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Salmonella hybrids containing genic material of multiple origins*

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SUMMARY

Bacterial hybrids were produced to contain genetic material of *Salmonella typhimurium*, *Escherichia coli*, *S. montevideo* and *S. abony* origins. Analyses by transduction provide evidence that 6% of the original *typhimurium* genome has been replaced in the production of these hybrids. Although a number of biosynthetic pathways are affected by this gene substitution, the growth rate of these hybrids in minimal medium is unchanged. Supporting evidence for the close relatedness between *S. typhimurium* and the other three species is not observed in recombination studies. Available results favour the concept that differences in base sequences are responsible for the low frequency of recombination obtained in heterologous crosses.

1. INTRODUCTION

For several years our laboratory has been concerned with studies on *Salmonella typhymurium–Salmonella montevideo* hybrids, i.e. *S. typhimurium* strains that possess some genic material of *S. montevideo* origin (Glatzer, LaBrie & Armstrong, 1966; Armstrong, 1967; Atkins & Armstrong, 1969). These investigations, therefore, have involved hybrids with genomes derived from two sources. To expand our knowledge of bacterial hybrids, attempts to produce hybrid strains that possess genetic material from several sources were undertaken. A protocol was developed to produce hybrids with chromosomes that contain genic material not only of *S. typhimurium* and *S. montevideo* origins but also of *S. abony* and *Escherichia coli*. Such hybrids were produced, then analysed by transduction to determine the regions of hybridity in each strain. The procedures employed to obtain these hybrids, as well as the results of genetic analysis and the conclusions derived from them, are presented in this report.

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2. MATERIALS AND METHODS

(i) *Production of hybrid strains*

The parental strain, TC-*ilvA12*, utilized in the study is a *S. typhimurium–E. coli* hybrid obtained from the late Dr M. Demerec. An *ilvC* (previously designated *ilvA*) strain served as the female parent and *E. coli* HfrCS–101 (Cavalli) as the male. A diagrammatic scheme of the procedure used to produce the hybrid strains is presented in Fig. 1. The mutagen, nitrosoguanidine, and the procedure described by Adelberg, Mandel & Chen (1965) were employed to obtain an arginine-requiring strain of TC-*ilvA12*. Enzymic analysis, kindly carried out by Dr E. E. Jones, established that this auxotrophic hybrid was deficient in arginosuccinase activity, i.e. an *argF* mutant. The *argF* hybrid was crossed with *S. montevideo* SU475 SH672 *pur-258* F^+, using the technique described by Glatzer *et al.* (1966). Prototrophic hybrids were selected, and these strains were arbitrarily assigned the designation 'TCM', i.e. hybrid strains that possess genic material of *S. typhimurium*, *E. coli* and *S. montevideo* origins. Transductional analyses (Glatzer *et al.* 1966) were carried out on six of the TCM strains to determine the region(s) of hybridity, and one strain was selected for further study. Leucine-requiring strains of the selected TCM hybrid were obtained by the procedure for mutagenesis referred to above. One of the *Leu* strains isolated was crossed with *S. abony* SU468 SH640 *his-1018* Hfr. Prototrophic recombinants derived from this cross were given the designation 'TCMA' (*typhimurium-coli-montevideo-abony*). Six hybrids were selected for study, and the results obtained with two (isolates nos. 4 and 5) are presented in this report. Because of the novelty of these hybrid strains, no attempt has been made to develop a formal nomenclature for them.

(ii) *Analysis of hybrid strains*

The procedures for the transductional analysis of hybrids are presented and discussed in detail in Glatzer *et al.* (1966). Additional information can be found in Armstrong (1967) and Atkins & Armstrong (1969). The following mutant strains of *S. typhimurium* were used as recipients in P22-mediated transduction: *cysC1021; aroB34; cysE396; pyrE125; pdx-4; ilvE16; ilvC8; metE338; rha-51; metF185; argF50; purD55; metA53; purA65; serB10; pyrA81; leuD657 ara-9 gal-1205; leuA124 ara-9;* and *proB25*. In crosses with *rha-51*, the minimal medium was prepared with 0·4 % (w/v) L-rhamnose instead of glucose.

3. RESULTS

(i) *Transductional analyses of hybrids*

Results of analyses by transduction of the hybrid strains are presented in Table 1. These recombination data are presented as 'per cent homology', which is the term that has been adopted to express the recombination observed in a cross between a hybrid and a given *S. typhimurium* marker as a per cent of the recombination observed in a homologous *typhimurium* cross (Glatzer *et al.* 1966).

A low value of homology indicates a locus of foreign origin in the chromosome of the hybrid, and a high value is recognized as a region of *S. typhimurium* origin. Analysis of the parental strain, TC-*ilvA12*, reveals that only the *ilv* region is of *coli* origin in this hybrid. Results obtained with strain TCM (product of the TC hybrid × *montevideo* cross) disclose low values of homology for three regions of the chromosome (*cysE-pyrE*, *ilv-metE* and *metF-argF-purA-metA*). Double and triple incorporations are not unusual observations in *typhimurium* × *montevideo*

Table 1. *Results of analysis of hybrid strains*

Hybrid strain	108* *aroB*	117 *cysE*	117 *pyrE*	120 *pdx*	122 *ilvE*	122 *ilvC*	123 *metE*	127 *rha*	128 *metF*	128 *argF*
TC-*ilvA12*†	98	119	91	108	*8*	*10*	115	114	114	95
TCM	81	*12*	*35*	82	*9*	*5*	*20*	109	*12*	*17*
TCMA no. 4	111	*17*	*35*	111	*13*	*8*	*31*	119	*21*	*17*
TCMA no. 5	129	*16*	*27*	111	*11*	*11*	*28*	117	*27*	*19*

Hybrid strain	129 *purD*	129 *metA*	136 *purA*	1 *serB*	2 *pyrA*	4 *leuD*	4 *leuA*	10 *proB*
TC-*ilvA12*†	120	130	99	96	93	114	92	107
TCM	*11*	*22*	89	101	88	88	85	95
TCMA no. 4	*18*	*36*	132	99	*30*	*11*	*17*	106
TCMA no. 5	*19*	*33*	124	125	*27*	*19*	*22*	106

Values expressed as 'per cent homology'.

* Map position, in minutes (Sanderson 1970). Markers included in a single map position are known to be located on the same transducing fragment.

† TC = *typhimurium-coli* hybrid; TCM = *typhimurium-coli-montevideo* hybrid; TCMA = *typhimurium-coli-montevideo-abony* hybrid.

crosses (Glatzer *et al* 1966). The incorporation of *montevideo* genic material at the *metE* locus, which is adjacent to the *ilv* cluster, raises the possibility that in the TCM hybrid *montevideo* genes have replaced the *coli* genic material that was originally present in the *ilv* cluster of the TC-*ilvA12* chromosome. Transductional analysis does not allow for an evaluation of this possibility. Subsequent to the TCM × *abony* cross, two isolates (TCMA nos. 4 and 5) were obtained that show an incorporation of genic material of *abony* origin in the *pyrA-leu* section of the chromosome. Thus, these TCMA hybrids contain the following chromosomal regions of foreign origin: *cysE-pyrE* (*montevideo*); *ilv* (*coli* or *montevideo*); *metE* (*montevideo*); *metF-metA* (*montevideo*); and *pyrA-leu* (*abony*).

(ii) *Supplementary studies on the hybrids*

When grown in minimal medium on a rotary shaker at 37 °C, the four hybrid strains listed in Table 1 and wild-type *S. typhimurium* LT2 were found to have a generation time of 48 min. Hence, the rate of growth for all of these strains in minimal medium is the same.

To investigate the possibility that the strains produced in this study are stable heterogenotes, rather than hybrids, the following cotransduction test was performed. Strain TCM (derived from an *argF* mutant of TC-*ilvA12*) was used as donor in a cross with *S. typhimurium metF185*. The *metF* and *argF* loci are located on the same transducing fragment (see Table 1). A total of 1100 recombinants obtained from the cross was screened for donor-type recombinants (arginine-requiring strains), and none were detected. If the TCM hybrid were a stable heterogenote (harbouring the mutant *argF* allele of its TC-*ilvA12* parent), a frequency of 20% donor-type recombinants would be predicted (Armstrong, 1967). These cotransduction results provide evidence that an incorporation of *montevideo* material had occurred in the *met-arg* region of the chromosome.

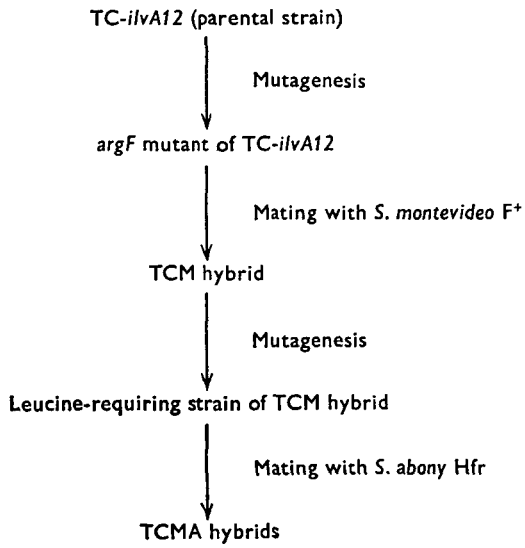

Fig. 1. Diagrammatic presentation of procedure used to produce the hybrid strains.

4. DISCUSSION

This study has involved the production and genetic analyses of hybrid strains whose genomes are derived from several sources. The hybrids with the greatest genetic diversity (TCMA) are those which, by experimental design, were produced to possess an *S. typhimurium* genome containing some genes derived from *E. coli*, *S. montevideo* and *S. abony*. As mentioned previously, it is uncertain whether these TCMA hybrids have *ilv* genes of *E. coli* or *S. montevideo* origin. Transductional analyses show that 6% of the chromosome of each of these TCMA hybrids has been replaced by foreign genetic material. This estimate is probably a minimal one because this type of genetic analysis is limited in scope to the placement of known *S. typhimurium* loci. For example, in Table 1 the chromosomal region between *metE* (123 min) and *rha* (127 min) or *leu* (4 min) and *proB* (10 min) remains unanalysed because of the sparcity of suitable *typhimurium* markers. The

percentage of substituted genic material observed is not unusually high because previous studies on hybrids (Falkow, Rownd & Baron, 1962; Demerec & Ohta, 1964; Glatzer *et al.* 1966) have reported comparable or larger amounts of incorporation. Once again, thoroughness of the transductional analyses performed influences any conclusions made. If the *rha* marker had been omitted in this study, the incorporation of *montevideo* material would have appeared to be much greater than it actually is, i.e. 6 min instead of 2 min for this region of the chromosome. Although the analyses *per se* have not yielded any unusual observations, it is of interest to consider other aspects of the study. A correlation of the results presented in Table 1 with the available knowledge about the loci involved in hybridity (Sanderson, 1970) furnishes the following information. When the metabolic processes of the TCMA hybrids are considered, it is apparent that gene substitution can be directly associated with the syntheses of isoleucine, valine, leucine, methionine, arginine, cysteine, purines and pyrimidines, i.e. a total of seven biosynthetic pathways. In the basically *typhimurium* environment of the hybrid cell, there are 25 biosynthetic enzymes that are not of *typhimurium* origin. This situation is not reflected in the ability of the hybrids to grow because, in minimal medium, these TCMA strains grow as well as wild-type *S. typhimurium*. Substitution of enzymes is not hampering the efficiency of the metabolic processes of the cell. These observations on hybridity and growth serve to illustrate the relatedness among the *coli* and *Salmonella* species used to produce the hybrids. It is recognized, however, that this evaluation is made about hybrids that are obtainable. Other patterns of hybridity may result in the production of non-viable strains, which would never be isolated.

The preceding paragraph emphasizes the relatedness of the four bacterial species used in the study. This relatedness, however, is not evident when recombination in a heterologous cross is examined. On the basis of recombination data, it appears that there is only limited relatedness between the genetic materials of *S. typhimurium* and the other species. Indeed, this low efficiency of recombination (low values of homology) serves as the criterion for the determination of regions of hybridity. These observations have been explained as due to differences in the base sequences (microhomology) of the two DNAs participating in recombination (Zinder, 1960; Demerec & Ohta, 1964). Enomoto & Yamaguchi (1969) have provided evidence that the reduced frequency of recombination in heterologous transduction is due in part to differences in genetic composition of the chromosomal fragments carried by the phage. Therefore, differences (size, genes carried) in comparable transducing fragments produced by *S. typhimurium* and the hybrid strains could account for some of the reduced frequency of recombination noted. An unpublished study from this laboratory has considered this possibility. P22 mediated co-transduction studies that utilized *ilvC* strains that grow suboptimally on a valine supplement as donors in crosses with *ilvE* strains were carried out under homologous and heterologous conditions. The following crosses were done: homologous *typhimurium ilvE* × *ilvC*; homologous *montevideo ilvE* × *ilvC*; *ilvE* (*typhimurium*) × *ilvC* (*montevideo*); and *ilvE* (*montevideo*) × *ilvC* (*typhimurium*). In

each cross, a frequency of 85–90 % wild-type recombinants was scored; thus, the origin of the *ilv* transducing fragment (*typhimurium* or *montevideo*) had no effect on the co-transduction observed. These results provide no evidence that there are any significant differences in the composition of these two *ilv* fragments. These conclusions, based on the analysis of one transducing fragment, cannot be extrapolated to include all fragments. However, the evidence accumulated in our studies on hybrids supports the explanation of differences in microhomology as the major factor involved in the reduced frequency of recombination in heterologous crosses. As proposed by Demerec & Ohta (1964), the degeneracy of the code makes it plausible that analogous genes of these closely related bacterial strains can possess differences in base sequences yet produce enzymes that are very similar, if not identical, in their amino acid sequences.

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