Reprint from GENETICS, Vol. 48, No. 3, March, 1963. *Printed in U.S.A.*

HFR MALES IN SALMONELLA ABONY[1]

PIRJO HELENA MÄKELÄ[2]

Department of Genetics, Stanford University School of Medicine, Palo Alto, California and Department of Serology and Bacteriology, University of Helsinki, Helsinki, Finland

Received November 21, 1962

IN a previous communication (MÄKELÄ, LEDERBERG and LEDERBERG 1962) the introduction of the sex fertility factor, F, from *Escherichia coli* K-12 to Salmonella species was reported. Both the wild type F of K-12 and the mutant F particles, F′ or F-*prime* (JACOB and ADELBERG 1959, HIROTA 1959), containing an attached piece of the host chromosome, could be transferred to a number of Salmonellas, and further from Salmonella to Salmonella or to *coli*. The efficiency of F infection was very much better in most homologous combinations than *e.g.* from *coli* to Salmonella or from one Salmonella species to another. In all instances, F infection could be demonstrated by back transfer to *E. coli*.

The motive for the F infection studies was the hope of establishing a sexual recombination that would allow a full genetic analysis in Salmonella. While the first reports of conjugation events with Salmonella involved *E. coli* as the other partner (BARON, CAREY and SPILMAN 1959; MIYAKE and DEMEREC 1959; ZINDER 1960a), there has recently been reported sexual recombination within a Salmonella species, although in a preliminary fashion (ZINDER 1960b; DEMEREC, LAHR, BALBINDER, MIYAKE, ISHIDSU, MIZOBUCHI and MAHLER 1960). It appears as if naturally occurring Salmonella strains were F^-, that is, capable of participating in sexual recombination as recipients of genetic material but not possessing the fertility factor, F, and thus unable to initiate conjugation and to act as genetic donors. After introduction of F they would be converted to F^+ and behave like F^+ *coli* strains. Our first experience of the F^+ Salmonellas, as reported, was promising: they did act as genetic donors to suitably marked stocks of Salmonella, although the frequency of recombinants obtained was low, around 5×10^{-7}. The present paper describes the elaboration of an effective sexual recombination system with Hfr-males in Salmonella, starting from *S. abony* infected with *coli* F.

MATERIALS AND METHODS

All Salmonellas used were derivatives of SW 803, *S. abony* 74 (EDWARDS and BRUNER 1942) with the antigenic formula 4, 5, 12:b:enx. *coli* strains both for F and F′ source and otherwise, were derivatives of K-12 (LEDERBERG and TATUM 1946).

[1] This work has been supported in part by grants from the National Science Foundation, by a Research Training Grant in Genetics (2G-295) and a Research Grant (C-4496) from the National Institutes of Health, U.S. Public Health Service (to Joshua Lederberg), and by a grant from Sigrid Jusélius Foundation.

[2] Present Address, Department of Serology and Bacteriology, University of Helsinki, Helsinki, Finland.

Media and most cultural procedures were described by LEDERBERG (1950). F infection of *S. abony* was described by MÄKELÄ *et al.* (1962). For crosses, the parent bacteria grown in "Penassay" broth (antibiotic medium 3 of Difco), were either spread or dropped together on a plate of selective medium after zero to two hours time of contact in broth. The final crossing technique adopted will be described in the text.

The following locus symbols will be used: *arg*-arginine, *aro*-phenylalanine + tyrosine, *his*-histidine, *ileu*-isoleucine, *leu*-leucine, *met*-methionine, *pro*-proline synthesis, *gal*-galactose fermentation, with + standing for synthetic or fermentative ability, − for inability. S^s = streptomycin sensitive; S^r = streptomycin resistant.

EXPERIMENTAL

The frequency of recombinants in *Salmonella abony* $F^+ \times F^-$ crosses—approximately 5×10^{-7}—observed in the previous paper, was lower than in the corresponding *E. coli* K-12 crosses (10^{-5}-10^{-6}). It was, however, found possible to increase this frequency ten to hundredfold by following a more rigorous experimental schedule.

Fresh cultures of both donor and recipient bacteria were found necessary. It did not, however, make any appreciable difference whether the cultures were in exponential growth or grown overnight, if other conditions were optimal.

Gentle aeration of the recipient (in 20 ml of Penassay broth in a 200 ml Erlenmeyer flask on a rotatory shaker) increased the frequency of recombinants four- to tenfold in many, but not all, experiments. The same relatively mild aeration of the donor did not have an effect on recombination.

The time of contact between donor and recipient bacteria was the most important factor in determining the frequency of recombinants observed. Best results were obtained by leaving the mating mixture undisturbed for two to three hours.

The same optimal conditions were found valid for both $F^+ \times F^-$ and Hfr $\times$ F^- crosses. Thus the following schedule was adopted: Exponentially growing gently aerated Penassay cultures were mixed, usually at densities of approximately 2×10^7 bacteria/ml of donor, 4×10^8 of recipient, and this mating mixture incubated at 37°. The mixture was then plated onto suitable selective media at appropriate dilutions, with the help of 3 ml of minimal medium with 0.6 percent of agar and without a carbon source. Plating with the agar-layer technique was found to give higher and more consistent recombinant counts than spreading with a glass rod. Recombinants were counted after two days incubation, and expressed as a fraction of input minority parent (usually donor). For all quantitative work, streptomycin was used as the selective agent against the donor.

With this technique recombinants were obtained from $F^+ \times F^-$ crosses at a frequency of around 10^{-5} with great regularity and irrespective of the marker selected. Some few F^- stocks give, however, up to 100 times fewer recombinants without the reason being readily apparent. They behave similarly towards different donor strains, also Hfr donors.

Several first attempts to produce Hfr Salmonella donors from F^+, by the method of ultraviolet irradiation and subsequent selection by replica plating of plates seeded with the irradiated culture as described for *E. coli* (TAYLOR and ADELBERG 1960), were futile. It was concluded that either F does not become permanently attached to the foreign Salmonella chromosome, or that this happens at much lower frequency than in K-12.

A more efficient selection for Hfr was therefore devised, making use of the sib selection method as described by CAVALLI-SFORZA and LEDERBERG (1956). The procedure, which proved very useful, is as follows: The exponentially growing F^+ culture is irradiated with ultraviolet light to leave approximately one percent survivors. The cell suspension is then diluted, and drops (*e.g.* with a Pasteur pipette) with approximately 100 surviving bacteria are distributed in 100 test tubes each containing 1 ml of broth. These are incubated overnight. The following day, indicator plates (*e.g.* minimal agar-streptomycin plates to be used with auxotrophic streptomycin-resistant F^- bacteria, if the F^+ was streptomycin-sensitive) are spread with 0.2 ml of exponentially growing recipient bacteria. Several recipients with markers presumably from different parts of the genome can be used at a time, thus increasing the probability of detecting Hfr. After these plates have dried, drops or large loopfuls from the 100 donor tubes are placed on all the different recipient plates (plus one plate without recipient to rule out streptomycin resistant mutants of the donor). Twenty donor tubes can easily be scored on one plate. After two days incubation, most drops usually contain approximately ten growing colonies, while some have from forty to hundreds of them. The tubes corresponding to these drops contain a high proportion of Hfr bacteria, and by scoring some 1000 colonies derived from a tube (replica plating can now be used), it is usually possible to isolate the Hfr. This method seems to have two main advantages: it gives a preliminary enrichment of the Hfr mutants after UV, and it makes the screening of recombination rates more effective.

By this method several Hfr donors of *S. abony* were isolated, as listed in Table 1.

The frequency of recombinants obtained in crosses of these Hfr's with the "high" markers has ranged from 0.02 to two percent of input donor. It is thus somewhat lower than with K-12, where frequencies of up to 40 percent are given (JACOB and WOLLMAN 1961).

TABLE 1

List of the first Salmonella abony *Hfr's*

Strain number	Genetic markers	Markers transferred at high frequency	Highest recombinant frequency	Infectious F	After removal of F	After reinfection with F
SW 1391	*met*$^-$*aro*$^-$*S*^{r}	*leu, pro*	2 percent	not present		
SW 1403	*met*$^-$*aro*$^-$*S*^{r}	*his, gal*		present	F^-	Hfr of original type
SW 1418	*pro*$^-$*S*^{r}	*leu*				
SW 1446	*S*^{s}	*met, arg*	0.2 percent			
SW 1452	*gal*$^-$*S*^{s}	*pro, leu*	0.02 percent			
SW 1454	*S*^{s}	*his*				
SW 1462	*S*^{s}	*his, ileu*	1 percent			

Somewhat surprisingly, most of the Hfr's still proved to contain an infectious F agent. On further tests all these were shown to resemble the δ_3 type of *E. coli* K-12 (RICHTER 1961): 1. When the F from them is infectively transferred to an F^- culture, the converted bacteria are F^+, not Hfr; 2. When F is removed from them (*e.g.* by passage through semisolid agar) (SKAAR, RICHTER and LEDERBERG 1957), they will behave as F^-; 3. When these F^- bacteria are reinfected with F from an F^+ culture, they will become Hfr of the original type. This is interpreted to mean that they have a stable sex factor affinity (*sfa*) locus in their chromosome; the F will alternate between an autonomous and a fixed state at this locus.

These Hfr's lose their F rather easily, as do F^+ Salmonellas (MÄKELÄ *et al.* 1962). This, of course, is a technical handicap when working with them; upon reinfection they have, however, in the course of two years always behaved like the original Hfr. The alternation of the F between chromosomal and free state probably occurs rather rapidly in these strains: replica plating of a recently infected culture always shows very nearly 100 percent colonies giving high numbers of recombinants like Hfr, and at the same time, capable of transferring F to an F-detector system (RICHTER 1961, MÄKELÄ *et al.* 1962) like F^+. Why so many of the Salmonella Hfr isolates were of this type, we do not know. Only one Hfr, SW 1391, behaves like a standard-type Hfr. It is not infectious, and only after numerous passages in semisolid medium was a culture behaving as F^- obtained once; it could not be reinfected. SW 1391 has been a very stable Hfr, and it also is one of the best in respect to numbers of recombinants produced.

In K-12, the Hfr bacteria are believed to inject their chromosome in the recipient cell in an orderly fashion, starting from the origin and proceeding at a constant rate until the whole chromosome is transferred in about two hours time. The chromosome can break, and usually does so, at any time during this process. Thus a gradient of transfer of markers is observed, those nearest to the origin being transferred at highest frequencies. The mating can be experimentally interrupted, *i.e.*, the chromosome broken at different times giving information about times of entry of different markers; this also gives the most exact chromosome map of *E. coli* K-12 (JACOB and WOLLMAN 1961).

TABLE 2

Gradient of transfer of different markers by the Hfr's

	leu	*pro*	*gal*	*his*	*ileu*	*met*	*arg*
SW 1391*	>1000 (200)	>1000 (50)	1000	100	100	...	20
SW 1403	20	100	1000	>1000	>1000	...	10
SW 1418	1000	...	2	5	500	500	500
SW 1446	5	5	20	20	1000	>1000	>1000
SW 1452	500	500	3	2	20	60	100
SW 1454	500	500	1000	>1000	200	200	500
SW 1462	200	100	5	>1000 (100)	500 (0.6)	200	400

* The figures represent approximate numbers of recombinant colonies observed in a simple drop test with the donor culture diluted ten- or hundredfold. The figures in parentheses are numbers of recombinants per 10^4 Hfr obtained in separate experiments with double mutant recipients.

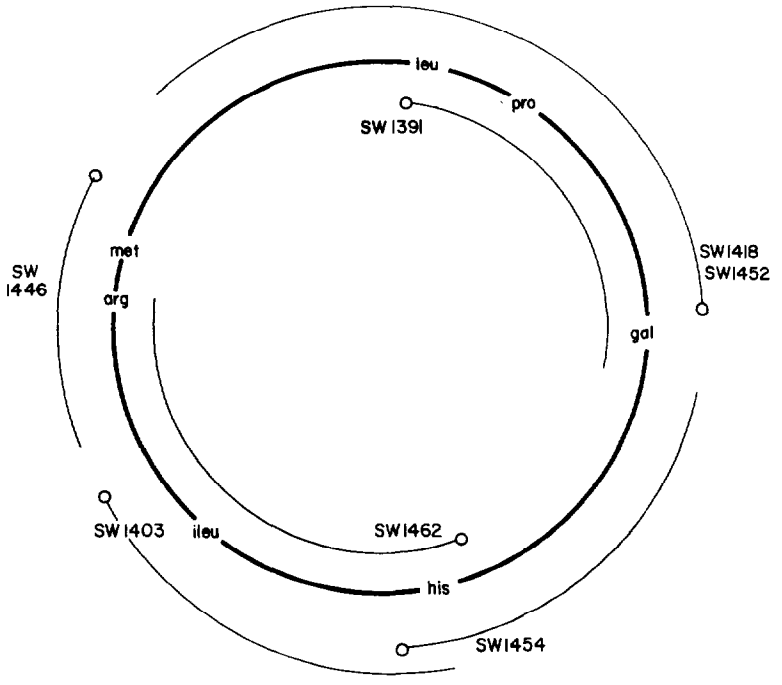

FIGURE 1.—The circular chromosome of *Salmonella abony* and the origin, O—, and proximal segment of each of seven Hfr types. The figure is based on gradients of transfer by these Hfr's.

A gradient of transfer was also observed with *S. abony* Hfr donors, indicating an analogous process of transfer. This view was further supported by interrupted mating experiments, the results of which will be the subject of another paper (MÄKELÄ and ZIEGLER, in preparation). The gradient gives an indication of the order of markers along the chromosome. Although there are major objections to this crude way of mapping, primarily due to differences in the mating efficiency of different recipient bacteria, it is of considerable use, and especially so for a preliminary characterization of Hfr's. Some of the data with *S. abony* Hfr's are given in Table 2; Figure 1 is constructed on the basis of these. It is notable that the data give a circular chromosome, and that the order of markers *leu-pro-gal-his-ileu-(met, arg)* is the same as in K-12 (JACOB and WOLLMAN 1959). A preliminary report on the mapping was published by MÄKELÄ (1962).

DISCUSSION

It has thus been possible to transfer the sexual recombination system originally detected in *E. coli* K-12 to another member (or genus) of the enteric bacteria, *Salmonella abony*. The process of mating as far as studied seems surprisingly similar in these two species. The F agent of *coli* origin apparently is able to establish a stable association with the Salmonella chromosome, giving rise to a standard Hfr, as well as to undergo recombination with the chromosome, giving rise to "sex factor affinity" loci. This kind of recombination between the

chromosome and the F episome was postulated by ADELBERG and BURNS (1960) as the basis for the formation of episomes, with an affinity for a specific site in the chromosome (F-*primes*), and of bacteria with chromosomes specifically altered to attract F to a specific site (*sfa* loci).

That recombination events can occur between the F of *coli* and the chromosome of *Salmonella abony*—even the formation of F-*primes* in the latter has later been observed (MÄKELÄ, to be published)—indicates a rather high degree of homology between them. Indeed, one would be inclined to believe that a true integration of genetic material has taken place in these cases, even if a homology between *Salmonella (typhimurium)* and *E. coli* could not be detected with a more rigorous test for homology by hybridization of DNAs (SCHILDKRAUT, MARMUR and DOTY 1961). On the other hand, as F represents only a small fraction of the total DNA in a bacterium (MARMUR, ROWND, FALKOW, BARON, SCHILDKRAUT and DOTY 1961), a hybrid formation caused by it could probably have remained undetected in this test.

SUMMARY

The fertility of *Salmonella abony* strains infected with F of *Escherichia coli* K-12 was examined. The F^+ strains gave recombinants at a frequency of 10^{-5}. From them several Hfr strains were isolated, one of which was of the usual noninfective type known in K-12, while six others still contained F (plus a sex factor affinity locus). They gave recombinants at frequencies of up to two percent, and had different origins and directions of transfer. A preliminary map constructed on the basis of gradients of transfer is given; it is circular, and the order of seven markers is the same as in K-12.

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