EPISOMIC TRANSFER BETWEEN *SALMONELLA* TYPHOSA AND *SERRATIA* MARCESCENS

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Received March 23, 1961

THE isolation of a culture of *Salmonella typhosa*, strain ST-2, which possessed the unusual characteristic of utilizing lactose (*lac*⁺) was recently reported (BARON, CAREY and SPILMAN 1959). When this strain was grown in mixed culture with many species and strains of *lac*⁻ *Escherichia*, *Salmonella* and *Shigella*, it transferred *lac*⁺ with relatively high efficiency (10^{-3} per ST-2 cell). The recipient cells which received the *lac*⁺ character were then able to transmit *lac*⁺ to other *lac*⁻ strains. Kinetic studies using the blender technic of WOLLMAN and JACOB (1955) revealed that *lac*⁺ and donor ability were transferred simultaneously and almost immediately after strain ST-2 and the recipient cells were mixed. Strain ST-2 and hybrids derived from this strain were relatively stable although *lac*⁺ and donor ability were lost spontaneously at low frequencies. The ability to transfer *lac*⁺, however, was not eliminated by treatment of ST-2 cells with acridine orange under the conditions described by HIROTA (1960) which were suitable for the removal of the fertility factor, F, from *Escherichia coli*, K-12. Cells receiving *lac*⁺ and donor ability by conjugation with strain ST-2 did not show the inheritance of any other genetic characters. Likewise, F⁻ cells of *E. coli* K-12 receiving the characteristics of strain ST-2 did not acquire the ability to transfer their chromosomal markers to other F⁻ strains. While the *lac* locus injected by strain ST-2 into a recipient may undergo homologous pairing with the chromosome, replication has been found to take place independently of the host's genome (FALKOW and BARON 1961). It is not necessary to assume that the *lac*⁺ character itself is transmissible, more likely it is carried by an unknown transmission agent probably similar to the F factor of *Escherichia*. The process observed for strain ST-2 would seem to involve the incorporation of a small segment of genetic material into a transmission agent resulting in a single unit of replication. This genetic element can then be transferred to other strains of bacteria where it may replicate independently of the host genome. The characteristics of strain ST-2, therefore, correspond closely to those of organisms harboring episomic elements (JACOB and WOLLMAN 1958). This class of genetic factors

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are not essential constituents of the cell, and when present, they can behave as an independent unit or be attached to the host chromosome.

While the process observed for strain ST-2 shows similarities to specialized transduction by phage (JACOB and WOLLMAN 1959), conjugation, a characteristic of sexual transfer in bacteria, has been found to be an essential requirement for transfer of the episome. At least two mechanisms act to limit genetic transfer by conjugation. The first of these is differences in mating polarities. The second is divergent structure of genetic material affecting chromosomal pairing and integration. Some of our experiments (to be reported in greater detail elsewhere) have demonstrated that mating polarities essential for the transfer of chromosomal elements in *E. coli* K-12 do not necessarily effect the transfer of the episomic element of strain ST-2. Moreover, the autonomous replication of the episome would seem to overcome some of the difficulty affecting incorporation of the transferred genetic material. It was decided, therefore, to study the possibility of episomic transfer between strain ST-2 and strains of other genera for which there is no positive sign of a common system of mating polarity and which differ appreciably in the base composition of their desoxyribonucleic acid (DNA).

Serratia marcescens has been reported to differ significantly from *Salmonella*, *Escherichia* and *Shigella* in the base composition of its DNA (BELOZERSKY and SPIRIN 1960). In addition, repeated attempts to demonstrate chromosomal transfer between Hfr and F⁺ *Escherichia* or *Salmonella* and *Serratia* have been unsuccessful in this laboratory. We therefore, selected this species to test with strain ST-2.

A stable *lac*⁻ prototrophic strain of *S. marcescens*, SM-6, was grown in mixed culture with strain ST-2. Since strain ST-2 requires cystine and tryptophan for growth, minimal lactose agar was used to detect hybrids. *lac*⁺ progeny, otherwise identifiable as *Serratia*, were observed at the low frequency of 4×10^{-8} per donor cell. Matings between strain ST-2 and *lac*⁻ *S. marcescens*, strains USC, and A-12 (nonpigmented) gave essentially the same results.

While bacterial DNA preparations have proved to be highly homogeneous in base composition, nevertheless, measurable compositional heterogeneity does occur (MARMUR and DOTY 1959). In order to be certain that the *Serratia* strains used in our experiments did, indeed, differ significantly from strain ST-2, we determined their guanine-cytosine (GC) content by estimation of the thermal denaturation temperature of their DNA (MARMUR and DOTY 1959). The GC content of these *Serratia* strains was found to be 58 percent as compared to a figure of 50 percent for strain ST-2 and *E. coli* K-12. These estimations are in good agreement with those previously reported in the literature (MARMUR and DOTY 1959; BELOZERSKY and SPIRIN 1960).

In order to simplify the selection and further purification of the *Serratia* hybrids, a number of streptomycin resistant mutants of strain SM-6 were isolated by plating dense suspensions of cells on nutrient agar containing 600 µg/ml of streptomycin. Some of these streptomycin resistant (S^r) clones showed slight increases in fertility with strain ST-2, while one clone, SM-6-S^r-11, mated at a

frequency of 10^{-4} per ST-2 cell. This clone was unchanged in GC content and still failed to yield progeny when plated with F⁺ and Hfr cultures of *E. coli*.

lac⁺ hybrids of *Serratia* were picked and tested for their ability to transfer the *lac*⁺ determinant to *lac*⁻ strains of *Salmonella*, *Shigella* and *Escherichia*. All of the hybrids tested were able to transfer *lac*⁺ although the frequencies observed in these experiments varied considerably. Some of the *Serratia* progeny acted as donors only at low frequency (10^{-8} per donor cell), but the majority were competent donors at a frequency of about 10^{-5} . A summary of the mating experiments is shown in Table 1.

These results should be considered in view of the concept developed recently (LANNI 1960; MARMUR and SHILDKRAUT and DOTY, in preparation) regarding genetic homology in relation to the DNA base ratios of the microorganisms involved. In all instances of genetic transfer between bacteria established to date, the organisms involved have similar DNA base ratios. This infers that strains differing appreciably in their DNA base ratios should not be genetically homologous. While this hypothesis may hold true for the integration of chromosomal DNA, our data indicate that small, autonomous units of genetic material can be transferred between organisms with dissimilar DNA base ratios. This observation has been extended with organisms carrying other episomic elements. We have observed that a strain of *E. coli* harboring an episome, F-*lac*, consisting of the fertility factor and *lac* locus (JACOB and ADELBERG 1959) and a strain of *Salmonella* carrying an episome conferring resistance to several antibiotics (MITSUHASHI, HARADA and HASHIMOTO 1960) can transfer their episomic elements to *Serratia*.

The divergence between the DNA base ratios of the original strains carrying episomes and *Serratia* led us to consider the possible demonstration of molecular heterogeneity in DNA prepared from *Serratia* hybrids. Using the cesium chloride gradient technic (MESELSON, STAHL and VINOGRAD 1957) it was found that the DNA isolated from the *Serratia* and *Salmonella* parental cultures had different buoyant densities estimated from their banding positions. In contrast, the DNA isolated from the *lac*⁺ *Serratia* hybrids gave rise to two bands in the analytical ultracentrifuge, one corresponding to the density observed for the

TABLE 1

Episomic transfer between Salmonella typhosa, ST-2 and Serratia marcescens

<i>lac</i> ⁻ recipient organism	Percent	Frequency of hybrid formation selection for <i>lac</i> ⁺ with		
		<i>S. typhosa</i> ST-2 (50%)	<i>E. coli</i> W1895 (Hfr) (50%)	<i>Serratia</i> * SM-6 <i>lac</i> ⁺ (58%)
<i>Shigella dysenteriae</i>	(50)†	5×10^{-3}	1×10^{-4}	1×10^{-5}
<i>Salmonella typhimurium</i> LT-7	(50)	2×10^{-3}	1×10^{-7}	2×10^{-5}
<i>Escherichia coli</i> K-12	(50)	1×10^{-3}	1×10^{-3}	2×10^{-5}
<i>Serratia marcescens</i> SM-6	(58)	4×10^{-8}	0	
<i>S. marcescens</i> SM-6-S ^r -11	(58)	1×10^{-4}	0	5×10^{-5}

* Derived from a cross with strain ST-2.

† Percentage figures correspond to the guanine-cytosine content of the organisms.

parental *Serratia* and another, comprising about one percent of the total DNA, corresponding to the density observed for the *Salmonella* parent (MARMUR, ROWND, FALKOW, BARON, SCHILDKRAUT and DOTY 1961). At present, we assume that the small amount of DNA with the buoyant density of the *Salmonella* parent represents the transferred episome.

SUMMARY

The transfer of lactose utilization and donor ability from *Salmonella typhosa*, strain ST-2, to *Serratia marcescens* via an episomic mechanism is described. This transfer is of particular significance because of the divergent DNA base ratios of the parental strains and the lack of a demonstrable common system of mating polarity.

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