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GENE TRANSFER BY CONJUGATION BETWEEN P. PSEUDOTUBERCULOSIS F-LAC AND P. PESTIS

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UNITED STATES ARMY
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GENE TRANSFER BY CONJUGATION BETWEEN *P. PSEUDOTUBERCULOSIS* F-*LAC* AND *P. PESTIS*

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FOREWORD

This work was performed during a one-year visit of W.D. Lawton to Microbiological Research Establishment, Porton, Salisbury, Wilts, United Kingdom. The junior authors are on the staff of M.R.E.

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ABSTRACT

Introduction of the F-*lac* episome from *Escherichia coli* into *Pasteurella pseudotuberculosis* yielded a strain of *P. pseudotuberculosis* F-*lac* that could transfer its genes by conjugation to *Pasteurella pestis*. The selected *P. pseudotuberculosis* marker arginine$^+$ was transferred at frequencies between 10^{-6} and 10^{-7} per donor cell. Unselected donor characters such as melibiose or rhamnose fermentation, phage sensitivity, and urease production appeared at different frequencies in the recombinants. The transferred markers were unrelated to the transfer of F-*lac* or its subsequent loss from the recipient cell.

I. INTRODUCTION

Introduction of the F-lac episome from Escherichia coli into Pasteurella pseudotuberculosis established a gene transfer system between P. pseudotuberculosis F-lac and various auxotrophs of P. pseudotuberculosis.[1] Since P. pseudotuberculosis is closely related to Pasteurella pestis, we investigated the possibility of extending the F-lac - mediated gene transfer system to include P. pestis.

II. MATERIALS AND METHODS

A. BACTERIAL STRAINS

The donor strain of P. pseudotuberculosis 32/IV F-lac cys^-, pur^-, thi^- was described previously.[1] The recipient strain of P. pestis was obtained as follows: (i) a methionine-, valine-, isoleucine-independent isolate[2] was selected from the virulent Alexander strain; (ii) after treatment with ultraviolet irradiation, an arginine E^- mutant was obtained; (iii) an isolate resistant to 1000 μg of streptomycin sulfate per ml was picked; (iv) a VW $antigen^-$ calcium $requirement^-$[3] avirulent colony was isolated. The final strain, labeled P. pestis 370, required cysteine (or thiosulfate), phenylalanine, and arginine for growth, was streptomycin-resistant and pigmented, produced fraction I antigen and pesticin I, and had the same gross antigens, phage sensitivity, and carbohydrate fermentation reactions as a classical strain of P. pestis.

B. MEDIA

Tryptic digest meat broth (TMB) or agar (TMA), Difco heart infusion broth (HIB), and Difco blood agar base (BAB) were employed as complete media. The basic minimal medium employed[1] was supplemented with the following (final concentrations expressed in millimoles in parentheses): cysteine (0.4); phenylalanine (0.5); methionine (0.2); valine (0.2); isoleucine (0.5); glycine (1.0); and sodium metabisulfite (0.5).

C. ABBREVIATIONS

The following abbreviations are used in this report: lac, lactose; arg, arginine; cys, cysteine; phe, phenylalanine; met, methionine; val, valine; ile, isoleucine; gly, glycine; mel, melibiose; rha, rhamnose; ure, urease; ϕIV^s, sensitivity to a bacteriophage originating as a

host-range mutant of a phage culture on *P. pestis*, serially propagated on *P. pseudotuberculosis* strain 32/IV; $T7^r$, resistance to phage T7; Sm^s, sensitivity to 1000 μg of streptomycin sulfate per ml; pig^{Pstb}, forming a large light-brown colony on hemin agar[4] characteristic of *P. pseudotuberculosis* in contrast to the compact, dark-brown colonies formed by *P. pestis*; *pes I*, pesticin I;[5] *fib*, fibrinolysin.[6]

D. MATING PROCEDURE

Essentially, the recipient strain was spread on a minimal agar medium selective for arg^+ recombinants, and, after ultraviolet irradiation, the donor strain was spread over the recipient lawn. Details of the mating procedure are described in previous publication.[1]

III. RESULTS

A. GENE TRANSFER

The frequency of arg^+ transfer from *P. pseudotuberculosis* F-*lac* to *P. pestis* 370 ranged from 10^{-6} to 10^{-7} per donor cell, which was about two logs less than we had observed for the transfer of four different markers between *P. pseudotuberculosis* strains.[1] This low frequency, resulting in only 5 to 50 recombinant colonies on the selective plates, was workable because of the complete absence of donor or recipient reversions on control plates.

Selection for cys^+ or phe^+ recombinants yielded no colonies.

B. EFFECT OF MEDIUM ON MATING

Although the recipient strain was cys^- phe^- arg^-, no arg^+ recombinants appeared in a minimal agar medium containing only *cys* and *phe*. The addition of *met*, *val*, *ile*, and *gly* (the typical growth requirements of *P. pestis*) apparently was necessary for mating. The arg^+ recombinants grew well on minimal agar containing only *cys* and *phe*.

As we had observed with the *P. pseudotuberculosis* X *P. pseudotuberculosis* mating system,[1] no recombinants resulted from broth matings.

C. ANALYSIS OF UNSELECTED MARKERS

Although *P. pseudotuberculosis* and *P. pestis* are similar, several easily measured properties can be used to distinguish the two species. Our donor strain of *P. pseudotuberculosis* was *arg*$^+$, *mel*$^+$, *φIV*^{s}, *rha*$^+$, *ure*$^+$, T^{7r}, *pla*^{Pstb}, *Sm*^{s}, *pes*$^-$, *fib*$^-$, *phe*$^+$; the recipient strain of *P. pestis* was the opposite for these characters. Analysis of 118 recombinants, obtained from six separate experiments, revealed that *mel*$^+$, and *φIV*^{s} were transferred at frequencies of 39% and 5%, *rha*$^+$ and *ure*$^+$ were transferred at a frequency of approx 1%, and none of the other donor markers were detected in the recombinants (Table 1).

TABLE 1. ANALYSIS OF *arg*$^+$ RECOMBINANTS FOR UNSELECTED MARKERS

P. pseudotuberculosis Donor Characters					Analysis of 118 *P. pestis* Recombinants[a]	
arg$^+$	*mel*$^+$	*φIV*^{s}	*rha*$^+$	*ure*$^+$	No.	%
+	-	-	-	-	62	52.5
+	+	-	-	-	46	39.0
+	+	+	-	-	6	5.1
+	+	+	+	-	1	0.85
+	+	-	+	-	1	0.85
+	-	-	+	-	1	0.85
+	+	-	-	+	1	0.85

a. Obtained from six separate experiments.

D. F-*lac* TRANSFER

Twelve of the 118 recombinants were *lac*$^+$, segregating *lac*$^-$ clones at different frequencies. The *lac*$^+$ marker was distributed at random among the recombinant classes and loss of the *lac*$^+$ marker did not change any other character.

E. BASE COMPOSITION

The T_m of DNA[7] extracted from the donor strain of *P. pseudotuberculosis* was 88.5 C (46.8% G + C). The T_m of *P. pestis* 370 measured at the same time was 88.4 C (46.6% G + C).

IV. DISCUSSION

The data presented indicate that the episome F-*lac* can promote chromosome transfer between *P. pseudotuberculosis* and *P. pestis* similar to that mediated by F-*lac* in *E. coli*.[8] The frequency of transfer observed so far is low (10^{-6} per donor cell) and only *arg*$^+$ has been used successfully as a selected marker. The detection of four unselected donor characters in the *arg*$^+$ recombinants in decreasing frequencies can be interpreted in terms of classical chromosome transfer.

The conversion of *P. pestis* to *P. pseudotuberculosis* has been reported by several Soviet investigators (reviewed by Pollitzer[9]), but never with genetically marked strains, and the experiments described have not dispelled all doubt. If this conversion occurs, it might be expected that the distinguishing properties between the two species would be controlled by a single gene locus. Successful gene transfer of one of these properties, e.g. *mel*$^+$, should be correlated with the transfer of other properties such as *rha*$^+$ and *ure*$^+$. The fact that these properties are not jointly transferred (Table 1) suggests that the main properties distinguishing *P. pestis* and *P. pseudotuberculosis* are not controlled by the same gene locus.

Investigation of the genetics of virulence requires a reproducible disease syndrome plus a gene transfer system. Plague can be considered a "model" acute disease because the human symptoms occur in common laboratory animals after injection of a few microorganisms. The etiological agent, *P. pestis*, has been the subject of extensive research and a great deal is known about virulence factors and immunity in this pathogen.[9-11] The extension of a gene transfer system to include *P. pestis* should lead to a resolution of some of the problems concerning virulence in this pathogen and eventually contribute toward a better understanding of virulence at the genetic level.

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