GENETIC RECOMBINATIONS IN INTESTINAL BACTERIA. COMMUNICATION 1. HYBRIDIZATION BETWEEN *Escherichia coli* AND *Shigella flexneri*

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The discorvery of a sexual process in bacteria [6, 11] and the isolation of Hfr strains of E. coli with a high frequency of recombinations [3, 5] have greatly extended the possibilities of studying the principles of the transfer of genetic material from one bacterial cell (donor) to another (recipient) within the limits of a single species. By analogy with the results of experiments on the transformation and transduction of cells, it appeared that this type of genetic transfer may also be possible in bacteria belonging to different species and genera. In fact, hybridization has been produced between E. coli and various species of Salmonella, between salmonellae and Bacillus subtilis, and between salmonellae and Vibrio cholerae [1, 2, 4]. However, the production of hybrids by crossing bacteria of different species has so far been attended with considerable difficulty, as shown by the unsuccessful attempts of many workers to cross E. coli with dysentery bacteria hybrids of which have been described only by Luria and Burrous [9]. Meanwhile, further attempts to find ways of crossing intestinal bacteria, especially pathogenic, are of considerable importance not only for the understanding of general problems (the evolution of natural populations, the graded transmission of signs, the classification of bacteria), but also for the solution of many special problems, such as the causes of the development of serotypes, of atypical strains, and so on.

In the present paper we describe the results of experiments on the hybridization of E. coli and Sh. flexneri, carried out for the purpose of determining the possibility of crossing these microorganisms and the extent to which this depended on the Hfr strains of E. coli used in the crosses.

EXPERIMENTAL METHOD

As donors, the following strains were used: W 1485 F+ (Me-, lac+, gal+, $T1^S$), HfrH (B_1-, lac+, gal+, $T1^S$, λ-, Hfr R_1) prototroph, lac+, gal+, $T1^r$, HfrC (Me-, lac+, gal+, $T1^S$), and HfrH (B_1-, lac+, gal+, $T1^S$, λ+)*, derived from E. coli K-12. All were sensitive to streptomycin.

As recipients 35 strains of Sh. flexneri were tested; these were obtained from the L. I. Tarasevich State Control Institute and from the department of microbiology of Tashkent Medical Institute. Streptomycin-resistant variants of these strains, isolated by picking off individual colonies grown after seeding on to MPA with the addition of 100 i.u. streptomycin/1 ml of medium.

For the crossing procedure, an 18 h broth culture of E. coli was diluted 1:3 with physiological saline. A suspension was made from an agar culture of Sh. flexneri, resistant to streptomycin, in physiological saline with a concentration of 250 million cells/ml. Samples of 1 ml of each culture were mixed together in a separate tube, the mixture incubated at 37° for 60 min, and then seedings of 0.1 ml of it and its dilutions made on a selective medium, consisting of minimal lactose medium [6] to which were added 0.4 g/liter yellow eosin, 0.065 g/liter methylene blue, and 100 i.u. streptomycin/ml. By means of this medium it was possible to select Lac + S^r recombinants,

*Me- unable to synthesize methionine; B_1 - unable to synthesize thiamine; lac+ fermentation of lactose; gal+ fermentation of galactose; $T1^S$ - sensitivity to phage T1; λ+ carries prophage; λ- prophage absent.

Results Showing Fertile Combinations of Strains of Sh. flexneri with Different Strains of E. coli Hfr

Strains of Sh. flexneri	Fertility of combinations of donors and recipients Strains of E. coli			
	HfrH (λ^-)	$HfrR_1$	HfrC	HfrH (λ^+)
621	+	—	—	—
628	+	—	—	—
3584	+	—	—	+
2048	+	—	—	—
2046	+	+	—	—
2055	—	+	+	—
2047	+	+	+	+
2049	—	+	—	—
2044	+	—	—	—
75/2	+	—	—	—
2050	+	+	—	+
5008	+	+	—	—
5030	+	+	—	—
828	—	—	—	+
845	+	—	—	—
970	—	—	—	+

i.e., recombinant strains of bacteria fermenting lactose and resistant to streptomycin.

As controls to these experiments, separate seedings were made of E. coli and Sh. flexneri on to a similar minimal medium containing lactose, indicator, and streptomycin.

EXPERIMENTAL RESULTS

To produce crossing, samples of bacteria of each of the 5 strains of E. coli were mixed with samples of all 35 strains of Sh. flexneri, and the experiments were repeated five times. In positive cases, recombinant colonies (resistant to streptomycin and fermenting lactose) appeared on the dishes of selective medium, seeded with a mixture of E. coli and Sh. flexneri, after 3-5 days of cultivation at 37°. In the control seedings no colonies were observed to grow, for development of E. coli was prevented by streptomycin and the streptomycin-resistant Sh. flexneri could not utilize the lactose contained in the medium as a source of carbohydrate.

Although the experiments were repeated many times, recombinant colonies were found only on those dishes with selective medium seeded with a mixture of Sh. flexneri and E. coli Hfr. So far as E. coli strain W 1485 F+ was concerned, attempts to cross it with Sh. flexneri did not lead to the production of recombinants, for no colonies developed on the selective medium. After crossing the 4 strains of E. coli Hfr with each of the 35 test strains of Sh. flexneri, combinations with only 16 strains proved fertile. In this way 183 cultures of recombinants were isolated. It may be concluded from these results that about half the test strains of Sh. flexneri could be identified as F- (recipient) strains.

The ability of Sh. flexneri to act as recipient cells in crossings was displayed to an unequal degree when all 4 strains of E. coli Hfr were crossed with donor cells. This is clear from the results showing fertile combinations of 4 strains of E. coli Hfr and 16 strains of Sh. flexneri (see table).

It is easy to see that fertile combinations with all 4 strains of E. coli Hfr were found only in the case of strain No. 2047 of Sh. flexneri. Strains Nos. 3584 and 2055 of Sh. flexneri gave fertile combinations with 2, and No. 2050 with 3 and 4 strains of E. coli Hfr used for crossing. In the remaining cases, the strains of Sh. flexneri gave fertile combinations with 2 or with only 1 strain Hfr. Whatever the strains used, the frequency of recombination was on the whole low, compared with that usually observed during crossing of Hfr strains with strains of E. coli F-

The results demonstrate that hybridization is possible between sexually differentiated strains of E. coli and Sh. flexneri, i.e., between bacteria characterized by considerable taxonometric differences.

By testing the strains of Sh. flexneri for fertility with different standard strains of E. coli Hfr by means of selection of Lac+ S^r recombinants, it was possible to isolate a relatively large number of dysentery strains behaving as F- strains in crossings with E. coli. From the quantitative point of view, these results are in agreement with those obtained by intra-species crossings of E. coli, and also by crossings of E. coli with salmonellae, when a quarter, or even half, of the various strains of E. coli possessed the fertility factor [8, 10].

Data relating to the genetic structure of the isolated recombinants (183) and a discussion of the results will be presented in later communications.

SUMMARY

Investigations carried out demonstrated that a recombination phenomenon takes place on mixing E. coli and Sh. flexneri cells. Sh. flexneri behave as females.

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