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Genetic Transfer of the Vi Antigen from *Salmonella typhosa* to *Escherichia coli*

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The Vi antigen was expressed in a strain of *Escherichia coli* after transfer of the *via*B locus from a *Salmonella typhosa* Hfr donor.

Salmonella typhosa produces an envelope antigen termed Vi, which is also found in *S. paratyphi* C, *Citrobacter ballerup*, and certain uncommon strains of *Escherichia coli* (3). In *S. typhosa*, genetic studies have revealed that at least two widely separated gene loci, designated *viaA* and *viaB*, are required for Vi antigen expression (5, 6). The *viaA* gene has been mapped in the chromosomal region adjacent to the determinant of histidine biosynthesis, *his* (6), whereas the *viaB* locus is situated near *purA* (5), a gene involved with adenine biosynthesis. These genetic studies have shown also that *S. typhimurium*, which does not produce Vi antigen, possesses a native, functional *viaA* determinant and is capable of Vi antigen expression after genetic transfer of the *viaB* locus from *S. typhosa* (5).

From the results of matings between an *E. coli* Hfr strain and an *S. typhosa* strain having a defective *viaA* determinant (unable to produce serologically detectable Vi antigen), we were able to demonstrate that a native, functional *viaA* gene is present in *E. coli* (Johnson, *unpublished data*). As in the case of *S. typhimurium*, therefore, it seemed reasonable to expect that transfer of the *S. typhosa viaB* gene (or gene complex) to *E. coli* would result in Vi antigen expression in that organism. A number of *E. coli* strains (none of them producing serologically detectable Vi antigen) of various serotypes, including *E. coli* K-12, were examined for this purpose, but most of them did not behave as suitable genetic recipients in crosses with the *S. typhosa* Hfr. However, one *E. coli* strain, WR3991, was capable of accepting and integrating portions of the *S. typhosa* Hfr chromosome. In the present communication, we describe mating experiments performed with *E. coli* WR3991 as the genetic recipient.

The pertinent characteristics of the bacterial strains are listed in Table 1. The derivation and characterization of the *S. typhosa* Hfr strain WR4000 (formerly designated TD-7), which produces the Vi antigen, have been described previously (4). Chromosome transfer by this Hfr is in the order origin—*pro*—*ara*—*xyl*; its counterselection in the mating experiments was accomplished by the omission of cystine and tryptophan from the selective medium. *E. coli* WR3991 is a derivative of *E. coli* W3442, an antigen test strain (O antigen 102, K antigen of the B type) previously described by Ørskov and Ørskov (7); it is a spontaneous mutant which has lost the K(B) antigen, as determined by its translucent colonial appearance [distinguishable from the more opaque colonies of the K(B)-producing strain] and its loss of ability to inhibit agglutination by O(102) antiserum. The minimal selective medium and the mating procedures were the same as those we employed in previous studies (5, 6). Hybrids were tested for Vi antigen expression by slide agglutination, by use of Vi antiserum prepared, as described by Edwards and Ewing (3), against *C. ballerup*.

In genetic crosses with *S. typhosa* Hfr WR4000, in which selection was made for the met^+ marker of the Hfr, *E. coli* WR3991 produced met^+ recombinants at a frequency of 10^{-7} per donor cell. One-hundred met^+ hybrids were examined for unselected inheritance of the Vi antigen, as well as for inheritance of the donor markers fuc^-, xyl^-, tna^-, ara^-, and pro^+. Inheritance of the *viaB* locus (which is situated between *met* and *ara*) and expression of the Vi antigen occurred in 22% of the hybrids (Table 2). Unselected marker inheritance in this cross appeared to be somewhat similar to that which we observed in interspecies *Salmonella* crosses (5, 6). Markers located proximal to the selected character (in this instance, *ara* and *pro*) were inherited rarely, and distal marker inheritance (*fuc*) was not observed; significant percentages of unselected marker inheritance occurred only with those genes located near the selected marker (in the present cross, *viaB*, *xyl*, and *tna*).

TABLE 1. *Characteristics of the bacterial strains*[a]

Strain	Auxotrophic characters	Carbohydrate utilization			Tna	Vi antigen	Mating polarity
		Ara	Xyl	Fuc			
Salmonella typhosa WR4000	Cys, Trp	−	−	−	−	+	Hfr
Escherichia coli WR3991	Pro, Met	+	+	+	+	−	Recipient

[a] Abbreviations and symbols: Cys, cystine; Trp, tryptophan; Pro, proline; Met, methionine; Ara, arabinose; Xyl, xylose; Fuc, fucose; Tna, production of indol; +, utilized or produced; −, not utilized or not produced.

TABLE 2. *Unselected marker inheritance by E. coli WR3991 hybrids obtained from matings with S. typhosa Hfr WR4000*

Selected marker	Per cent unselected markers[a]					
met$^+$	*fuc*$^-$	*xyl*$^-$	*tna*$^-$	Vi	*ara*$^-$	*pro*$^+$
All	<1	34	35	22	8	1

[a] Percentages are based on the examination of 100 *met*$^+$ hybrids. Abbreviations: *ara*, arabinose; *fuc*, fucose; *pro*, proline; *tna*, indole production; *xyl*, xylose.

The expression of the negative *Salmonella* donor alleles *xyl*$^-$, *tna*$^-$, and *ara*$^-$ by a high proportion of these *E. coli* hybrids, indicating that they are haploid with regard to those genes, is not typical of the hybrids which we observed in other intergeneric matings (2). For example, in crosses in which *S. typhosa* is used as the recipient and the *E. coli* donor is of the K-12 line, the transferred genetic material of the donor is maintained as a partial diploid and only rarely replaces the allelic region of the recipient (1, 2). At the present time, our limited knowledge of the factors which produce the partial diploid state in bacterial genetic hybrids precludes explanation of this difference. Further experimentation with the mating system employed in this study and its addition to other systems available for analysis of bacterial diploidy should prove useful in investigating this phenomenon.

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