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IncP2 group of *Pseudomonas*, a class of uniquely large plasmids

IN *Pseudomonas* bacteria, the plasmids of the P-2 incompatibility group (IncP2) are notable for several reasons: they include both antibiotic resistance plasmids of nosocomial origin (R plasmids) and degradative plasmids that allow dissimilatory oxidative metabolism of unusual carbon sources; they are a naturally occurring high frequency transfer system with a host range restricted primarily to *Pseudomonas*; they are reported to be the most frequent incompatibility group of R plasmids found in nosocomial *Pseudomonas aeruginosa* isolates; and they show a wide geographical distribution[1–4]. Until now, their classification as plasmids has primarily been on the basis of genetic evidence: cotransfer of plasmid phenotypic markers independent of chromosome transfer; and co-curing of plasmid markers by another IncP2 plasmid. We have developed a novel method to permit routine physical isolation of these plasmids[5]. Using this method, a survey of the sizes of 12 different IncP2 plasmids reveals them to be notable in another way: they constitute an unique group of very large bacterial plasmids, generally having a homogeneous, unprecedented size range between approximately 280 and 312 megadaltons.

Our plasmid isolation method (summarised in Fig. 1) was developed from the hypothesis of Kline *et al.*[6] that quantitative nonintegrative attachment of stringently replicating plasmids to the bacterial folded chromosome acts as both the site and the mechanism of replication and segregation of the plasmid[6]. We therefore reasoned that the problem in isolating large plasmids was twofold: they must be separated from the folded chromosome and the procedures must be gentle to avoid hydrodynamic shearing. The inclusion of heat pulses, high sodium dodecyl sulphate concentration, and alkali denaturation in a gentle lysis procedure, permitted isolation of large plasmid DNA and its visualisation by agarose gel electrophoresis. Fortuitously, the denaturation step also reduces the amount of broken chromosomal DNA as seen in agarose gels[5]. Plasmids that are not covalently-closed, circular DNA, or plasmids that have alkali-sensitive relaxation complexes would not be recovered by our procedure.

In Fig. 1, two IncP2 plasmids, pMG1 and pMG5, were co-electrophoresed on a single agarose slab gel with four other smaller plasmids. The sizes of pMG1 and pMG5 listed in Table 2 were measured by electron microscopy of satellite DNA taken from an ethidium bromide–caesium chloride density equilibrium gradient. Agarose gel electrophoresis of that satellite DNA gave bands that comigrated with plasmid DNA prepared by our lysis method; thus, the IncP2 plasmid bands seen on agarose gel electrophoresis are considered to be supercoiled, covalently closed circular DNA[5]. In Fig. 1, the plasmid bands have migrated in accordance to size: the smallest plasmid, the LT2 cryptic (60 megadaltons) is furthest from the origin (well *a*), and the largest plasmid, pMG1 (312 megadaltons) has migrated the least distance (well *f*). A standard curve of the six plasmids in Fig. 1 constructed in the method of Meyers *et al.*[7] (log relative mobility against log molecular weight) does not appear linear for plasmids above about 140 megadaltons[5]. But we considered that we could use pMG5 and pMG1 as relative size standards in agarose slab gel electrophoresis to test whether the IncP2 plasmids were all of a similar size.

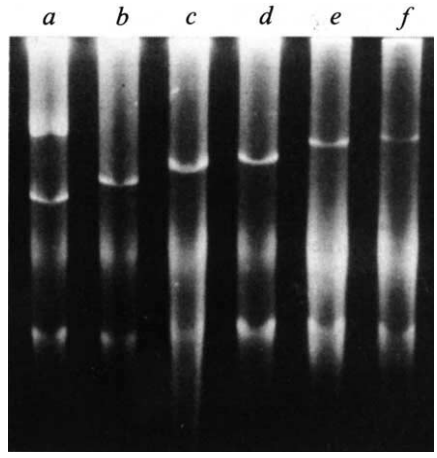

Fig. 1 Preparation of DNA for agarose gel electrophoresis is described in detail in ref. 5. Cells were grown in 40 ml complex broth medium to late log phase, washed and resuspended at high density in buffered 25% sucrose (*p*H 8·0). All subsequent mixing was done by slow, gentle inversion. To lyse cells, we added lysozyme and ethylenediaminetetraacetate, and then sodium dodecyl sulphate (SDS) to 4% final concentration. Eight repeated cyles of heat pulse and mixing produced a clear, viscous lysate. DNA was denatured at *p*H 12·1–12·3 by adding 3 M NaOH and mixing for 3 min at room temperature. Then, tris(hydroxymethyl)aminomethane (*p*H 7·0) was added to return the *p*H below 9·0. We added SDS to 4% final concentration, NaCl to 1·0 M, and mixed by 20 inversions; after 6 h at 4 °C, the salt-precipitated chromosome–membrane complexes were pelleted by centrifugation at 17,000*g* (4 °C, 30 min). The supernatant was mixed with polyethylene glycol 6000 to 10% concentration. After 6 h at 4 °C, the tubes were centrifuged at 700*g* (4 °C, 5 min). Resuspension of the resulting pellets in 0·15 ml cold buffer gave 'plasmid-enriched DNA solution'. Agarose slab gel electrophoresis, for 3 h at 100 V through 0.7% agarose (w/v), was carried out using the method of Meyers *et al.*[7]. Each well contained 25 μl of 'plasmid-enriched DNA solution' mixed with 10 μl Meyers tracking dye[7]. Gels were stained with ethidium bromide solution and visualised on an ultraviolet transilluminator. The distinct bands seen are plasmid DNA. The species of the bacterial strains used as input for DNA preparation are indicated by the beginning letters of the strain designation: AA or LT are *Salmonella typhimurium*, DT is *Escherichia coli*, and PAO is *P. aeruginosa*. Input strains for the different wells were: *a*, *LT2*; *b*, AA2103(F'80*his-gnd*); *c*, DT41(R27); *d*, DT78(TP116); *e*, PA038(pMG5); *f*, PA038(pMG1). LT2 contains the cryptic plasmid. DNA migration was from top to bottom. The photograph is a single slab gel, with multiple wells; thus, relative size comparisons can be made within the gel.

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Table 1 Selected plasmids of the P-2 incompatibility group

Plasmid	Relevant phenotype*	Species that originally harboured plasmid†	Geographical location and source of original plasmid-containing strain (date of earliest report‡)	Ref.
pMG1§	Tra^+TerHgSmSuGmUv	*P. aeruginosa*	Capetown, South Africa; burn unit clinical isolate; 1974	12¶
pMG2§	Tra^+TerHgSmSuGmUv	*P. aeruginosa*	Atlanta, Georgia; burn unit clinical isolate; 1974	12
pMG5§	Tra^+TerHgSuPmaKmTmAkBt	*P. aeruginosa*	Japan; clinical isolate; 1972	12
pMG6§	Tra^+TerHgSmSuCmGmKmTm	*P. aeruginosa*	Boston, Massachusetts; clinical isolate; 1978	13
Rms159§	Tra^+TerHgSmTcPmaCm	*P. aeruginosa*	Japan; 1976	12
R38§	Tra^+TerHgSmSuTcPma	*P. aeruginosa*	Matsumoto, Japan; urinary tract infection clinical isolate; 1972	12
R931**	Tra^+TerHgSmTcUv	*P. aeruginosa*	Alberta, Canada; clinical isolate; 1972	12
R3108**	Tra^+TerHgSmSuTcPma	*P. aeruginosa*	Alberta, Canada; clinical isolate; 1973	12
RPL11‖	Tra^+TerHgSmSuTcPmaCmGmCb	*P. aeruginosa*	Cincinati, Ohio; urinary tract infection clinical isolate; 1975	12
RPL11-JH4	RPL11 derivative sensitive to Gm, Tc			This lab.
Cam**	Tra^+TerUvCam	*P. putida*	Reported from Urbana, Illinois; 1959	12
OCT**	Tra^-Alk	*P. putida*	Ann Arbor, Michigan††; soil; 1963	12

* Phenotypes for fertility inhibition towards plasmids RP1 and FP2, and phage-growth inhibition of various *Pseudomonas* phages, are not included, but see ref. 12 for a partial list. The Ter phenotype comes from ref. 14; other phenotypes are from ref. 12. Phenotypic designations are as follows: mediating conjugation, Tra; biodegradation of camphor, Cam; biodegradation of alkanes, Alk; and resistances to telluri(a)te, Ter; mercuric ion, Hg; streptomycin, Sm; sulphonamide, Su; tetracycline, Tc; phenyl mercuric acetate, Pma; chloramphenicol, Cm; gentamicin, Gm; ultraviolet light, Uv; kanamycin, Km; carbenicillin, Cb; tobramycin, Tm; amikacin, Ak; butirosin, Bt.

† None of the plasmids used in this work were contained in their original hosts.

‡ The date of the earliest report of the strains from which the various IncP2 plasmids originated necessarily lags behind the actual date of isolation.

§ Plasmids obtained from G. A. Jacoby, Massachusetts General Hospital.

¶ This reference is a review compilation that includes citations of original research papers.

** Plasmids obtained from J. A. Shapiro, University of Chicago.

‖ Plasmid obtained from J. C. Loper, University of Cincinnati.

†† Personal communication, M. J. Coon, University of Michigan, Ann Arbor, Michigan.

The comparison of 12 different IncP2 plasmids on two agarose slab gels is seen in Fig. 2. Both gel 1 and gel 2 contain pMG5 and pMG1 as size standards (wells 1*b*, 1*i*, 2*e* and 2*f*). When PA038 does not contain either pMG5 or pMG1, the plasmid band is absent (well 1*a*). A similar control with a plasmid-free PU21 is seen in well 2*g*. Plasmid-free controls of PF0141 and PA02 also show no bands (unpublished data). The *P. putida* strains, PpS731, Pp1142 and Pp1169, are isogenic for chromosome, but differ in the IncP2 plasmids that they contain: variations in plasmid band mobility correlated with the presence of different plasmids. Thus, the bright bands seen in the two slab gels in Fig. 2 are IncP2 plasmid DNA (the lower diffuse fluorescence is the region of chromosomal pieces). In Fig. 2, gel 1, we see that the plasmids R931, R3108, RPL11 and RPL11-JH4 all migrated about as far as pMG5, whereas the Cam plasmid seemed to be closer in size to pMG1. In gel 2, we see that the IncP2 plasmids pMG2, pMG6, R38 and Rms159 all seem to migrate between pMG5 and pMG1. Measurement of relative mobilities in repeated gels confirms this visual inspection. Therefore, it seems that pMG1 and pMG5 conveniently define approximate upper and lower bounds for at least nine other IncP2 plasmids.

The exception to this was the OCT plasmid (Fig. 2, well 1*f*) which migrated faster than pMG5. Estimates of OCT's size by comparison on gels with the standards in Table 2 were subject to error, as the standard curve was non-linear and we do not have size standards between 143 and 280 megadaltons. But OCT is larger (migrates more slowly) than TP116 (unpublished data) and its probable size is between 180 and 230 megadaltons. OCT's smaller size compared with other IncP2 plasmids correlates with its transfer deficient (Tra^-) phenotype (see Table 1). There is evidence that the original parental OCT may have dissociated into three plasmids, OCT, MER and K (ref. 8). The K plasmid is Tra^+ and will mobilise the transfer of OCT; estimates of its size vary from 65–85 megadaltons[5,8,9]. Interestingly, if K and OCT were combined, that cointegrate would fit the size range of the other IncP2 plasmids we have observed. Fennewald *et al.*[10] also have evidence for the large size of IncP2 plasmids. They were not able to isolate unbroken plasmid DNA, but used restriction digests of the linear pieces of plasmid DNA obtained to establish a minimum size estimate of 200 megadaltons for several IncP2 plasmids.

There are reports of plasmids of smaller size isolated from strains that include various IncP2 plasmids, such as R931, Cam, OCT and pMG1 (refs 1, 5, 9). If smaller plasmids are present in our lysates, they must remain a minor proportion, as they are not evident in agarose gels (Figs 1 and 2). We feel that the large plasmids discussed here are the predominant and

Table 2 Plasmids used as size standards in agarose gel electrophoresis

Plasmid	Incompatibility group	Molecular size (megadaltons)	Refs
LT2 cryptic*	Unknown	60	15
F'80*his-gnd**	F_I	79	15
R27†	H1	112	16
TP116†	H2	143	16
pMG5‡	P2	280±15	5
pMG1‡	P2	312±17	5

* Plasmids obtained from H. J. Whitfield, University of Michigan Medical School.

† Plasmids obtained from D. E. Taylor, Hospital for Sick Children, Toronto, Ontario, Canada.

‡ See Table 1.

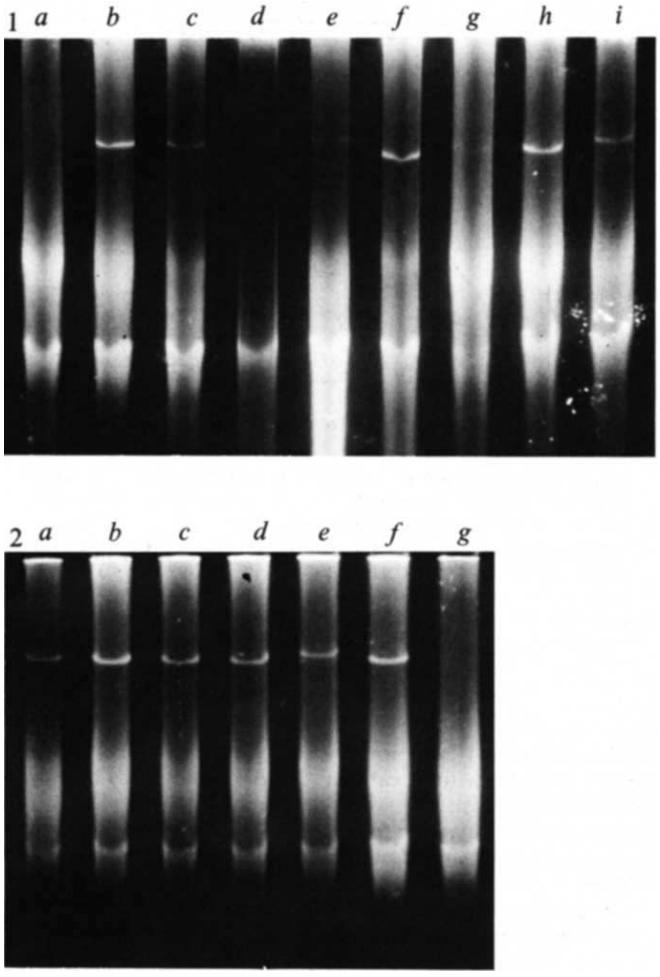

Fig. 2 Each well was loaded with 25 μl 'plasmid-enriched DNA solution' and 10 μl tracking dye. For preparation of DNA and electrophoresis conditions, see Fig. 1. The species of bacterial strains used as input for DNA preparation are indicated by the beginning letters of the strain designations: PA0 and PU are *P. aeruginosa*, PFO is *P. fluorescens*, and PpS is *P. putida*. Input strains for the different wells were: 1*a*, PA038; 1*b*, PA038(pMG5); 1*c*, PpS731 (contains R931 plasmid); 1*d*, PpS733 (contains R3108 plasmid); 1*e*, PpS1142 (contains Cam plasmid); 1*f*, PpS1169 (contains OCT plasmid); 1*g*, PF0141(RPL11); 1*h*, PA02(RPL11-JH4); 1*i*, PA038(pMG1); 2*a*, PU21(pMG2); 2*b*, PU21(pMG6); 2*c*, PU21(R38); 2*d*, PU21(Rms159); 2*e*, PA038(pMG1); 2*f*, PA038(pMG5); and 2*g*, PU21. PA038 and PU21 do not contain any IncP2 plasmids. Each photograph is a single slab gel, with multiple wells; thus, relative size comparisons can be made within each gel.

usual form. But it may well be that IncP2 plasmids can transiently produce smaller derivatives through occasional dissociation.

Inspection of Table 1 demonstrates the geographical diversity (spanning three continents) in origins of IncP2 plasmids. Notably, the two degradative plasmids, OCT and Cam, differ from the IncP2 R plasmids in two ways: they originated in a different species, a saprophytic soil pseudomonad, *P. putida*, and reports of their isolations predates those of the R plasmids by 10 years.

The molecular weight of the *P. aeruginosa* chromosome has been estimated at $2{\cdot}1\times10^9$ (ref. 11); thus the IncP2 plasmids are 10–15% of the magnitude of their host's genome. Several questions exist about this prototypical class of very large plasmid. Does not the comparison of their size with their known phenotypes suggest that the plasmids carry many other genes (perhaps 300 or more) the function of which is as yet unknown? Why is size so widely conserved among the IncP2 plasmids? What natural advantage accounts for their frequent appearance in nosocomial isolates? What is the relationship between IncP2 plasmids from soil isolates and those from the hospital? How much homology exists among the IncP2 plasmids?

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Chloroplast DNA codes for tRNA from cytoplasmic polyribosomes

CHLOROPLAST DNA (ctDNA) from *Euglena gracilis* contains 25–27 cistrons for tRNA[1,2] and two cistrons each for 16S and 23S tRNA[3], and 2.8% of the DNA codes for non-ribosomal RNA containing polyadenosine[4]. The tRNA from purified chloroplasts complements 18 cistrons, whereas whole cells contain species of tRNA complementing 7–9 additional cistrons[1]. Chromatography on DBAE-cellulose separates the tRNA into two fractions[1]. Uniquely modified bases are found in the fraction II tRNA. Isolated chloroplasts do not contain fraction II tRNA but this tRNA does hybridise to 7 or 8 cistrons on the ct DNA[1]. The present results demonstrate that fraction I and fraction II tRNAs are coded by separate and distinct cistrons and that cytoplasmic polyribosomes contain the fraction II tRNA. Further, the aminoacyl acceptor species in fraction II tRNA from whole cells and from cytoplasmic polyribosomes are the same, but the relative abundance of each species varies in the two samples.

A saturating level of total cellular tRNA was hybridised to ctDNA and competed with total cellular tRNA, 16S and 23S rRNA, or fraction I tRNA, (Fig. 1). All hybridisation reactions saturated the ctDNA within a precision of ±10% in 4–20 repetitions of the experiments. Typical results of competition reactions are shown in Fig. 1. The zero point, 100%, is equal to 25 cistrons. The total cellular tRNA competes to the theoretical level (91%, for a 10-fold excess of competing RNA) and validates the hybridisation reactions. The 16S and 23S rRNA (present in equivalent amounts) did not affect the hybridisation

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