The Transfer of Motility and Tyrosine Requirement to *Escherichia coli* Strain B by Recombination with *E. coli* Strain K12

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SUMMARY: Motile recombinants may be obtained by crossing *Escherichia coli* strain K12, F+, with the non-flagellated strain *E. coli* strain B, F-. The flagella of the recombinants have the same antigenic pattern as those of the F+ parent and the locus controlling motility is linked to loci controlling tyrosine requirement and fermentation of arabinose and galactose (*gal*₅).

Motility may be transduced to non-motile strains of *Salmonella* by phage grown on motile *Salmonella* strains (Stocker, Zinder & Lederberg, 1953). The majority of the motile clones thus obtained produce flagella having the antigenic composition of the motile parent stock from which the non-motile recipient strain was derived by mutation. Transduction therefore normally reveals their inherent antigenic pattern. By using suitable selective techniques, in some cases, a minority of motile recombinants having flagella of the antigenic pattern of the bacteria on which the phages were propagated (donor strain) can be demonstrated.

These observations suggested that motility might similarly be transferred by recombination between mutants of *Escherichia coli* strain K12 and mapped as another marker on the bacterial chromosome. However, all mutants of strain K12 were found to become motile on inoculating Craigie tubes. *E. coli* strain B, on the other hand, never mutated to motility but acquired motility when incubated with motile strain K12 F+ mutants (Furness & Rowley, 1955). All the motile progeny had flagella of the same antigenic pattern as the F+ parent but linkage with other characters could not be shown. Linkage has been further investigated by using auxotrophic strains of *E. coli* strain B. This paper reports the results of experiments in which it has been found that motility is linked to other markers on the bacterial chromosome, namely tyrosine requirement and ability to ferment arabinose and galactose.

METHODS

Strains of Escherichia coli strain B. Both strains were F-, non-motile, resistant to 500 µg. streptomycin/ml., fermented lactose, arabinose, galactose, xylose and mannitol but not maltose (*lac*⁺, *arab*⁺, *gal*⁺, *xyl*⁺ *mtl*⁺, *mal*⁻) and were sensitive to all of the T series of phages. *E. coli* B/S^BF- grows on minimal medium (Davis & Mingioli, 1950). *E. coli* B mutant 615/S^BF- was kindly made available to us by Dr de Haan and was auxotrophic for tyrosine and methionine (de Haan, 1955).

Strains of Escherichia coli K12. Strain W677 was auxotrophic for threonine, leucine and vitamin B₁ (T⁻L⁻B₁⁻), did not ferment lactose, arabinose, galactose, maltose, xylose and mannitol (*lac*⁻, *arab*⁻, *gal*_s⁻, *mal*⁻, *xyt*⁻, *mtl*⁻) and was resistant to phages T₁ and T₅ (V_{1,5}^R; Clowes & Rowley, 1954; Lennox, 1955). It was streptomycin sensitive and F⁺, mating with *E. coli* B strains. To ensure that all organisms were fully motile it had been repeatedly passaged through Craigie tubes.

Media. Minimal medium was that of Davis & Mingioli (1950) and the formula of Lederberg (1950) was used for eosin + methylene-blue sugar-containing media. The minimal medium was supplemented with 20 µg./ml. of tyrosine or methionine when required. Craigie tubes were prepared as described by Tulloch (1939) using nutrient broth solidified with 0.5% Difco agar. Semi-solid gelatin agar was made by the method of Stocker *et al.* (1953). All streptomycin media contained 500 µg. streptomycin/ml.

Recombination techniques. Using overnight cultures 3 ml. of coli B parent and 6 ml. F⁺ parent were mixed, incubated ½ hr. at 37°, diluted with 45 ml. broth and incubation continued for a further 3½ hr. 8 ml. of the mixture and 5 ml. of each parent, as controls, were centrifuged. Further stages of the technique depended on the recombinants to be selected, as follows.

(a) *Selection of motile recombinants.* The deposits after centrifugation were spread by loop in arcs (Stocker *et al.* 1953) on plates of semi-solid gelatin agar containing streptomycin to eliminate the F⁺ parent. The plates were incubated at 37° for 24 hr. and left at room temperature for a further 72 hr.

(b) *Selection of mono-auxotrophic recombinants.* The organisms were washed three times in minimal salts, resuspended in approximately one-tenth the original volume and 0.1 ml. amounts were spread on minimal medium plates supplemented as necessary. All plates were incubated at 37° for 42 hr.

Purification of recombinants. Motile or mono-auxotrophic recombinants were purified by picking a discrete colony after plating out on nutrient agar. After checking that the purified strain still showed the recombinant characters, it was tested for the unselected markers by standard methods for testing sugar fermentations, streptomycin resistance, phage resistance and nutritional requirements; motility was tested by microscopic examination and ability to grow through a Craigie tube within 24 hr.

'H' agglutination technique. Agglutinating serum obtained from a rabbit immunized with a saline suspension of motile *Escherichia coli* strain K12 was inactivated by heat. Standard methods were used for the preparation of 'H' and 'O' agglutinable suspensions and for the Dreyer agglutination test (Mackie & McCartney, 1948).

RESULTS

Recombination using motility as selective marker of the F⁺ parent

Ninety-eight motile streptomycin-resistant recombinants from the mating W677 F⁺ × Coli B/S^RF⁻ were picked from discrete flares in the semi-solid streptomycin gelatin agar and tested for the unselected markers of the F⁺ parent, i.e., T⁻, L⁻, B₁⁻, V_{1,5}^R, *lac*⁻, *arab*⁻, *gal*_s⁻, *xyt*⁻, and *mtl*⁻. These recom-

binants had all the unselected markers of the F⁻ parent. Control plates of semi-solid gelatin agar containing streptomycin when inoculated with W 677 F⁺ alone showed no growth and when inoculated with coli B/S^BF⁻ alone never yielded flares, indicating that the F⁺ parent had not mutated to streptomycin resistance, nor the F⁻ parent to motility.

'H' agglutinable suspensions were prepared from these recombinants and from the strain K12 culture used for the preparation of the agglutinating serum. All suspensions including the K12 suspension agglutinated to the same titre with the serum prepared against the motile K12 parent; this showed that the flagella of the recombinants and of the F⁺ parent had the same antigenic composition. Using this technique, neither 'O' agglutinable suspensions of K12 nor saline suspensions of coli B/S^B agglutinated with this serum.

On mating W 677 F⁺ and 615S^BF⁻ by the same method it was found that the unselected marker *tyr*⁺ had been transferred from the F⁺ parent to 10.7% of the 84 motile, streptomycin-resistant recombinants tested. One of these motile *tyr*⁺ recombinants was also *arab*⁻, *gal*₅⁻ (see Table 1). No other marker from the F⁺ parent was found in any of the recombinants. It is concluded that *motility* is linked to *tyr* and, less closely, to *arab* and *gal*₅.

Table 1. *Transfer of unselected markers*

From F⁺ parent to recombinants on mating *E. coli* W 677 F⁺ motile (T⁻L⁻B₁⁻V₁₅^B, *lac*⁻, *arab*⁻, *gal*⁻, *xyl*⁻, *mtl*⁻) with *E. coli* B 615S^BF⁻ non motile (Tyr⁻, M⁻, *lac*⁺, *arab*⁺, *gal*⁺, *xyl*⁺, *mtl*⁺) on streptomycin semi-solid gelatin agar using motility as selective marker of the F⁺ parent

Unselected F ⁺ markers transferred			No. of recombinants	%
<i>tyr</i> ⁺	<i>arab</i> ⁻	<i>gal</i> ⁻		
—	—	—	74	88.1
+	—	—	9	10.7
+	+	+	1	1.2
Total motile recombinants			84	

+ Indicates transfer of unselected markers from F⁺ parent. The unselected markers T⁻L⁻B₁⁻V₁₅^B, *lac*⁻, *xyl*⁻, *mtl*⁻ were not transferred from the F⁺ parent to any of the recombinants total.

The rate of recombination was estimated at 1 motile recombinant in 10⁸ *Escherichia coli* strain B cells, but an accurate determination could not be made by the technique of streaking centrifuged deposits on plates.

Recombination using tyr⁺ as selective marker of the F⁺ parent

Samples of a mixture of W 677 F⁺ and 615S^BF⁻ were spread on minimal medium supplemented with methionine, and also on minimal medium supplemented with methionine and streptomycin (to examine the effect of counterselection for streptomycin-sensitivity). It was found that the inclusion of streptomycin reduced the number of recombinants from 1 in 5 × 10⁸ 615S^BF⁻ cells to 1 in 5 × 10⁶. Control plates seeded with either W 677 F⁺ or 615S^BF⁻ alone gave no colonies.

On testing the unselected markers of the recombinants recovered from the methionine minimal plates it was found that only 3.9% of the recombinant

clones were streptomycin-sensitive, which suggests that contra-selection was not the sole reason for the reduction in the yield of recombinants when streptomycin was included. Moreover, the presence of streptomycin not only reduced the yield of *tyr*⁺ recombinants but, with the exception of *motility*, also significantly effected the relative frequency of the various classes of recombinants (see table 2).

Table 2. *Transfer of unselected markers*

From F+ parent to recombinants on mating *E. coli* W 677 S^s motile F⁺ (T⁻ L⁻ B₁⁻, V_{1,5}^B, *lac*⁻, *arab*⁻, *gal*₅⁻, *xyl*⁻, *mtl*⁻) with *E. coli* B 615 S^BF⁻ non-motile (Tyr⁻, M⁻, *lac*⁺, *arab*⁺, *gal*⁺, *xyl*⁺, *mtl*⁺) on minimal medium supplemented with methionine, or methionine and streptomycin, *tyr*⁺ being the selective marker of the F+ parent.

Unselected markers transferred from F+ parent										Supplements to medium			
										Methionine		Methionine and streptomycin	
V _{1,5} ^B	<i>lac</i> ⁻	<i>motility</i>	<i>arab</i> ⁻	<i>gal</i> ₅ ⁻	S ^s	<i>xyl</i> ⁻	<i>mtl</i> ⁻	M ⁺		No.	%	No.	%
-	-	-	-	-	-	-	-	-		80	62.5	124	72.5
-	-	-	+	-	-	-	-	-		9	7.0	6	3.5
-	-	-	+	+	-	-	-	-		10	7.8	8	4.6
-	-	-	-	+	-	-	-	-		1	0.8	3	1.8
-	-	+	-	-	-	-	-	-		9	7.0	25	14.6
-	-	+	+	-	-	-	-	-		5	3.9	3	1.8
-	-	+	+	+	-	-	-	-		7	5.4	2	1.2
+	+	+	+	+	+	+	+	+		1	0.8	.	.
-	-	-	+	+	+	+	+	+		1	0.8	.	.
+	-	+	+	+	-	-	-	-		1	0.8	.	.
+	+	-	-	-	-	-	-	-		1	0.8	.	.
-	-	-	-	-	+	+	-	-		1	0.8	.	.
-	-	-	-	-	+	+	+	-		1	0.8	.	.
-	-	-	+	+	+	+	-	+		1	0.8	.	.
Total recombinants tested										128		171	

+ indicates transfer of unselected marker from F+ parent.

When *tyr*⁺ was used as the selective marker from the F+ parent the only frequent (> 2 %) class of recombinants obtained were: those with no unselected marker from the F+ parent (62.5 % or 72.5 %); those with the unselected markers *motility* (7 % or 14.6 %); *arab* (7 % or 3.5 %); *arab* and *gal*₅ (7.8 % or 4.6 %); *motility* and *arab* (8.9 % or 1.8 %); *motility*, *arab* and *gal* (5.4 % or 1.2 %); the percentages for each class being stated for recombinants selected in the absence and in the presence of streptomycin. These frequent classes can be explained as resulting from double, rather than multiple, cross-overs in the region of the *tyr* locus if the order of the loci concerned is *motility-tyr-(arab, gal*₅*)*, with none of the other unselected markers tested lying between *motility* and (*arab, gal*₅*)*.

DISCUSSION

Electron micrographs have shown that the cells of *Escherichia coli* strain B are without flagella and attempts to obtain motile mutants have failed. As a result, it is impossible to find the latent flagellar antigens, if any, of *E. coli* B for comparison with those of *E. coli* K12. The flagella of the motile progeny

have the antigenic pattern of K12, the F+ parent. This is in contrast to the salmonellas, where, when motility is transduced to a non-flagellated strain, the latent genes determining the H antigens of the recipient strain are usually expressed.

From the present data the loci concerned appear to be in the order *motility-tyr-(arab, gal_s)*. De Haan (1955) using the streptomycin-sensitive parent of this 615S^BF- strain has reported that the linkage results in recombination experiments can be markedly altered by the cultural conditions of the F- strain and also by the addition of methionine to the medium used for selecting recombinants. It is possible that streptomycin has a similar effect. The action of added streptomycin, in altering the apparent closeness of linkage of *arab* and of *gal_s* to *motility* and *tyr*, cannot at present be explained.

I wish to thank Dr P. G. de Haan for the auxotrophic mutants of *Escherichia coli* strain B, Dr D. Rowley for his interest and encouragement and Miss Margaret Ham for her excellent technical assistance.

REFERENCES

- CLOWES, R. C. & ROWLEY, D. (1954). Some observations on linkage effects in genetic recombination in *Escherichia coli* K12. *J. gen. Microbiol.* 11, 250.
- DAVIS, D. B. & MINGIOLI, E. S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B₁₂. *J. Bact.* 60, 17.
- FURNESS, G. & ROWLEY, D. (1955). Transfer of motility by *Escherichia coli* K12 to *E. coli* B. *J. gen. Microbiol.* 12, v.
- HAAN, P. G. DE (1955). Genetic recombinations in *Escherichia coli* B. III. The influence of experimental conditions on the transfer of unselected markers. *Genetica*, 27, 364.
- LEDERBERG, J. (1950). Isolation and characterisation of biochemical mutants of bacteria. *Meth. med. Res.* 3, 5.
- LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. *Virology*, 1, 190.
- MACKIE, T. J. & MCCARTNEY, J. E. (1948). *Handbook of Practical Bacteriology*, 8th ed. Edinburgh: E and S. Livingstone, Ltd.
- STOCKER, B. A. D., ZINDER, N. D. & LEDERBERG, J. (1953). Transduction of flagellar characters in Salmonella. *J. gen. Microbiol.* 9, 410.
- TULLOCH, W. J. (1939). Observations concerning bacillary food infection in Dundee during the period 1923-1938. *J. Hyg., Camb.* 39, 324.

(Received 24 January 1958)