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TRANSFER OF EPISOMIC ELEMENTS TO *PROTEUS*

II. NATURE OF *LAC*+ *PROTEUS* STRAINS ISOLATED FROM CLINICAL SPECIMENS

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ABSTRACT

FALKOW, STANLEY (Walter Reed Army Institute of Research, Washington, D.C.), J. A. WOHLHIETER, R. V. CITARELLA, AND L. S. BARON. Transfer of episomic elements to *Proteus*. II. Nature of *lac*+ *Proteus* strains isolated from clinical specimens. J. Bacteriol. **88**:1598–1601. 1964.—Strains of *Proteus mirabilis* exhibiting the unusual property of utilizing lactose (*lac*+) have been reported in clinical material. A genetic examination discloses that the *lac*+ determinants in these *Proteus* strains are associated with an infectious element, P, which is distinct from the sex factor of *Escherichia coli* K-12. The composite genetic element, P-*lac*, is readily transmissible to other enteric species and possesses properties which conform to those of an episomic element of the transfer variety. CsCl density-gradient studies of deoxyribonucleic acid (DNA) extracted from *lac*+ *P. mirabilis* indicate that the P-*lac*+ element did not arise in this species, but was acquired from an organism possessing a markedly different DNA base composition.

Naturally occurring *Proteus* species capable of utilizing lactose (*lac*+) have been sufficiently infrequent to warrant special mention when encountered. Recently, however, Sutter and Foecking (1962) isolated a large number of *lac*+ *Proteus* strains from clinical material. Since the *lac*+ strains isolated by these workers represented all members of the genus, it seemed unlikely that a simple mutational event could account for the sudden appearance of *lac*+ *Proteus* species, but that some genetic transfer mechanism was involved. In this communication, we report that the *lac*+ determinants carried by *Proteus* are associated with an infectious element and are readily transmissible to other enteric organisms.

MATERIALS AND METHODS

Bacterial strains. The *lac*+ and *lac*− strains of *Proteus mirabilis* employed in this study were isolated from clinical material and sent to us by V. Sutter. The strains of *Escherichia coli* K-12, *Salmonella typhosa*, and *Serratia marcescens* have been described previously (Falkow and Baron, 1962).

The media, mating procedures, and methods for deoxyribonucleic acid (DNA) isolation and CsCl density-gradient centrifugation have been reported in a previous communication (Falkow et al., 1964).

RESULTS

Two *P. mirabilis* strains, PML and PML_3, received from V. Sutter did not differ phenotypically from the usual representatives of this species (Breed, Murray, and Smith, 1957), other than in their ability to ferment lactose. On MacConkey Agar (Difco) there were no discernible *lac*− segregants. When, however, these *lac*+ strains were mated with *lac*− *Escherichia* and *Salmonella*, *lac*+ hybrid clones were detected at a frequency of 4×10^{-4} per *Proteus* cell in the mating mixture. The *lac*+ hybrids purified from these matings were tested with a variety of *lac*− organisms, including *Proteus*, for their ability to act as donors for *lac*+. All hybrids tested proved to be donors, and Table 1 shows the results of a series of matings performed with a *lac*+ hybrid of *S. typhosa* 643.

Escherichia and *Proteus lac*+ strains are quite stable, whereas *Salmonella* and *Serratia* hybrids segregate *lac*− clones at a frequency of 10^{-4} – 10^{-5}. All hybrid strains, as well as the original *lac*+ *Proteus* strains, however, may be "cured" of their *lac*+ and donor property to some extent by growth in broth containing acridine orange (Table 2). Interrupted mating experiments show that *lac*+ and donor ability are transferred simultaneously within 10 min after mating suspensions are mixed.

These data are consistent with the view that the *lac*$^+$ determinants in *Proteus* are associated with an infectious element with which they replicate and are transmitted as a single unit. The properties of this unit conform to that of an episomic element of the transfer variety, as defined by Campbell (1962). We have termed this unit P-*lac*$^+$.

The P-*lac*$^+$ element may be transferred to cultures carrying either the autonomous or integrated sex factor, F, of *E. coli* K-12, although the frequency of transfer is generally of a lower order than to F$^-$ strains (Table 1). The transfer of P-*lac*$^+$ is repressed in cultures carrying F to a frequency of 10^{-5} to 10^{-6} per donor cell; the characteristic functions of F, however, remain unaltered. On the other hand, F$^-$ (P-*lac*$^+$) *E. coli* K-12 cells exhibit a low frequency of chromosomal determinant transfer (Table 3). F$^-$ (P-*lac*$^+$) cells are not agglutinated by F$^+$ antiserum (Orskov and Orskov, 1960), nor are they lysed by an F specific phage (Loeb, 1960). F$^+$ (P-*lac*$^+$) and Hfr (P-*lac*$^+$) cells remain fully agglutinable and phage-sensitive. The fertility component of P-*lac*$^+$ is distinct, therefore, from the sex factor of *E. coli* K-12, although it possesses similar properties.

The DNA of *P. mirabilis* possesses an overall base composition of 39% guanine + cytosine (GC) which differs markedly from that of most other representatives of the Enterobacteriaceae (Falkow, Ryman, and Washington, 1962). If, therefore, P-*lac*$^+$ originated in *P. mirabilis*, it is reasonable to assume that the DNA of this genetic factor would resemble the DNA of this organism. On the other hand, if the P-*lac*$^+$ element did not arise in *Proteus*, but was acquired in vivo through genetic interaction with a *lac*$^+$ enteric species, then it should be possible to

TABLE 1. *Transfer of P-lac*$^+$ *to lac*$^-$ *strains**

lac$^-$ recipient	Per cent transfer
Escherichia coli K-12 2340 F$^-$	18
E. coli K-12 W2586 F$^-$	25
E. coli K-12 W1895 Hfr	12
E. coli K-12 58–161 F$^+$	7.5
Salmonella typhimurium TM-9	5
Serratia marcescens SM-S^r-11	0.1
Proteus mirabilis PM-1	0.001

* A *lac*$^+$ hybrid of *S. typhosa* 643 isolated from a cross with *P. mirabilis* PML$_3$ was mated in Penassay Broth (Difco) with each of the indicated *lac*$^-$ strains for 60 min at 37 C. Mating suspensions were diluted and plated on minimal lactose medium selective for hybrids. The *S. typhosa* *lac*$^+$ strain was contraselected by streptomycin.

TABLE 2. *Effect of acridine orange on P-lac*$^+$ *strains**

Strain	Concn of acridine orange (μg/ml)	No. of colonies examined	No. *lac*$^-$	Per cent *lac*$^-$
PML$_3$	0	1,271	0	0.0
	20	770	5	0.7
	50	692	8	1.1
W2586 P-*lac*$^+$	0	652	0	0.0
	10	655	3	0.5
	20	Inhibited		

* Overnight broth cultures of the specified strains were diluted 2×10^{-5} and 0.1 ml (about 10^4 cells) was inoculated into 1 ml of Penassay Broth (Difco) (pH 7.6) containing various concentrations of acridine orange. The tubes were incubated for 16 hr at 37 C and plated on MacConkey Agar.

TABLE 3. *Fertility of F*$^-$, *F*$^+$, *and Hfr strains after acquisition of P-lac*$^+$*

Cross	Selection	Frequency
Escherichia coli Hfr × *E. coli* F$^-$	*thr*$^+$ + *leu*$^+$	5×10^{-3}
E. coli Hfr (P-*lac*$^+$) × *E. coli* F$^-$	*thr*$^+$ + *leu*$^+$	4×10^{-3}
E. coli Hfr (P-*lac*$^+$) × *E. coli* F$^-$	*lac*$^+$	5×10^{-6}
E. coli F$^+$ (P-*lac*$^+$) × *E. coli* F$^-$	*lac*$^+$	1×10^{-5}
E. coli F$^-$ (P-*lac*$^+$) × *E. coli* F$^-$	*lac*$^+$	2×10^{-2}
E. coli F$^-$ (P-*lac*$^+$) × *E. coli* F$^-$	*thr*$^+$ + *leu*$^+$	3×10^{-8}

* Cultures of *lac*$^-$ *E. coli* Hfr (Cavalli) and F$^+$ (58–161) were obtained by ultraviolet irradiation. These strains and the F$^-$ *lac*$^-$ strain W2586 were infected with P-*lac*$^+$. The specified matings were performed with an F$^-$ *lac*$^-$ threonine + leucine-requiring (*thr*$^-$ + *leu*$^-$) *E. coli* stock, W1117. Frequencies are expressed as the number of hybrids per donor cell in the mating mixture. Streptomycin or methionine contraselections were employed.

demonstrate molecular heterogeneity in *P. mirabilis lac*$^+$ strains.

DNA was prepared from the original PML and PML_3 strains and examined in the Spinco model E analytical ultracentrifuge with the CsCl density-gradient technique. Normally, strains of *Proteus* exhibit only a single band of DNA in ultraviolet-absorption photographs, one which has a buoyant density of 1.698 g/cm^3, equivalent to 39% GC. The *lac*$^+$ *Proteus* strains, however, both exhibit a striking molecular heterogeneity and show two distinct bands of DNA corresponding to buoyant densities of 1.698 g/cm^3 and 1.710 g/cm^3. The band of 1.710 g/cm^3 density is equivalent to a GC composition of 50% and represents about 10% of the total extracted DNA. The association of the P-*lac*$^+$ element with the 1.710 g/cm^3 band was shown further by its appearance in strains of *Proteus* after infection (Fig. 1). These results suggest that the P-*lac*$^+$ element probably did not originate in *P. mirabilis* but was acquired from an organism possessing a DNA base composition of 50%. A complete molecular and enzymatic analysis of the P-*lac*$^+$ element will appear elsewhere (Wohlhieter et al., 1964).

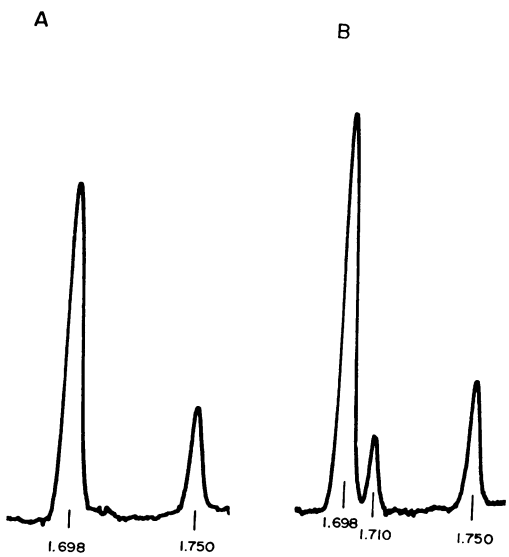

FIG. 1. *Molecular heterogeneity of Proteus DNA after acquisition of P-lac*$^+$. *Microdensitometric tracings of ultraviolet-absorption photographs taken after equilibrium was reached at 44,770 rev/min. The band of buoyant density 1.750 g/cm*^{3} *(deuterated Escherichia coli K-12 DNA) is the density standard. (A) DNA extracted from PM-1 strain before infection with P-lac*$^+$. *(B) DNA extracted from PM-1 strain after infection with P-lac*$^+$.

DISCUSSION

In a previous report (Falkow et al., 1964), we described the transfer of F-linked chromosomal determinants, including *lac*$^+$, to strains of *Proteus*. These *Proteus lac*$^+$ strains were heterogenotes, genetic donors, and could be "cured" with acridine orange. The acquisition of F-linked genes by *Proteus* was correlated with the appearance of a DNA fraction of *Escherichia*-like base composition (50% GC). We now report essentially the same results, with the major exception that the *Proteus lac*$^+$ strains were not derived from a laboratory manipulation but represent cultures isolated in vivo. In this instance, the *lac*$^+$ determinants are associated with an infectious element, P, which is distinct from the sex factor of *E. coli* K-12, although it exhibits similar properties. The origin of P-*lac*$^+$ is unknown, except that it probably was derived from an organism with a 50% GC DNA base composition.

The P-*lac*$^+$ factor, therefore, is an addition to a group of elements, such as resistance-transfer factor (Watanabe, 1963) and F_0-*lac*$^+$ (Falkow and Baron, 1962), which were recognized initially because of the determinants they carried, before their episomal nature was suspected. In each of these cases, an organism possessing an unusual property—*lac*$^+$ or multiple drug resistance—was isolated unexpectedly from an in vivo source and subsequently identified as being associated with an infectious element. The identification of these elements in naturally occurring microbial populations represents the best evidence to date for genetic transfer mechanisms operating in vivo (Marmur, Falkow, and Mandel, 1963).

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