ELECTROPHORETIC STUDY OF *SALMONELLA TYPHIMURIUM*- *SALMONELLA MONTEVIDEO* HYBRIDS¹

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PREVIOUS analyses of *Salmonella typhimurium*-*Salmonella montevideo* hybrids from this laboratory have relied on P22-mediated transduction to determine the region of hybridity in the chromosome of individual hybrid strains (GLATZER, LABRIE and ARMSTRONG 1966; ARMSTRONG 1967). For these studies, the hybrid strains serve as the donor, and *S. typhimurium* auxotrophs as recipients. Transducing fragments that possess genetic material of *S. montevideo* origin display a frequency of recombination that is considerably lower than that observed in a homologous (*S. typhimurium* × *S. typhimurium*) cross; hence, by using appropriate *S. typhimurium* auxotrophs, it is possible to determine the portion(s) of the hybrid chromosome that is composed of genic material of *S. montevideo* origin. Such recombination data have been presented as "percent homology," which is a convenient way of expressing the recombination observed in a cross between a hybrid and a given *S. typhimurium* marker as a percent of the recombination observed in a homologous cross. Low values of homology (5–30%) indicate that the locus under investigation is of *S. montevideo* origin in the chromosome of the hybrid, and, conversely, high values (70–100%) are recognized as a region of *S. typhimurium* origin. A third class of values, termed "intermediate" (percentages that fall between the low and high values for a given locus), are interpreted to mean that: 1) the locus, although not of *S. montevideo* origin, is adjacent to a region that is (as reflected by a decrease in recombination) or 2) a small incorporation of *S. montevideo* genetic material has occurred within the locus. This latter interpretation is more valid if the results are obtained with the locus of hybrid selection. Thus, it is these three classes of percent homology that have served as the basis for the conclusions that have been made about the hybrid strains in our collection.

To obtain a biochemical evaluation of the interpretations derived from the genetic analyses, advantage was taken of the observation of a difference in the electrophoretic mobility of the reductoisomerase activities of *S. typhimurium* and *S. montevideo* when cell-free extracts are analyzed by starch gel electrophoresis. The reductoisomerase is the product of the *ilv C* gene, which is located in the *ilv*

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(isoleucine-valine) cluster of the *Salmonella* chromosome (ARMSTRONG and WAGNER 1964). Because a majority of the hybrids have been selected at the *ilv* region, there is available a collection of strains that is well-suited for an adequate study of hybridity of the *ilv C* locus. The following report is concerned with the analysis of cell-free extracts of *S. typhimurium*-*S. montevideo* hybrid strains by starch gel electrophoresis to determine if the observed mobility of the reductoisomerase activity of an individual strain corresponds to the genetic origin that has been assigned to its *ilv C* locus by transductional analysis.

MATERIALS AND METHODS

Details concerning the production, isolation, and transductional analysis of *S. typhimurium*-*S. montevideo* hybrids can be found in GLATZER, LABRIE and ARMSTRONG (1966). The two wild-type parental strains used in this study as standard references were *S. typhimurium* LT2 and *S. montevideo* SH672.

The procedure utilized for starch gel electrophoresis is essentially that described by BECKMAN and JOHNSON (1964) for studies with *Drosophila*. In this study, 0.1 M borate buffer, pH 7.8, was used in the preparation of 11% gels, which were made 1 mM with respect to both MgSO_4 and β -mercaptoethanol. The bridge solution was 0.3 M borate buffer, pH 8.1.

Bacterial strains were grown in minimal medium (ARMSTRONG and WAGNER 1964) on a rotary shaker at 37°C. When mid-log phase of growth was attained, the cells were harvested by centrifugation at $700 \times g$ for 10 min in a Sorvall model RC-2 refrigerated centrifuge. The cells were washed with 0.1 M potassium phosphate buffer, pH 7.4, recentrifuged, resuspended in the above-mentioned phosphate buffer (at a ratio of 3 ml per g wet weight of cells), and subjected to sonic disruption (Branson Sonifier). The sonified cell preparation was centrifuged at $3400 \times g$ for 10 min, and the resultant supernatant fraction served as the cell-free extract. To apply the sample to the gel, squares (7 mm) of Whatman # 3 MM filter paper were dipped into the cell-free extracts and inserted in a slit that had been cut across the width of the gel (at a distance of 5 cm from the base). Twelve to fourteen such filter paper squares were used per gel. Electrophoresis was carried out at room temperature for a period of 5 hr at a voltage gradient of 20 per cm. To keep the gels cool, a pan of ice was placed on the glass plate that covered the gel.

The assay for reductoisomerase activity was carried out according to the procedure described in ARMSTRONG and WAGNER (1961). This assay capitalizes on the fact that the reduced form of triphosphopyridine nucleotide (TPNH) fluoresces under ultraviolet light; whereas, the oxidized form (TPN) does not. Hence, the reaction mixture containing TPNH is spread on the surface of a gel that is illuminated under a long wave ultraviolet light (360 m μ). Areas of reductoisomerase activity (TPNH $\rightarrow$ TPN) appear as dark bands against a bright background. A photograph of a developed gel can be found in ARMSTRONG and WAGNER (1961). Each hybrid utilized was subjected to a minimum of three separate electrophoretic analyses, and each gel was scored independently by no fewer than three individuals.

RESULTS

Figure 1 depicts a tracing of a photograph of bands of reductoisomerase activity as they appear on the starch gel. As seen, *S. typhimurium* activity migrates a greater distance from the origin than does that of *S. montevideo* and, for simplicity in labeling, the *S. typhimurium* migration pattern is referred to as "high," and the *S. montevideo* pattern as "low."

A representative sample of the results obtained with hybrid strains is presented in Table 1. The results of transductional analysis of the *ilv* region and the closely linked *met E* locus (ROTH and SANDERSON 1966) are listed, as well as the electro-

FIGURE 1.—Tracing of a photograph of bands of reductoisomerase activity as they develop on a gel. The gel was photographed with Kodak Tri-X film through the magnifying viewer of a Burton ultraviolet lamp.

phoretic patterns observed for reductoisomerase activities. Three of the strains listed represent the patterns of hybridity routinely observed in this region of the chromosome (TM-*ilv* E32 = *ilv* cluster only; TM-*met* E50 = *met* E locus only; and TM-*ilv* D31 = *ilv* and *met* E loci). The fourth strain (TM-*met* F18) is representative of a hybrid strain with no incorporation of *S. montevideo* material in this region. The electrophoretic mobility of the reductoisomerase activity in each of these hybrids, as seen in Table 1, corresponds to that of the species origin assigned the *ilv* C locus by genetic analysis. Similar results were obtained in the analysis of a total of 26 such hybrids.

Listed in Table 2 are the results obtained with hybrid strains that yield an intermediate value for percent homology for the *ilv* C locus. In the case of TM-*met* E11, the *ilv* C locus is the only *ilv* locus that shows an intermediate value, and this is interpreted to mean that although the *ilv* C locus is of *S. typhimurium* origin, it is located next to a region of *S. montevideo* origin (*met* E locus). These results were used as evidence of the orientation of the *ilv* cluster with respect to

TABLE 1

Genetic and electrophoretic results on TM hybrids representing four types of hybridity

Representative strain	<i>ilv</i> E	<i>ilv</i> D	<i>ilv</i> A	<i>ilv</i> C	<i>met</i> E	migration of reductoisomerase
TM- <i>ilv</i> E32*	6.2†	5.0	6.5	8.0	89	low
TM- <i>met</i> E50	75	78	124	108	26	high
TM- <i>ilv</i> D31	5.4	2.9	3.3	2.6	11	low
TM- <i>met</i> F18	117	94	119	86	102	high

Values expressed as "percent homology" (see text paragraph one).

* TM-*ilv* E32 = 32nd. *S. typhimurium*-*S. montevideo* hybrid isolated by selection at the *ilv* E locus of the *S. typhimurium* parent.

† Italicized values indicate loci of *S. montevideo* origin.

TABLE 2

Results on TM hybrids with intermediate values for percent homology at the ilvC locus

Hybrid strain	<i>ilv E</i>	<i>ilv D</i>	<i>ilv A</i>	<i>ilv C</i>	<i>met E</i>	migration of reductoisomerase
TM- <i>met E11</i>	99	91	83	60	17	high
TM- <i>ilv E34</i>	33	27	48	50	100	high
TM- <i>ilv D27</i>	70	58	57	55	..	high

Values expressed as "percent homology" (see text paragraph one).

the *met E* locus (GLATZER, LABRIE and ARMSTRONG 1966). Hybrids TM-*ilv E34* and TM-*ilv D27* represent strains with small incorporations of *S. montevideo* material in the region of hybrid selection; the results of which can affect the recombination frequency observed with all *ilv* loci. In these three hybrids, it can be concluded that the *ilv C* locus in each is of *S. typhimurium* origin. As listed in Table 2, the reductoisomerase activities, upon electrophoretic analysis, exhibit a mobility comparable to that of *S. typhimurium* (high). Two other strains with patterns of hybridity similar to those of TM-*ilv E34* and TM-*ilv D27* also produced analogous results.

DISCUSSION

This study was initiated in an attempt to obtain biochemical data that could be correlated with the results of genetic analyses that had previously been carried out on *Salmonella typhimurium*-*Salmonella montevideo* hybrids. A discernible difference in the electrophoretic mobility of the reductoisomerase (product of the *ilv C* gene) of the two parental *Salmonella* species served as the basis for a qualitative test for the study. From the available collection of hybrids, a total of thirty-one strains, selected for genetic information pertaining to the *ilv C* locus, were analyzed by starch gel electrophoresis to determine the electrophoretic mobility of the reductoisomerase activity of each strain. As presented in Tables 1 and 2, the interpretations made about high, intermediate, and low values of percent homology for the *ilv C* locus were supported by the electrophoretic results. The observed mobility, in each strain, was faithful to that of the species origin that had been assigned its *ilv C* locus. Although this biochemical study is limited to an analysis of the product of one gene, it nevertheless provides evidence that the analysis of hybrids by transduction does yield valid information about the regions of hybridity in the *Salmonella* chromosome.

SUMMARY

The migration patterns observed for the reductoisomerase activities of 31 *S. typhimurium*-*S. montevideo* hybrid strains, when cell-free extracts were subjected to starch gel electrophoresis, correlate with the species origin assigned to individual *ilv C* loci by genetic analysis. Thus, these data support the validity of previous interpretations that have been made concerning regions of hybridity in the *Salmonella* chromosome.

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