* phase-1 antigen respond equally to this control, even to an extent which permits similar rates of variation between the two phases.

One would also have liked to make flagellar antigenic hybrids by introducing the corresponding portions of *Escherichia coli* genome into *Salmonella*, and to test more *Salmonella* *Escherichia* combinations. Many such interspecies crosses are, however, poorly or not at all fertile. Attempts to obtain *his*⁺ recombinants by crossing several different *Hfr* strains of *E. coli* K 12 or 025 to *Salmonella abony* or *S. typhimurium his*⁻ recipients have failed (Mäkelä, unpublished). A successful cross between the *S. abony* donors used in this work and an *E. coli* 020 : 17 : *his*⁻ recipient gave *his*⁺ recombinants with the *Salmonella* flagellar antigen b.

The same linkage of genes which determine the flagellar antigens to the locus for histidine synthesis has been demonstrated in many *Escherichia coli* serotypes (Ørskov & Ørskov, 1962) and in many *Salmonella* species (Mäkelä, unpublished). Furthermore, the different specificities of the single flagellar antigen of *E. coli* have been shown to be determined by a series of alleles at one locus, *H* (Ørskov & Ørskov, 1962), while the different phase-1 antigens of many *Salmonella* species are determined by a series of alleles of a locus, *H1* and the phase-2 antigens by another series at *H2* (Lederberg & Edwards, 1953). Even if the experiments reported in this paper were mainly made with one pair of *E. coli* and *Salmonella* strains, it can probably be concluded that the *H* genes of *E. coli* and the *H1* genes of *Salmonella* represent a series of alleles of the same locus.

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