Transduction of *Myxococcus virescens* by Coliphage P1CM: Generation of Plasmids Containing both Phage and *Myxococcus* Genes

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Chloramphenicol-resistant *Myxococcus virescens* were obtained by infecting myxococci with *Escherichia coli* specialized transducing phage P1CM. The drug-resistant myxococci were phenotypically unstable. They contained more than one type of plasmid; these plasmids were not found in the parent strain. Chloramphenicol-resistant *E. coli* were obtained by transformation with either a fraction of myxococcal DNA containing the plasmids or with P1CM prophage DNA. These transformants contained plasmids. *Escherichia coli* transformed by DNA from the myxococci contained both P1CM and myxococcal genes. Individual transformant clones differed in the genetic make-up of their plasmids. Among the myxococcal genes expressed in these plasmid-harboring *E. coli* strains were a capacity for self-transmissibility and a pattern of phage sensitivity characteristic of R factor incompatibility group W. *Escherichia coli* transformed with P1CM prophage contained incomplete P1CM genomes; none of the chloramphenicol-resistant transformants produced P1CM phage particles. The significance of these findings for an understanding of mechanisms for the generation of R factors is discussed.

INTRODUCTION

Members of the genus *Myxococcus* (Myxococcaceae, Myxobacterales) are Gram-negative rods which demonstrate gliding motility and aggregate to form simple fruiting bodies. Myxococci can acquire drug resistance by three different types of process. (i) Unstable resistance to chloramphenicol may be induced in *M. xanthus* by exposure of vegetative organisms to low concentrations of the drug (Burchard & Parish, 1975). (ii) Unstable resistance to a variety of drugs (including chloramphenicol) may be acquired following incubation of mixtures of strains of either *Escherichia coli* or *Pseudomonas fluorescens* harbouring R factors with strains of either *M. xanthus* or *M. fulvus* (Parish, 1975). (iii) Chloramphenicol resistance may be acquired by *M. xanthus* following infection of vegetative cells with the specialized *E. coli* transducing phage P1CM (Kaiser & Dworkin, 1975). P1 phage is not propagated by the myxococci and, as in (i) and (ii), the chloramphenicol-resistant myxococci are very unstable.

The last two types of procedure for the acquisition of drug resistance by myxococci imply that exogenous DNA (of plasmids or prophage) can survive in a myxobacterial host. In the present work we have examined the nature of the bacterial replicon which includes genes encoded in the exogenous DNA and present in the myxococcal host. We have chosen not to use the strains whose resistance is seemingly derived from R factors because of their extreme instability. Indeed, when such strains were examined after prolonged subculture in the

presence of the drug, they appeared to have lost any R factor as judged by DNA:DNA hybridization (Morris, unpublished data). We conclude that this is because low intracellular levels of the drug eventually induce an endogenous resistance mechanism [process (i)] which results in loss of the selective advantage for retaining the R factor in the presence of the drug. Kaiser & Dworkin (1975) did not characterize the DNA of their transductants and they were unable to obtain P1 phages from the chloramphenicol-resistant myxococci. It is therefore possible that their transductants were abortive and that the resistance was due to transient partial detoxication of the drug by PICM and consequent induction of the system described by Burchard & Parish (1975).

In this paper we describe work to characterize the nature of the replicon which includes the genes for chloramphenicol resistance in transductants of *M. virescens* by phage PICM. *Myxococcus virescens* is closely related to *M. xanthus* but differs in details of its morphogenetic cycle (Parish, Wedgwood & Herries, 1976). It was chosen for this work because there is no evidence of an inducible, endogenous chloramphenicol resistance system of the type found in *M. xanthus*.

METHODS

Organisms, phages and growth. Strains of bacteria and phages used in this work are listed in Table 1. Following an agreed international nomenclature for myxobacterial strains (Wireman & Dworkin, 1977), the *M. virescens* parent strain has been designated HP300. This organism was previously referred to as strain v2 (Parish *et al.*, 1976). New plasmids have been numbered from pBL1001 using the recommendations of Novick *et al.* (1976) for plasmid nomenclature. Plasmid Sa appears in the earlier literature as S-a. Bacteriophage K7 is a newly isolated double-stranded DNA phage with an icosahedral head and a long tail (Morris, Virrankoski-Castrodeza & Parish, unpublished).

Bacteria were grown in shake culture at 30 °C. Medium for *M. virescens* was CTYE which contained 1% (w/v) Bactocasitone and 0.5% (w/v) yeast extract; medium for *E. coli* was L broth which contained 1% (w/v) tryptone, 0.8% (w/v) NaCl and 0.8% (w/v) yeast extract.

The PICM strain used was the same as that employed by Kaiser & Dworkin (1975); it carries a temperature-sensitive mutation in a gene required for the maintenance of lysogeny. Cultures of lysogens can therefore be grown at the permissive temperature (30 °C) and induced at the non-permissive temperature (42 °C). Like all P1 phages, the prophage state is a stable plasmid which does not integrate into the *E. coli* chromosome (Ikeda & Tomizawa, 1968).

Phages were assayed by standard procedures. Underlay contained 1.2% (w/v) agar and overlay contained 0.8% (w/v) agar. Overlay agar contained (in addition to the nutrients described for the growth media) 2.5 mM-CaCl₂, 1 mM-MgSO₄ and (for P1 phages only) 0.1% (w/v) glucose. Plates for phage assays were incubated at 37 °C. Chloramphenicol (25 µg ml⁻¹) was included in media for selection and maintenance of chloramphenicol-resistant strains.

For conjugation of *E. coli*, mixtures of donor and recipient strains were incubated at 30 °C in stationary culture prior to plating.

Escherichia coli strains were labelled with ³H or ¹⁴C by growth in minimal A medium (Miller, 1972) supplemented with appropriate amino acids (each 4 µg ml⁻¹), deoxyadenosine (25 µg ml⁻¹) and either [methyl-³H]thymidine (47 Ci mmol⁻¹, 1.0 mCi ml⁻¹) or [methyl-¹⁴C]thymidine (50 mCi mmol⁻¹, 50 µCi ml⁻¹). *Escherichia coli* was labelled with ³²P by growth in the buffered salts of Garen & Levinthal (1960), supplemented with glucose (1% w/v), sodium β-glycerophosphate (0.14%, w/v), appropriate amino acids (each 4 µg ml⁻¹) and [³²P]orthophosphate (carrier free, 1 mCi ml⁻¹). *Myxococcus virescens* was labelled by growth in bovine serum albumin (1%, w/v; Fraction V; Sigma) supplemented with the basal salts medium of Brockman (1973), sodium β-glycerophosphate (0.14%, w/v), spermidine.3HCl (0.0024%, w/v) and radiochemicals in the same amounts as were used for *E. coli*.

Indole production was detected by the method of Baker (1967).

* Abbreviations: *str*, *cml*, *kan*, *amp*, *sul*, *tet*, *tmp* and *gen* indicate resistances to streptomycin, chloramphenicol, kanamycin, ampicillin, sulphonamides, tetracycline, trimethoprim and gentamycin, respectively; *hsdR hsdM*⁺, unrestricted but modifying with respect to the κ12 restriction-modification system; *drd*, depressed; Clr, clear plaque; *clr100* is a temperature-sensitive mutation.

† In some strains of phages P1 and λ, the strain designation incorporates a partial definition of the genotype. In this paper, we have followed this practice in order to facilitate cross-reference to the rest of the literature and the phenotype (Clr) has been entered under genotype. Phages K7 and PR4 only plaque on *E. coli* strains harbouring R factors of certain incompatibility groups.

‡ Letters in parentheses identify the incompatibility groups of the plasmids (Datta & Hedges, 1972).

Table 1. *Bacterial strains, plasmids and phages*

	Relevant genotype*	Source
Bacteria		
<i>Myxococcus virescens</i>		
HP300		Laboratory stock strain
HP312	<i>cml</i>	This work
<i>Escherichia coli</i> K12		
802	<i>hsdR hsdM⁺ met</i>	W. J. Brammar
w3110		Laboratory stock strain
TLA02	<i>trp lac arg F⁺</i>	S. Baumberg
J53		Laboratory stock strain
M5086	<i>str pon</i>	S. Baumberg
C600	λ^-	Laboratory stock strain
Phages†		
P1CM	<i>clr100 cml</i>	J. Rosner
P1c4.32	Clr	J. Rothman Scott
P1 am1	Clr	J. Rothman Scott
P1 am4	Clr	J. Rothman Scott
P1 am13	Clr	J. Rothman Scott
P1 am21	Clr	J. Rothman Scott
P1 am31	Clr	J. Rothman Scott
λ_{vir}	Clr	Laboratory stock strain
K7	Host range	See text
PR4	Host range	D. E. Bradley
Plasmids‡		
R386 (FII)	<i>amp str cml kan sul</i>	S. Baumberg
R1-19 (FII)	<i>cml kan amp str sul drd</i>	S. Baumberg
R124 (FIV)	<i>tet</i>	S. Baumberg
R64 (I α)	<i>str tet</i>	S. Baumberg
R144-3 (I α)	<i>kan drd</i>	S. Baumberg
R483 (I β)	<i>str tet</i>	S. Baumberg
R621a (I γ)	<i>tet</i>	S. Baumberg
R721 (I δ)	<i>tmp</i>	S. Baumberg
RA1 (A)	<i>tet sul</i>	S. Baumberg
R57b (C)	<i>cml amp kan sul gen</i>	S. Baumberg
R27 (H)	<i>tet</i>	S. Baumberg
R371 (J)	<i>kan</i>	S. Baumberg
R387 (K)	<i>str cml</i>	S. Baumberg
R471a (L)	<i>amp</i>	S. Baumberg
R446b (M)	<i>str tet</i>	S. Baumberg
R46 (N)	<i>amp str tet sul</i>	S. Baumberg
R390 (N)	<i>cml amp str sul tet</i>	S. Baumberg
R16 (O)	<i>amp str tet sul</i>	S. Baumberg
RP4 (P)	<i>amp tet kan</i>	S. Baumberg
R478 (S)	<i>tet cml kan</i>	S. Baumberg
Rts-1 (T)	<i>kan</i>	S. Baumberg
Sa (W)	<i>cml kan str sul</i>	S. Baumberg
R7K (W)	<i>amp</i>	R. W. Hedges
R6K (X)	<i>amp str</i>	S. Baumberg
SSu (-)	<i>str sul</i>	S. Baumberg
pBL1003	<i>cml</i>	This work
pBL1005	<i>cml</i>	This work
pBL1009	<i>cml</i>	This work
pBL1010	<i>cml</i>	This work
pBL1011	<i>cml</i>	This work
pBL1013	<i>cml</i>	This work
pBL1014	<i>cml</i>	This work
pBL1016	<i>cml</i>	This work
pBL1017	<i>cml</i>	This work
pBL1021	<i>cml</i>	This work
pBL1024	<i>cml</i>	This work

For footnotes to table see opposite page

Transduction with bacteriophage P1CM. *Escherichia coli* w3110(P1CM) was grown to exponential phase at 30 °C in L broth and lysed by incubation for 90 min at 42 °C in the presence of 1 mM-Mg²⁺. Chloroform (1 to 2 ml) was then added, and the lysate was clarified by centrifugation and assayed using *E. coli* c600 as indicator.

Transduction of *M. virescens* HP300 with bacteriophage P1CM was performed as described for *M. xanthus* by Kaiser & Dworkin (1975).

Preparation of plasmid DNA. P1CM prophage DNA was isolated from *E. coli* w3110(P1CM) by the method of Clewell & Helinski (1969). Otherwise, plasmid DNA was isolated from 'cleared lysates' of plasmid-carrying strains of *M. virescens* and *E. coli* by a modification of the method of Currier & Nester (1976). A culture in exponential phase (500 ml) was harvested by centrifugation, washed twice in TES buffer (0.05 M-Tris/HCl, 0.5 M-NaCl, 0.005 M-EDTA, pH 8.5) and lysed by incubating for 30 min at 37 °C in the presence of 0.5 mg pronase ml⁻¹ (Sigma, predigested by incubation at 37 °C for 90 min) and 1% (w/v) sodium lauryl sulphate. Chromosomal DNA was sheared by forcing the lysate through a syringe and needle (18 gauge) six times, denatured by raising the pH to 12.0 to 12.3 for 10 min and neutralized. Denatured DNA was removed by extraction with redistilled, water-saturated phenol, and the phenol was removed by extraction with chloroform/isoamyl alcohol (24:1, v/v). Plasmid DNA was precipitated by treatment with ethanol and magnesium phosphate at -20 °C (Dessev & Grancharov, 1973). The precipitate was redissolved in a minimum volume of 0.1 M-EDTA, pH 8.0, and dialysed against TES buffer. For density-gradient centrifugation, 5 ml portions were taken; CsCl and ethidium bromide (3.75 mg) were dissolved to achieve a density of 1.57 g ml⁻¹ (Flamm, Birnstiel & Walker, 1972). The samples were centrifuged for 60 h at 40 000 rev. min⁻¹ and 16 °C in a Beckman type SW50.1 rotor. Plasmid DNA was removed by fractionation from the bottom of the tube or by withdrawal of visibly stained zones with a syringe needle. Ethidium bromide was removed from DNA by extraction with isoamyl alcohol and CsCl was removed by dialysis.

For fractionation of DNA from cleared lysates by column chromatography, the solution (in 0.1 M-EDTA, pH 8.0) was first passed through a column of Sepharose 4B to remove protein and RNA (Šatava, Zadrazil & Šormová, 1973). The fractions containing DNA were pooled and fractionated by chromatography on hydroxyapatite in the presence of ethidium bromide (Pakroppa, Goebel & Müller, 1975).

Electron microscopy of plasmid DNA. DNA samples were prepared by the aqueous technique of Davis, Simon & Davison (1971). Grids were stained in uranyl acetate and then rotary shadowed with platinum and examined with a Philips EM200 microscope at an operating voltage of 60 kV. The original magnifications were between $\times 5000$ and $\times 9200$ and pictures were recorded on 35 mm film. Certain negatives were printed. Otherwise they were projected, traced and contour lengths were measured with a map measurer.

DNA:DNA hybridization. For hybridization experiments, sheared unfractionated bacterial DNA was prepared by the method of Berns & Thomas (1965). Approximately 0.01 μ g ³H-labelled sheared denatured plasmid P1CM DNA (1000 to 3000 c.p.m.) was incubated with 200 μ g non-radioactive denatured sheared bacterial DNA, annealed in 0.42 M-NaCl at 60 °C for 16 h and digested with S1-nuclease to remove single-stranded DNA. Radioactivity was assayed in the remaining double-stranded DNA. Preparation of S1-nuclease and methods of sample preparation and assay of radioactivity were according to Crosa, Brenner & Falkow (1976).

Transformation. *Escherichia coli* was transformed with P1CM prophage plasmid DNA and preparations of plasmids from *M. virescens* HP312 by the method of Mandel & Higa (1970) with the following modifications. The CaCl₂ concentration was 0.1 M. The 'heat shock' consisted of incubating organisms at 42 °C for 2 min; then the bacteria were chilled for 90 min (0 °C). Deoxyribonuclease was not added to the mixture. The transformation mixture was diluted 1:10 in L broth and incubated for 4 to 6 h at 30 °C to permit phenotypic expression of chloramphenicol resistance prior to plating on selective medium.

RESULTS

Properties of transduced Myxococcus virescens

By transducing *M. virescens* HP300 with bacteriophage P1CM with a multiplicity of infection of 5, chloramphenicol-resistant organisms were produced at a frequency of approximately 5×10^{-6} per cell. This is comparable with the frequency obtained when *M. xanthus* was transduced in the same way (Kaiser & Dworkin, 1975). One such chloramphenicol-resistant transductant, *M. virescens* HP312, was used for further analysis.

The chloramphenicol resistance in *M. virescens* HP312 is extremely unstable. After transfer to unselective medium, chloramphenicol-sensitive segregants appeared after 5 h (Fig. 1). The erratic growth curve for the organism is due to the fact that *M. virescens* HP300 and its

Fig. 1

Fig. 2

Fig. 1. Instability of *M. virescens* HP312 in unselective medium. Organisms were grown in CTYE medium supplemented with chloramphenicol ($25 \mu\text{g ml}^{-1}$) and diluted into CTYE at time 0. At intervals, portions were removed, diluted and the numbers of colony forming units (c.f.u.) were measured by plating on CTYE agar containing chloramphenicol (●) and CTYE agar without chloramphenicol (○).

Fig. 2. Fractionation of DNA from 'cleared lysates' by hydroxyapatite-ethidium bromide chromatography (see Methods). DNA of cultures of *M. virescens* strains HP300 and HP312 was labelled with ^{14}C and ^3H , respectively. Cleared lysates were prepared and mixed so that equal amounts of ^{14}C and ^3H radioactivity were present, and the solution was then fractionated. Radioactivity (^{14}C , ○; ^3H , ●) was determined in each sample by liquid scintillation counting; quenching and overlap corrections were made. Plasmids were present in fraction 77.

derivatives grow in a semi-disperse form in liquid media and, as with certain other myxococci, small clumps accumulate and disperse during growth (Parish, 1975).

Analysis of DNA from a 'cleared lysate' of *M. virescens* HP312 revealed the presence of a DNA fraction which adsorbed strongly to hydroxyapatite in the presence of ethidium bromide. This is evidence for the presence of covalently closed DNA (Pakroppa *et al.*, 1975). Such material was not found in cleared lysates from strain HP300 (Fig. 2).

Electron microscopic examination of the fraction of HP312 DNA which adsorbed to the hydroxyapatite column revealed three types of molecule. Small plasmids (Fig. 3) were found; these had an apparent molecular weight (determined from contour length measurements) of 1.9×10^6 . Larger molecules were also found; these had an apparent molecular weight of 8.7×10^6 . Every molecule of this type was associated with a 'blob' of darkly staining material (Fig. 4). Careful examination of the photographic negatives suggested that the DNA molecule is probably covalently continuous, and the blobs are likely to be fragments of plasma membrane. This is supported by the constancy of the molecular weight of these molecules. However we have not rigorously ruled out the possibility that the molecules could be fragments from larger plasmids present in the eluate from the hydroxyapatite column and, indeed, there is evidence that much larger plasmids must be present in these preparations (next section). The electron microscope grids also contained DNA of an extremely tangled appearance with no visible ends. These structures were not apparently supercoiled and it has not been possible to measure their contour lengths nor to establish whether or not they are covalently closed.

Fig. 3

Fig. 4

Figs 3 and 4. Electron micrographs of plasmids from 'cleared lysates' of *M. virescens* HP312. Bar markers represent 0.2 μm .

Properties of E. coli strains transformed with DNA from M. virescens HP312
and P1CM prophage

A fraction of *M. virescens* HP312 cleared lysate DNA was enriched for covalently closed DNA by CsCl-ethidium bromide buoyant-density centrifugation. *Escherichia coli* 802 (an unrestrictive but modifying strain) was transformed with this DNA. By using 3 μg DNA ml^{-1} and 3×10^9 bacteria ml^{-1} in the transformation mixture, chloramphenicol-resistant *E. coli* transformants were obtained at a frequency of approximately 10^{-7} per cell. In a separate experiment using P1CM prophage plasmid DNA and the same conditions, chloramphenicol-resistant transformants were obtained at a frequency of approximately 10^{-6} per cell. In the description of plasmid-harboring *E. coli* strains obtained in this way, we refer to those in which the transforming DNA was obtained from HP312 as *cml*312 and to those in which the transforming DNA was obtained from P1CM prophage as *cml*P1CM. All the chloramphenicol-resistant transformants were stable and chloramphenicol-sensitive organisms have not segregated in unselective liquid medium at any measureable frequency.

None of the strains tested (four of the type *cml*P1CM and nine of the type *cml*312) produced P1CM phage following growth at the non-permissive temperature for maintenance of P1CM $\text{c}lr$ 100 lysogens. From this we conclude that none of these organisms contained a complete P1 genome. We believe that the failure to obtain intact P1CM genomes by transformation with P1CM prophage is due to the transformation event selecting for that proportion (approximately 20%) of P1 prophage plasmids which naturally delete extensive parts of their genome (Yun & Vapnek, 1977). We have independently confirmed that a proportion of the prophage plasmids obtained from our strain of *E. coli* w3110 (P1CM) are shorter by amounts comparable with the data of Yun & Vapnek (1977).

We have characterized the DNA from one transformant, *E. coli* 802(pBL1005) *cml*312. Analysis by ethidium bromide-CsCl buoyant-density centrifugation confirmed the presence of the plasmid pBL1005 in this organism (Fig. 5). Electron microscopic examination of DNA from the plasmid peak of gradients such as that illustrated in Fig. 5 showed two types of molecule which are the relaxed and supercoiled conformations of the plasmid (Figs 6 and 7). From contour length measurements of relaxed forms (such as Fig. 6), the apparent molecular weight of pBL1005 is $68.8 (\pm 0.45) \times 10^6$. This is significantly larger than any plasmid found in *M. virescens* HP312 (previous section). We therefore conclude that we have so far failed to observe in the electron microscope plasmids such as pBL1005 in the myxococci from which they were derived.

Plasmid pBL1005 was transferred from *E. coli* 802 to *E. coli* TLA02 by conjugation. Transconjugants appeared after 1 h (Fig. 8). This type of transfer was not demonstrated by *E. coli* 802(pBL1003) *cml*P1CM. The genetic exchange of chloramphenicol resistance demonstrated in Fig. 8 did not occur with culture supernatants from *E. coli* 802(pBL1005) *cml*312 and was therefore not due to transduction. This conclusion was confirmed by show-

Fig. 5. Fractionation of DNA from a 'cleared lysate' of *E. coli* 802(pBL1005) *cml312* by ethidium bromide-CsCl buoyant-density centrifugation. Organisms were labelled with [^3H]thymidine (see Methods); after centrifugation, fractions were assayed for radioactivity by liquid scintillation counting. Fraction 1 is the bottom of the gradient. Plasmid DNA was present in the peak in fraction 30; the peak in fraction 37 was *E. coli* chromosomal DNA. The peak at the bottom of the gradient was presumed to contain RNA-DNA-protein aggregates.

Fig. 6

Fig. 7

Figs 6 and 7. Electron micrographs of plasmids from 'cleared lysates' of *E. coli* 802(pBL1005) *cml312*. Bar markers represent 1.0 μm . See text for discussion.

ing that a mutant of *E. coli* which lacks a P1 receptor (strain m5086) is a successful recipient in such a transfer. As P1CM prophage is not a self-transmissible plasmid, the transfer function in pBL1005 must be of myxococcal origin. A second phenotypic change in *E. coli* 802 following acquisition of pBL1005 is that the cells become hosts for a newly isolated double-stranded DNA phage K7. (Details of the properties of this phage will be published elsewhere.) *Escherichia coli* 802 and 802(P1CM) are not hosts for the phage and therefore K7-sensitivity must rely upon expression of myxococcal genes in pBL1005. Twenty-five *E. coli* strains harbouring R factors (Table 1) of different incompatibility groups were tested

Fig. 8. Conjugal transfer of chloramphenicol resistance from *E. coli* 802(pBL1005) to *E. coli* TLA02. At intervals, portions of culture were removed, diluted and plated, and numbers of colony forming units (c.f.u.) of strains 802(pBL1005) (○), TLA02 (●) and TLA02(pBL1005) (□) were determined using suitable selective media. The chloramphenicol-resistant transconjugant colonies were tested on lactose indicator medium and were found to be *lac*. This constituted a test for an unselected marker in the transconjugants because strain 802 is *lac*⁺ and TLA02 is *lac*.

Table 2. Comparison of the phenotypes of *E. coli* 802 strains harbouring plasmids

Plasmid pBL1003 is of the type *cmI*P1CM; all other pBL plasmids in this table are of the type *cmI*312 (see text).

Plasmid	Sensitivity to phage K7*	P1CM <i>hsp</i> system assayed with λ†	Indole production§	Rescue of P1 markers					
				<i>c4.32</i>	<i>am1</i>	<i>am4</i>	<i>am13</i>	<i>am21</i>	<i>am31</i>
None	—	u	++	—	—	—	—	—	—
P1CM	—	r	++	<i>i</i>	<i>i</i>	—	<i>i</i>	—	<i>i</i>
pBL1003	—	u	++	—	—	+	—	+	—
pBL1005	+	‡	—	+	+	+	+	+	+
pBL1009	+	u	++	—	—	+	+	—	—
pBL1010	+	u	+	NT	NT	NT	NT	NT	NT
pBL1011	+	u	++	+	—	—	—	—	—
pBL1013	+	u	++	—	+	+	+	+	—
pBL1014	+	u	++	—	—	—	—	—	—
pBL1016	+	u	—	+	+	+	+	+	+
pBL1017	+	u	++	—	—	—	—	—	—
pBL1021	+	u	++	—	—	—	—	—	—
pBL1024	+	u	++	—	+	—	—	—	—

* +, Sensitive; —, insensitive.

† r, Restrictive; u, unrestrictive.

‡ *Escherichia coli* 802(pBL1005) is not a host for phage λ.

§ ++, Indole production in nutrient qualitatively similar to wild type; +, indole production just detectable but significantly reduced; —, no indole detectable.

|| +, Marker rescued (i.e. plaques turbid); —, marker not rescued (plaques clear); *i*, immune; NT, not tested.

as possible hosts for phage K7. The only hosts [apart from *E. coli* (pBL1005) and strains harbouring other *cmI*312 plasmids] were *E. coli* (Sa) and *E. coli* (R7K). Both of these last two plasmids belong to incompatibility group W (Watanabe, Furuse & Sakaizumi, 1968; R. W. Hedges, personal communication). Genes for this or a related incompatibility group are therefore present in *M. virescens* HP300 and are presumptively associated with the transfer function. The presence of the determinants of such an incompatibility group was independently confirmed by testing with phage PR4. This phage is specific for *E. coli* strains with either W or P group pili (Bradley, 1976; Bradley & Cohen, 1977). Of four *cmI*312 plasmids tested (pBL1009, pBL1010, pBL1013 and pBL1016), all were hosts for phage PR4.

Escherichia coli 802(pBL1005) does not produce indole when grown on a catabolite-free

nutrient medium. The reason for this is unknown but we believe that it may be due to an inhibition of the activity of the catabolite repressible protein (CRP) because indole production in *E. coli* is due to the expression of the *tna* operon and expression of this operon shows a strong catabolite effect (Khairul Bashar, Parish & Brown, 1971). Strain TLA02(pBL1005) does produce indole. The difference may reflect a dependence upon the genetic background of the *E. coli* strain or may be due to segregation of pBL1005 following conjugative transfer.

Several independently isolated strains of *E. coli* containing *cml312* plasmids were compared to establish whether they were identical and to estimate the part of the P1CM genome present in these plasmids. For this latter purpose, we attempted to rescue defined P1 genes by superinfecting the strains with P1 phages carrying conditional mutations in *c* genes. These mutations define genes required for products needed for the maintenance of lysogeny. The phages carry amber mutations and were passaged through *E. coli* 802. The phages selected for the study all form clear plaques on *E. coli* 802 as indicator and the mutations in *c* genes in these phages are therefore not suppressed by the suppressor mutation (*supE*) present in this strain. When strains harbouring plasmids were used as indicators, certain phages produced turbid plaques. The phages in these plaques were not further characterized and we cannot say whether the maintenance genes were rescued by recombination or complementation. Many of the plaques were extremely small and 'turbidity' was recognized as the presence of lysogenic colonies seen in the plaques when these were examined under a microscope (35 $\times$ magnification). The pattern of rescue of maintenance genes differed markedly between different strains of *E. coli* harbouring different plasmids. These data are summarized in Table 2 together with other phenotypic properties of the strains.

The data show that all the new *cml312* plasmids confer K7-sensitivity and none of them confers the P1 restriction-modification system. The other phenotypes vary. The effect on indole production is only manifest in three plasmids of those tested (pBL1005, pBL1010 and pBL1016). The greatest variation in the plasmids occurs in the region which contains P1 genes. These results show that a culture of *M. virescens* HP312 must be heterogeneous with respect to its plasmid complement and that the myxococcal-P1CM hybrid plasmids in these organisms form a mixture undergoing genetic recombination.

The data of Table 2 imply that extensive parts of the P1CM genome are present in pBL1005 and other *cml312* plasmids and hence, by implication, in *M. virescens* HP312. This was independently confirmed by DNA:DNA hybridization using radioactive P1CM phage DNA as a probe. Of the order of 70% of the P1CM sequences are present in the plasmids tested (pBL1005 and pBL1010) and of the same order of magnitude in *M. virescens* HP312 and also *M. virescens* HP315 *cml*. This latter organism is an independently isolated P1CM transductant of *M. virescens* HP300.

DISCUSSION

Our data show that recombinational events between phage P1CM DNA and an endogenous myxococcal replicon occurs within *M. virescens* HP312. Analysis of *E. coli* transformants with HP312 plasmid-enriched DNA generates a diverse set of plasmids as evidenced by the different properties of, for example, pBL1005, pBL1010 and pBL1024. This implies that strain HP312 contains an unstable population of plasmids. The transformation experiment effectively uses *E. coli* 802 to clone specific recombinants. Analysis of the P1CM markers present in the *cml312* plasmids shows that several recombinational events have occurred because there are gaps between P1 markers that can be rescued (Fig. 9).

In order to account for our findings we propose the following unified hypothesis for the fate of P1CM DNA in *M. virescens*. Strain HP300 is presumed to contain a replicon which can exist as a plasmid (possibly the plasmid is always present in HP300 cells; so far we have no experimental evidence on this point as no endogenous plasmid has been found). The replicon includes genes for incompatibility group W. Upon infection, P1CM recombines

Fig. 9. Summary of the presence or absence of certain P1CM genes in pBL*cml*312 plasmids. *hsdR* denotes restriction; other markers are defined by clear plaque mutations or chloramphenicol resistance. The order of the mutations is from Scott & Kropf (1977) and the positions on the physical map from Yarmolinsky (personal communication). A + indicates the presence and expression of a gene in a plasmid. A - indicates lack of expression. If it is assumed that this implies absence of the gene, there must be at least one region deleted between any + which are interspersed by one or more - in a horizontal line.

with the replicon by an unknown mechanism. If P1CM is unable to circularize in the unnatural host (which seems reasonable), then at least two recombinational events are required to generate a plasmid. This accounts in part for the fragmentation of the P1CM genome in the *cml*312 plasmids. Subsequent growth of HP312 and further site-specific recombination generates a mixture of segregants. There is direct evidence for this in the mixture of plasmid types in HP312 and the heterogeneity of the *cml*312 plasmids. We believe it is likely that the only plasmids which survive in *M. virescens* HP312 are those which use the myxococcal (as opposed to the P1) origin of replication. The instability of strain HP312 in the absence of chloramphenicol reflects the fact that chloramphenicol is selecting for those organisms in which there is association of the origin of the replicon in HP300 with the chloramphenicol gene of P1CM.

We have designed a method for cloning certain myxococcal plasmid genes in *E. coli*. The technique should prove of value in genetic studies on members of the genus *Myxococcus*. Of greater ecological importance is the process whereby previously independent incompatibility and resistance genes are brought together. Myxococci are widely distributed in surface soil, rotting vegetation and animal droppings. Plasmid pBL1005 *cml*312 and its relatives are new R factors constructed by association of a drug resistance element and certain linked genes (in this case P1 genetic markers) with a new incompatibility group, following genetic exchange (probably involving site-specific recombination) in such an organism. Bearing in mind the prevalence of myxococci in pasture land and the widespread use of antibiotic drugs in veterinary medicine, the mechanism is a clear indication of a likely process for the reassortment of drug resistance genes in the environment.

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