CHROMOSOME TRANSFER BETWEEN ESCHERICHIA COLI HFR STRAINS AND PROTEUS MIRABILIS

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Different genera classified in the Enterobacteriaceae have been readily hybridized by the conjugal transfer of episomic elements. *F-lac*, an F-merogenote incorporating the lactose operon of *Escherichia coli*, and other episomes (plasmids) have been transmitted between *Escherichia*, *Salmonella*, and *Shigella*.[1-4] Such episomal infections have not been limited to these three genera which have the same average guanine plus cytosine content (50% GC), but have been extended to genera whose DNA base compositions differ significantly from that of *E. coli* and, in two instances, have crossed family lines. Thus, *F-lac* and other episomes have been transferred by conjugation to *Serratia marcescens* (58% GC),[5, 6] *Proteus* species (39% GC),[7-9] *Vibrio comma*,[10] and *Pasteurella pestis*.[11]

The use of *E. coli* Hfr strains to mediate intergeneric hybridizations has also been reported. Chromosomal markers have been transferred by mating *E. coli* K-12 Hfr donors with *Salmonella typhimurium*,[12] *Salmonella typhosa*,[13] and *Shigella* species.[14, 15] Unlike episomes which are relatively promiscuous in their transfer among Enterobacteriaceae,[7] the transfer of chromosome from *E. coli* Hfr donors has been reported only for these two genera whose DNA is of the same average GC composition as that of *E. coli*. This transfer occurs usually at lower frequencies than that found in *E. coli* and generally results in the formation of unstable partial diploids.[12-14] There is, however, no *a priori* reason precluding chromosome transfer to genera with DNA of different base composition. The present communication describes experiments involving chromosome transfer from *E. coli* Hfr strains (50% GC) to *Proteus mirabilis* (39% GC) and the genetic and physicochemical characterization of the resulting hybrids.

Materials and Methods.—Bacterial strains: WR 2001 and WR 2002, two Hfr derivatives of *E. coli* K-12, were employed as donor strains. A nonswarming *P. mirabilis* strain, WR 11, served as the recipient. Also employed were strain WR 12, a *Proteus mirabilis* carrying an *F-lac* episome, and *E. coli* K-12 F^- strain WR 2300. The characteristics of these strains are presented in Table 1.

Bacteriophage: R-17, a male-specific RNA phage,[16] was grown on WR 2001 and used to test for male properties in *Proteus* hybrids.

Media: Antibiotic medium #3 (Penassay broth, Difco), brain heart infusion (Difco), and meat-extract agar were used for routine cultivation of organisms. The composition of minimal medium, used for the selection of recombinants, has been described previously.[12] Fermentation characters were scored on MacConkey indicator medium, consisting of MacConkey agar base (Difco) supplemented with 20 per cent solutions of appropriate carbohydrate (20 ml/liter). Urea broth (Difco) was employed in the differentiation of *Proteus* and *E. coli*. One-ml amounts of the urea broth were inoculated with cells and scored after overnight incubation as urease-positive (*Proteus*) or negative (*E. coli*).

TABLE 1

BACTERIAL STRAINS

Strain no.	Original designation	Characteristics
WR 2001	W1895 Hfr-3	*E. coli* K-12 Hfr, met^-, str^s *O*-lac^+-pro^+-ara^+ . . . . . gal^+
WR 2002	Hfr H	*E. coli* K-12 Hfr, str^s *O*-ara^+-lac^+-gal^+
WR 2300	A 252	*E. coli* K-12 F^-, lac^- (deletion) str^s
WR 11	—	*P. mirabilis*, nic^-, lac^-, ara^-, gal^- str^r
WR 12	PM-1-*F-lac*	*P. mirabilis*, nic^-, lac^-, ara^- *F*-lac^+ str^s

Met, methionine; *pro*, proline; *nic*, nicotinic acid; *lac*, lactose utilization; *ara*, arabinose utilization; *gal*, galactose utilization; *str*, streptomycin; *O*, origin.

Preparation of DNA and density-gradient centrifugation: The extraction and purification of bacterial DNA was accomplished by the method of Marmur.[17] The CsCl density-gradient techniques for observing *Proteus* and "satellite" DNA have been described previously.[7, 9]

Results.—Isolation of Lac$^+$ Proteus hybrids: The results of mating WR 2001 or WR 2002 with the WR 11 recipient indicated that Lac$^+$ *Proteus* hybrids are recovered at very low frequencies as compared to episome transfer to *Proteus*. In a typical experiment, 0.1 ml of the Hfr donor (containing 10^9 cells) and 0.1 ml of the recipient (with about 5×10^9 cells) were plated separately (for controls) and together (for mating) on minimal lactose medium supplemented with streptomycin, nicotinic acid, and casein hydrolysate. Under such conditions, lactose utilization served as the selective marker and streptomycin counterselected the Hfr donor. After two to five days' incubation at 37°C, a small number of colonies usually was present on the mating plates; these were purified by streaking on plates of the original selective medium.

Some of these colonies were not Lac$^+$ *Proteus* hybrids, but represented either streptomycin-resistant mutants of the Hfr parent or mixed growth of the original parents as a result of cross-feeding. These colonies could be readily excluded from further analysis. On repeated purifications and the examination of colonial morphology, *Proteus* hybrids yielded smooth-textured colonies, whereas those of *E. coli* K-12 appeared granular and irregular. The identifications made through such observations were confirmed by the urease test. By application of these methods, Lac$^+$ *Proteus* hybrids were recovered from matings with either WR 2001 or WR 2002 donors.

Characterization of Lac$^+$ Proteus hybrids: Instability of the Lac$^+$ character: Three Lac$^+$ hybrids were chosen for further analysis. One of these (WR 13) was derived from a mating with WR 2001, whereas the other two (WR 14, WR 15) resulted from a cross with WR 2002. After repeated platings of Lac$^+$ derivatives on MacConkey lactose agar, all three strains were found to be unstable for the Lac$^+$ character, segregating Lac$^-$ clones. This finding suggested that these hybrids were unstable Lac$^+$ heterozygous diploids consisting of a chromosomal segment unassociated with the F factor. It was essential, however, to consider the alternative explanation, namely, that the hybrids were harboring an *F-lac*$^+$ episome which may have been acquired during mating with the *E. coli* Hfr donor strains. To re-

solve these alternatives, the hybrids were tested for male properties known to be associated with the presence of the F factor.

Donor ability and male phage sensitivity: Of the three hybrids, only WR 13 could be tested for donor ability. This strain and for comparison WR 12, a *Proteus F-lac*$^+$ strain, were mixed with an *E. coli* K-12 *lac*$^-$ deletion mutant (WR 2300) on minimal lactose agar. Under these conditions, lactose was the selective marker and the absence of nicotinic acid counterselected the *Proteus* strains. No transfer of Lac$^+$ from WR 13 was detected, whereas the expected low levels of Lac$^+$ transfer (i.e., *F-lac*) from WR 12 occurred. Moreover, as further evidence against male properties in strain WR 13, no F-pili[18] could be detected by electron-microscopic examination.[19] Such structures are readily seen on WR 12.[18] Strains WR 14 and WR 15 could not be tested for donor ability, having lost their requirement for nicotinic acid, the only counterselective marker available.

Another test employed for determining male properties was the assay for sensitivity to the male-specific RNA phage, R-17.[16] The hybrids WR 13, WR 14, WR 15, and for comparison WR 12 (known to propagate the male phage[20]) were grown at 37°C overnight in Penassay broth supplemented with $CaCl_2$ (final conc. $M/500$). Phage R-17 was added (input ratio about one plaque-forming unit/1000 bacteria) and the mixtures were incubated without shaking at 37°C overnight. After centrifugation, the supernatant fluids were assayed by the soft agar method for plaque-forming units of R-17 using WR 2001 as host. No increase in the R-17 titer was detected in the case of hybrids; under similar conditions the R-17 titer increased 500–1000-fold with the male WR 12 strain.

Characterization of DNA from Proteus hybrids: Examination of the DNA from hybrids in a CsCl density gradient showed the presence of an additional DNA component. Lac$^+$ hybrid strain WR 13 was grown overnight in brain-heart infusion broth and the DNA was extracted from washed cells by the Marmur technique.[17] The DNA was then centrifuged to equilibrium in a CsCl density gradient. In Figure 1 the upper tracing is DNA of the parent *Proteus* strain WR 11 before mating. This DNA bands as a single homogeneous component characteristic of *Proteus* DNA. The lower tracing is of DNA extracted from the hybrid WR 13. This DNA contains a main component identical to the parent DNA and an additional or "satellite" band having a density of 1.710 gm/cm^3 which is the same density as *E. coli* DNA. Treatment with DNase eliminated both bands, and heat denaturation increased the density of both bands by 0.015 gm/cm^3. Thus, it was concluded that the satellite band is native DNA with the same GC content as the DNA of the *E. coli* donor. Since the area under the curve is proportional to the DNA concentration, it is possible to estimate the relative amount of DNA in the satellite band. In the WR 13 hybrid about 6 per cent of the total DNA isolated from the cells is in the satellite band. A similar additional band has been observed when the *F-lac*$^+$ episome is transferred to *Proteus* strains.[7] However, *F-lac* episomal DNA also having a 50 per cent GC content amounts to about 3 per cent of the total DNA.[7]

Backcrosses of the WR 13 hybrid: Using procedures similar to those described previously, the WR 13 hybrid was backcrossed with WR 2001 or WR 2002 for the selective markers Ara$^+$ or Gal$^+$. Although no detailed quantitative studies have been performed, recombination frequencies increased about tenfold, suggesting that

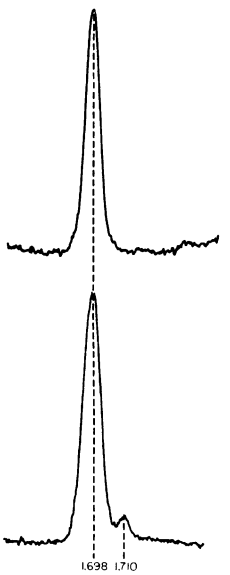

FIG. 1.—The upper curve is a densitometric tracing of the banding pattern of DNA from the parent *Proteus* strain WR 11 in a CsCl density gradient. The lower curve is a tracing of DNA from the *Proteus* Lac$^+$ hybrid (strain WR 13). The main band has the same density as the parent *Proteus* and, in addition, there is a satellite DNA band at a density of 1.710 gm/cm^3 which corresponds to the density of *E. coli* DNA.

the hybrid acts as a better recipient than the original *P. mirabilis* parent. An analysis of five such Ara$^+$ hybrids revealed that the Ara$^+$ marker was markedly unstable, i.e., Ara$^-$ Lac$^+$ and Ara$^-$ Lac$^-$ clones were being segregated; Ara$^+$ Lac$^-$ segregants were not observed. As in the case of the Lac$^+$ diploids, sensitivity to R-17 was not detected in these backcrossed hybrids. Similar results were observed in the case of the Lac$^+$ Gal$^+$ hybrids.

The satellite bands of Lac$^+$ Ara$^+$ (WR 16), Lac$^+$ Gal$^+$ (WR 17), and Lac$^+$ Ara$^+$ Gal$^+$ (WR 18) hybrids are considerably larger than the Lac$^+$ hybrids. They contain as much as one fourth of the total DNA isolated from the cell. Figure 2*a* shows the tracing of the DNA bands of the Lac$^+$ Gal$^+$ hybrid and Figure 2*b* shows

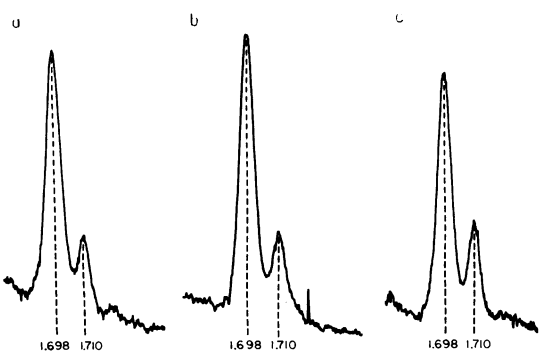

FIG. 2.—(*a*) A tracing of the DNA from the *Proteus* Lac$^+$ Gal$^+$ hybrid; (*b*) from the *Proteus* Lac$^+$ Ara$^+$ hybrid; (*c*) from the *Proteus* Lac$^+$ Ara$^+$ Gal$^+$ hybrid. The DNA extracted from these hybrids all had additional DNA bands that are the same density as *E. coli* DNA. From the relative area under the curves, estimates of the percentage of DNA in the satellite bands have been made (see Table 2).

the Lac$^+$ Ara$^+$ hybrid. The amount of DNA in the satellite band of the Lac$^+$ Ara$^+$ hybrid is 20 per cent of the total DNA extracted from the cell. The amount of DNA in the satellite band of the Lac$^+$ Gal$^+$ hybrid represents 16 per cent of the total DNA. Likewise, the Lac$^+$ Ara$^+$ Gal$^+$ hybrid (Fig. 2*c*) is about 26 per cent of the total DNA. All three have an average density of 1.710 gm/cm^3 corresponding to a GC content of 50 per cent. Segregants that have become Lac$^+$ Gal$^-$ and Lac$^+$ Ara$^-$ have bands that contain about 6 per cent of the total DNA similar to the original Lac$^+$ hybrids. Segregants that are Lac$^-$ Ara$^-$ and Lac$^-$ Gal$^-$ contain no observable satellite band. These results are summarized in Table 2.

Discussion.—Unstable Lac$^+$ *Proteus* hybrids have been recovered from matings between *E. coli* Hfr donors and *Proteus mirabilis* recipients. Their lack of sensitivity to the R-17 male-specific phage and, in the case of WR 13, the absence of donor ability and F-pili indicate that these hybrids do not harbor *F-lac* episomes. Although it is still conceivable that these strains contain defective *F-lac* merogenotes, the most likely explanation is that they are unstable heterozygous Lac$^+$ diploids. This interpretation is not unusual because intergeneric matings between *E. coli* Hfr donors and *Salmonella* recipients also are known to yield unstable partial diploids.[13]

Since *E. coli* and *Salmonella* have the same GC content, physicochemical study of such diploids required the annealing of the DNA from the *Salmonella* diploid with N^{15}-deuterated *E. coli* DNA. The amount of DNA formed in the "molecular hybrid" band reflected the amount of *E. coli* DNA present in the *Salmonella* hybrid.[22] On the other hand, differences in over-all base composition allow the direct observation of *E. coli* genes in *Proteus mirabilis*. Examination of the DNA of the *Proteus* hybrids shows physically recognizable DNA bands having the buoyant density typical of *E. coli* DNA. As more *E. coli* characters are transferred to *Proteus* by backcrossing of a Lac$^+$ hybrid, the amount of DNA in the satellite band appears to be a sum of the additional segments. By subtracting the 6 per cent satellite of the Lac$^+$ diploid from the per cent satellite DNA in the Lac$^+$ Ara$^+$ and Lac$^+$ Gal$^+$ diploids, values of 14 per cent for the Ara$^+$ segment and 10 per cent for the Gal$^+$ segment have been estimated. The Lac$^+$ Ara$^+$ Gal$^+$ diploid would be expected to have a satellite containing about 30 per cent of the total DNA. The observed size (26%), however, is somewhat less, probably because of the segregation of characters by some cells in the population employed for DNA extraction. Thus,

TABLE 2

AMOUNT OF DNA IN THE SATELLITE BAND OF *Proteus* HYBRIDS

Strain	Characteristics	Main band GC content (%)	Satellite Band GC content (%)	Satellite Band Amount* (%)
WR 11	Lac$^-$ *Proteus* parent	39	—	Nd†
WR 13	Lac$^+$ hybrid	"	50	6
WR 16	Lac$^+$ Ara$^+$ hybrid	"	50	20
	Lac$^+$ Ara$^-$ segregant	"	50	6
	Lac$^-$ Ara$^-$ segregant	"	—	Nd
WR 17	Lac$^+$ Gal$^+$ hybrid	"	50	16
	Lac$^+$ Gal$^-$ segregant	"	50	6
	Lac$^-$ Gal$^-$ segregant	"	—	Nd
WR 18	Lac$^+$ Ara$^+$ Gal$^+$ hybrid	"	50	26

* Per cent of total DNA extracted.
† None detected.

it appears that the amount of satellite is the sum of the chromosomal segments added by conjugation.

A large satellite band, containing 8 per cent of the total DNA, has been reported for the F′-13 episome[7] which carries the genes for lactose utilization and, in addition, the information for the synthesis of alkaline phosphatase, adenine, and T6 receptor. Some of the diploids described here have satellite bands which are considerably larger. An analysis correlating the size of these diploid bands with the total number of genetic markers has not yet been completed.

The low frequency of hybrid recovery and their unstable partial diploid character are presumably an expression of the lack of homology between *E. coli* and *Proteus* DNA as shown by molecular hybridization experiments.[23] The lack of homology between these strains is indicated by the diploid state, i.e., inefficient pairings in the *lac* regions without (or at least not detected) integration of the genes. Similar findings in hybrids between *E. coli* and *Salmonella* have been interpreted as an expression of poor molecular homology.[21]

Preliminary experiments backcrossing WR 13 with WR 2001 and WR 2002 for other characters suggest that this hybrid acts as a better recipient than the original strain, WR 11. Similar results reported for backcrosses of *Salmonella* hybrids[13, 23] have been explained in two ways. The fortuitous isolation of a high-frequency recipient mutant of *S. typhimurium* without prior mating experience[12] suggested that the low frequency of hybrid recovery in *E. coli* × *S. typhimurium* matings represented the selection of rare competent mutants from otherwise sterile populations. On backcrossing, these would be expected to yield higher recombination frequencies. In *S. typhosa*, however, the increased hybrid recipient ability has been reported to be due to the presence of integrated *E. coli* genetic material rather than the selection of high-frequency females.[24] The existence of such a phenomenon in *Proteus* hybrids is presently being investigated, with a consideration of the above propositions, as well as the possibility of host modification and restriction.

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