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Lysogenic Conversion of *Pasteurella* by *Escherichia coli* Bacteriophage P1 *CM*

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Bacteriophage P1 *CM* can convert *Pasteurella pestis* or *P. pseudotuberculosis* to chloramphenicol resistance and phage restriction, but no viable phage was induced from converted *Pasteurella* strains.

In searching for a transducing phage for *Pasteurella*, we noted that several strains of *P. pestis* and *P. pseudotuberculosis* were converted to chloramphenicol resistance (CM^r) upon incubation with P1 *CM* phage propagated on *Escherichia coli*. P1 *CM* is a derivative of the generalized transducing phage, P1 *kc*, which had acquired the CM^r marker by recombination with an RTF factor and which showed no detectable differences from the parent phage (2). Acquisition of the CM^r marker by the recipient cell was always accompanied by lysogenization with P1 *CM* (i.e., a lysogenic conversion).

Certain strains of *E. coli* and *Shigella dysenteriae* lysogenic for phage P1 restrict the heterologous phages T1, T3, T7, P2, and λ (1, 3). We relied on this restriction of heterologous phages to show that selected CM^r clones were carrying P1 *CM* phage.

A sterile lysate of P1 *CM* phage, prepared on *E. coli* strain W1485, was incubated with log-phase cultures at a multiplicity of 0.1 to 1 for 2 hr at 37 C. Selection for CM^r was made on Difco blood-agar base containing 25 μg of chloramphenicol/ml. With *E. coli*, only 5% of the cells survived phage treatment, and 20% of these survivors were converted to CM^r. With *P. pestis* and *P. pseudotuberculosis*, all the cells survived, and the conversion to CM^r was 0.7% for the former and 0.02% for the latter.

P1 *CM* phage was released from CM^r clones of *E. coli* upon incubation in L broth with or without ultraviolet induction. On the other hand, we were unable to detect by plating or spotting on lawns of *E. coli*, *P. pestis*, or *P. pseudotuberculosis* the release of phage particles from CM^r clones of *P. pestis* or *P. pseudotuberculosis*. However, these clones were apparently lysogenic for P1 *CM* because they restricted heterologous phages as shown in Table 1.

The efficiency of plating (EOP) of all three phages (T7, H, and φIV) on the nonlysogenic *E. coli* was 1.0, whereas the EOP on the P1 *CM* lysogen was 10^{-6} to 10^{-8}. Similarly with *P. pestis* and *P. pseudotuberculosis*, the EOP of these phages was restricted in the P1 *CM* lysogen in all cases where the phage was active on the parent nonlysogen.

TABLE 1. *Restriction of heterologous phages by P1 CM lysogenic strains*[a]

Strain (all F^-)	EOP of phage		
	T7	φH	φIV
Escherichia coli	1	1	1
E. coli (P1 *CM*)	10^{-8}	10^{-7}	10^{-6}
Pasteurella pestis	10^{-2}	1	NS
P. pestis (P1 *CM*)	10^{-9}	10^{-8}	NS
P. pseudotuberculosis	10^{-2}	10^{-1}	1
P. pseudotuberculosis (P1 *CM*)	10^{-6}	10^{-6}	10^{-6}

[a] EOP = efficiency of plating; NS = not sensitive.

Previously, we showed that only one strain of *P. pestis* (YpA-38) was sensitive to the male-specific phage MS2 (4). A derivative of this strain carrying F'*lac* was converted to CM^r with P1 *CM* phage. As shown in Table 2, the CM^r clones selected from this conversion (presumably P1 *CM* lysogenic) restricted MS2 (i.e., the EOP of MS2 was $<10^{-11}$ compared with an EOP of 10^{-1} on the nonlysogenic parent strain). CM^r clones selected from the conversion of an F'*lac* strain of *P. pseudotuberculosis* (YsD-20) also restricted MS2. However, CM^r derivatives of *E. coli* (F'*lac*, F^+ or Hfr strains) did not restrict MS2.

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Since true restriction of phage ribonucleic acid had never been demonstrated, we explored the possibility that the presence of P1 *CM* affected the adsorption of phage MS2. The results (Table 3) show that, although the percentage of *lac*$^+$ cells was approximately the same in both lysogenic and nonlysogenic cultures, only 6% of the MS2 phage was adsorbed by the P1 *CM* lysogens compared with 64% by the nonlysogen. By spot test, the P1 *CM* lysogen was MS2^r, but the parent strain was MS2^s. The parent strain was normally resistant to the female-specific phage T7, whereas the P1 *CM* lysogen became T7^s. However, the heterologous phage IV to which the parent was normally sensitive was restricted in the P1 *CM* lysogen.

These data show that the presence of P1 *CM* can prevent adsorption of the male-specific phage to an F'*lac* strain of *P. pseudotuberculosis*, possibly by blocking F pili formation.

TABLE 2. *Restriction of MS2 by F'lac strains of Pasteurella lysogenic for P1 CM*

Strain	EOP[a] of MS2 on	
	Nonlysogen	P1 *CM* lysogen
Pasteurella pestis F'*lac*	10^{-1}	$<10^{-11}$
P. pseudotuberculosis F'*lac*	10^{-1}	$<10^{-11}$
Escherichia coli F'*lac*	1	1
E. coli F$^+$	1	1
E. coli Hfr	1	1

[a] EOP = efficiency of plating.

TABLE 3. *Comparison of adsorption of MS2 by Pasteurella pseudotuberculosis (P1 CM) and its nonlysogenic parent*

Determinations	Nonlysogen[a]	P1 *CM* lysogen[a]
CM^r colonies	0	81
lac$^+$ colonies	90	95
MS2 adsorbed (MOI = 0.01)[b]	64	6
Phage sensitivity (spot test)		
MS2	S	R
T2	R	S
ϕIV	S	R

[a] Numbers are in %; S = sensitive; R = resistant.

[b] MOI = multiplicity of infection.

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