Application of Agarose Gel Electrophoresis to the Characterization of Plasmid DNA in Drug-resistant Enterobacteria

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A simple gel electrophoresis method has been described for the detection of plasmid DNA in bacteria (Meyers *et al.*, 1976). We investigated further the problems encountered in using this method for the analysis of plasmids in wild enterobacterial strains. The migration of open circular and linear plasmid DNA was examined, since these forms sometimes caused difficulty in the interpretation of the plasmid content of uncharacterized strains. Electrophoresis at different agarose concentrations was employed to resolve clearly plasmid DNA from the chromosomal DNA fragments in the crude preparations. Dissociation of some plasmids occurs in *Salmonella typhimurium*, and this was detected by electrophoresis.

The technique was applied to the study of drug-resistant strains of *S. typhimurium* phage type 208 from several Middle Eastern countries. The cultures carry a drug resistance plasmid of the F_{me} compatibility group, and at least two other plasmids which were detected and identified by gel electrophoresis. The studies supported and extended the genetic findings and provided information on the distribution of particular plasmids.

INTRODUCTION

Epidemiological studies of pathogenic bacteria require techniques for the identification and characterization of different strains. Important properties, for example drug resistance, are frequently coded for by plasmids and these can be studied by several different methods. Genetic analysis, such as compatibility grouping, has been widely used to classify bacterial plasmids. Similarities or differences between plasmids identified in different strains may also be detected by molecular characterization including DNA reassociation studies.

Recently, the technique of agarose gel electrophoresis has been applied to the study of covalently closed circular (CCC) plasmid DNA (Meyers *et al.*, 1976). The method is rapid, relatively inexpensive and technically simple and is suitable for the examination of a large number of bacterial cultures. The plasmid content of several bacterial species has been examined by this method (Meyers *et al.*, 1976; Crosa *et al.*, 1977). However, there are limitations in the application of this technique in epidemiological studies in which it is important to determine unambiguously the number and sizes of plasmids in different wild strains. Such strains frequently carry up to five or six plasmids of different types and sizes. We have investigated the conditions for gel electrophoresis in an attempt to extend its use in the analysis of wild strains. In particular, we have studied the interference of chromosomal DNA fragments and of multiple plasmid DNA forms in the detection and identification of plasmids.

We have examined some drug-resistant strains of *Salmonella typhimurium* which originated in several Middle Eastern countries. The cultures all carry resistance plasmids of the F_{me}

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Table 1. *Standard plasmids*

Plasmid*	Drug resistance† or other markers	$10^{-6} \times$ Molecular weight‡	Reference
ColE1	colicin E1	4.7	Fredericq (1956)
NTP16	AK	5.7	Anderson <i>et al.</i> (1977)
NTP7	ASSu	9.4	Smith <i>et al.</i> (1974)
NTP14	A, colicin E1, <i>EcoRI</i> restriction and modification enzymes	11.4	Smith <i>et al.</i> (1976)
NTP106	K	14.5	This laboratory
RP1	AKT	37.8	Datta <i>et al.</i> (1971)
R1-19K-	ACSSu	54.2	Meynell & Cooke (1969)

* Plasmids with the prefix NTP are non-autotransferring.

† Symbols for drug resistances: A, ampicillin; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulphonamides; T, tetracyclines.

‡ Molecular weight determined by electron microscopy in this laboratory.

compatibility group (Threlfall *et al.*, 1976; Anderson *et al.*, 1977) and other plasmids coding for drug resistance. The plasmids present in these strains have been detected on gels and the number and sizes of plasmids present in cultures from the different countries have been compared. A preliminary account of these investigations has been published (Willshaw *et al.*, 1977).

METHODS

Bacterial strains and plasmids. Table 1 lists the standard plasmids employed in this investigation, their relevant genetic markers, including drug resistance, and their molecular weights as determined by electron microscopy. The sizes of plasmids used in molecular weight determinations are listed in the legends to the figures.

For the preparation of plasmid DNA, the plasmids concerned were usually introduced into standard carrier strains. These were *Escherichia coli* K12 F⁻ (= K12) or its nalidixic acid-resistant derivative, and a line of *S. typhimurium* phage type 36, which has lost the plasmid MP10 (Smith *et al.*, 1973). The recipient strain for transformation experiments was *E. coli* K12 C600. The origin and properties of the wild *S. typhimurium* strains from which plasmid DNA was prepared are given in Table 2.

Isolation of partially purified plasmid DNA. Plasmid-carrying strains of *E. coli* K12, *S. typhimurium* type 36 or wild *S. typhimurium* were grown in 50 to 200 ml nutrient broth. Bacteria were usually lysed with a mixture of Brij 58 and sodium deoxycholate (Clewell & Helinski, 1969); lysis with sodium dodecyl sulphate (Guerry *et al.*, 1973) was sometimes employed for *S. typhimurium* cultures. The procedure of Meyers *et al.* (1976) was the basis for the subsequent partial purification of plasmid DNA.

Preparation of pure plasmid DNA. Radioactively labelled plasmid DNA for electron microscopy was prepared as described by Willshaw *et al.* (1978). Large quantities of pure plasmid DNA for electrophoresis were obtained from cleared lysates of plasmid-carrying strains; plasmid DNA was precipitated with polyethylene glycol 6000 and purified by caesium chloride-ethidium bromide dye-buoyant density gradient centrifugation (Humphreys *et al.*, 1975).

Agarose gel electrophoresis of plasmid DNA. Electrophoresis was performed in vertical slab gels (16 × 15 × 0.4 cm) which usually contained 0.75% (w/v) agarose (BDH electrophoresis grade). Some plasmid DNA preparations were analysed at several agarose concentrations, as indicated in the text. The electrophoresis buffer, pH 8 to 8.2, contained 89 mM-Tris, 2.5 mM-EDTA and 89 mM-boric acid (Greene *et al.*, 1974).

Crude ethanol-precipitated DNA (2 to 100 µl) or pure plasmid DNA was mixed with a solution of 60% (w/v) sucrose and 0.25% (w/v) bromophenol blue in 0.03 M-Tris/HCl pH 8.0 containing 0.05 M-NaCl and 0.005 M-EDTA. Samples loaded on to gels contained 6 to 10% (w/v) sucrose. Electrophoresis was at a constant voltage of 140 V, a voltage gradient of 8.7 V cm⁻¹, for 2.5 to 4 h at room temperature. Gels were soaked for 30 min in a solution of ethidium bromide (0.5 µg ml⁻¹, in water) and viewed under long and short wavelength ultraviolet light (Chromatovue cabinet, Ultra Violet Products). DNA bands were photographed on Polaroid type 52 Land film or Kodak Tri-X, using a ×7 red filter.

Electron microscopy of plasmid DNA. Techniques for the examination of plasmid DNA have been described

Fig. 1. Separation of plasmid and chromosomal DNA at different agarose concentrations. Partially purified preparations were subjected to electrophoresis for 3.5 to 4 h at room temperature. The same preparations were run in tracks 1 to 4 at agarose concentrations of (a) 0.75 %, (b) 0.65 % and (c) 1.0 %. Tracks: 1, NTP106 (mol. wt 14.5×10^6); 2, NTP14 (11.4×10^6); 3, NTP7 (9.4×10^6); 4, standard plasmids of molecular weights (all $\times 10^6$) 4.7, 5.7, 20.2, 31.7, 64.0. Abbreviation: chr, chromosomal DNA fragments.

previously (Grindley *et al.*, 1973). The contour length of plasmid DNA was determined and molecular weights were calculated on the assumption that $1 \mu\text{m}$ is equivalent to a molecular weight of 2.07×10^6 (Lang, 1970).

Transformation of *E. coli* K12. Partially purified preparations containing plasmid DNA were dialysed for about 36 h against two changes of 75 mM-calcium chloride. Transformation of CaCl_2 -treated cells of *E. coli* K12 C600 was performed by the method of Cosloy & Oishi (1973) as modified by Humphreys *et al.* (1979).

RESULTS AND DISCUSSION

Separation of plasmid and chromosomal DNA

Partially purified plasmid DNA obtained by ethanol precipitation of phenol-treated cleared lysates contained plasmid DNA molecules which migrated on agarose gels generally as single bands at a rate inversely related to their molecular weights (Meyers *et al.*, 1976). The preparations also contained varying amounts of fragmented chromosomal DNA not removed in the preparation of cleared lysates of plasmid-carrying strains, and this banded as a broad diffuse area (see Fig. 1). The brightness and size of this region might obscure plasmid DNA bands over a significant range of molecular size and therefore interfere with the detection of plasmids in uncharacterized strains. We examined the separation of plasmid and chromosomal DNA bands using plasmids of molecular weight 9×10^6 to 15×10^6 which were partially purified from *E. coli* K12. They were subjected to electrophoresis on gels of different agarose concentration in order to alter the mobility of the plasmids relative to that of the linear chromosomal DNA fragments. It has been shown that the separation of the linear and CCC forms of the same plasmid DNA is highly dependent on gel strength (Johnson & Grossman, 1977).

Figure 1(a) shows migration of NTP7 (mol. wt 9.4×10^6), NTP14 (11.4×10^6) and NTP106 (14.5×10^6) on 0.75 % agarose. NTP14 appeared as a band running within the chromosomal DNA region (track 2) and NTP106 and NTP14 were just separated from this region (tracks 1 and 3). On 0.65 % agarose (Fig. 1b), the mobility of CCC DNA molecules was increased

Fig. 2. Determination of molecular weights of plasmid DNA by agarose gel electrophoresis. The logarithmic plots show molecular weight as a function of relative mobility for plasmid DNA of known size, partially purified from *E. coli* K12. (a) 0.75% agarose, taking the mobility of the plasmid Sa (25.9×10^6) as 10. Sizes of other plasmids (all $\times 10^6$): 4.7, 5.7, 20.2, 31.7, 37.8, 64.0, 77.6, 100, 123, 144. (b) 0.65% agarose (○), taking the mobility of 5.7×10^6 plasmid as 10, and 1.0% agarose (●), taking the mobility of 20.2×10^6 plasmid as 10. Sizes of other plasmids (all $\times 10^6$): 3.1, 3.7, 4.7, 5.7, 9.4, 20.2, 31.7, 37.8, 48.0, 64.0, 77.6.

relative to that of chromosomal DNA fragments, which extended over a position corresponding to 12×10^6 to 17×10^6 . The plasmid NTP7 was therefore clearly resolved ahead of the chromosome (track 3) while NTP106 migrated within the chromosomal region (track 1). By electrophoresis on 1% agarose, both NTP106 and NTP14 could be separated from chromosomal fragments (Fig. 1c, tracks 1 and 2), whereas on this gel NTP7 migrated just behind the chromosomal region (track 3).

Analysis of plasmid DNA preparations from wild and other strains was initially performed on 0.75% agarose gels. These conditions gave a reliable estimate of molecular sizes over a wide range (3×10^6 to 80×10^6) for which molecular weight and relative mobility were linearly related (Fig. 2a). Within this range, plasmid sizes determined by electrophoresis were within 10% of the values obtained by electron microscopy. For larger plasmids, up to 144×10^6 , the linear relationship was no longer valid and the resolution of plasmid species was reduced. Preparations of plasmid DNA were subsequently run on 1.0 or 0.65% gels if the presence of plasmids within or near the chromosomal region was suspected. With 1% agarose, plasmid mobility was a linear function of molecular size from 3×10^6 to approximately 50×10^6 (Fig. 2b) and these conditions were not used for the determination of the size of large plasmids. Molecular weights in excess of 80×10^6 could be determined on 0.65% gels, but at this agarose concentration the mobilities of plasmids smaller than 10×10^6 were less than would be predicted from a linear relationship between molecular weight and relative mobility (Fig. 2b).

Recently an isolation procedure has been described which is specifically adapted to the preparation of large plasmids up to 300×10^6 (Hansen & Olsen, 1978). In this technique, further steps were introduced to release plasmid DNA from the folded chromosome and to reduce contamination of plasmid DNA with chromosomal fragments. However, the alkaline denaturation involved lowered the yield of some plasmids and this may limit the application of the method in the analysis of wild strains.

Detection of open circular and linear plasmid DNA

It has been shown that open circular (OC) DNA generally migrates more slowly than the covalently closed circular form (Aaij & Borst, 1972; Johnson & Grossman, 1977). The relative mobilities of CCC and linear forms of plasmid DNA depend on gel concentration,

Fig. 3. Electrophoresis of covalently closed circular and open circular plasmid DNA. DNA of NTP16 (5.7×10^6) and ColE1 (4.7×10^6) was purified from plasmid-carrying lines of *E. coli* K12 by centrifugation of polyethylene glycol-precipitated cleared lysates in caesium chloride gradients containing ethidium bromide (Humphreys *et al.*, 1975). Gradient fractions containing CCC or OC plasmid DNA molecules were dialysed against 0.01 M-Tris/HCl pH 8.0 containing 1 mM-EDTA. Samples were subjected to electrophoresis on 0.75% agarose for 3.5 h at room temperature; partially purified plasmid DNA preparations were run on the same gel. Tracks 1 to 3 show NTP16: 1, partially purified DNA; 2, OC DNA; 3, CCC DNA. Tracks 4 to 6 show ColE1: 4, partially purified DNA; 5, OC DNA; 6, CCC DNA. Abbreviations: lin, linear plasmid DNA; chr, chromosomal DNA fragments.

electric field strength and DNA size (Johnson & Grossman, 1977). During electrophoresis of freshly made DNA preparations from strains which carried a single plasmid of less than 10×10^6 in size, we frequently observed a slow-moving band in addition to the predominant fast-moving species which was presumably CCC plasmid DNA. Analysis of the plasmids in wild strains of *E. coli* and *S. typhimurium* also showed many bands moving ahead of the chromosomal DNA region. In this situation, it was important to determine the number of independent plasmids, and whether certain plasmids were present in the OC or linear configurations, as well as the CCC form. We therefore investigated the migration of different molecular forms of some standard plasmids under the conditions most commonly used for examination of wild strains.

Plasmid DNA of ColE1 (mol. wt 4.7×10^6) and NTP16 (5.7×10^6) was purified on caesium chloride-ethidium bromide density gradients and samples containing CCC or OC plasmid molecules were subjected to electrophoresis. Preparations of CCC DNA (Fig. 3, tracks 3 and 6) showed a bright band, which corresponded to the faster moving band in tracks 1 and 4 which contained partially purified preparations of ColE1 or NTP16. Samples of OC DNA (tracks 2 and 5) contained predominantly molecules which migrated at the same position as the slower band in tracks 1 and 4. In the ColE1 preparations (tracks 5 and 6), the faint band between OC and CCC forms contained linear plasmid molecules (see below). Thus, the OC form of plasmids within the size range 4×10^6 to 6×10^6 migrated ahead of chromosomal DNA fragments during electrophoresis of partially purified

Fig. 4. Mobility of linear plasmid DNA on 0.75% agarose. Both ColE1 (4.7×10^6) and RP1 (37.8×10^6) have a single site for cleavage by *Eco*RI endonuclease (Lovett *et al.*, 1974; Jacob & Grinter, 1975). Preparations of pure or partially purified DNA of these plasmids were dialysed against 0.01 M-Tris/HCl pH 8.0 containing 1 mM-EDTA. Samples were digested with *Eco*RI (Miles Laboratories) by the method of Greene *et al.* (1974). Enzyme-treated DNA was subjected to electrophoresis on 0.75% agarose together with samples of the undigested plasmid preparations. Tracks: 1, Control – phage λ DNA treated with *Eco*RI; 2, partially purified RP1 plus *Eco*RI; 3, partially purified RP1, untreated; 4, pure ColE1 plus *Eco*RI; 5, untreated pure ColE1; 6, partially purified ColE1 plus *Eco*RI; 7 and 8, partially purified ColE1, untreated. Abbreviation: lin, linear plasmid DNA.

preparations under our conditions. In this position, the band could correspond to the CCC form of a plasmid with a molecular weight of 8×10^6 to 11×10^6 . Since plasmids of 4×10^6 to 6×10^6 are common in wild strains (Smith *et al.*, 1974; Crosa *et al.*, 1977; see below) the presence of OC plasmid DNA is likely to complicate the interpretation of results.

In vitro techniques for the conversion of CCC to OC molecules, such as treatment with pronase, sodium dodecyl sulphate (SDS) or DNAase, have limited application to DNA preparations containing several plasmids. Electrophoresis in the presence of different concentrations of ethidium bromide has been proposed as a method for differentiation of CCC, linear and OC DNA (Johnson & Grossman, 1977). As the ethidium bromide level is increased, the relative migration of the DNA configuration changes. This effect depends on the molecular size of the plasmid studied, so that the technique would be difficult to apply to wild strains. The most useful approach we found in the identification of plasmids present in more than one form in wild strains was to correlate the loss of properties, such as drug resistance or phage restriction, with the disappearance of more than one of the observed bands.

In contrast to our observations on small plasmids, OC molecules were not usually detected during electrophoresis of plasmids larger than 20×10^6 , even after prolonged storage, repeated freezing and thawing of the preparations or treatment with SDS or pronase. Some plasmids, especially I-like R factors within the size range 50×10^6 to 60×10^6 , gave a low yield of CCC DNA. The DNA of these plasmids readily undergoes conversion from the CCC to the OC form (Clewell & Helinski, 1969; Humphreys *et al.*, 1972). These large OC molecules

Fig. 5. Dissociation of plasmid DNA in *S. typhimurium*. DNA of the plasmid R1-19K⁻ was partially purified from strains of *E. coli* K12 and *S. typhimurium* 36. Samples of the preparation from each host were subjected to electrophoresis on 0.85% agarose for 4 h at room temperature. Tracks: 1, standard plasmids of molecular weights (all $\times 10^6$) 4.7, 25.9, 37.8, 48.0, 77.6; 2, standard plasmids of molecular weights (all $\times 10^6$) 5.7, 20.2, 25.9, 31.7, 64.0; 3, R1-19K⁻ in *E. coli* K12; 4, transfer factor isolated in *S. typhimurium* after loss of R1-19K⁻ resistance markers; 5 and 6, R1-19K⁻ in *S. typhimurium* – track 6 also contains a reference plasmid of molecular weight 25.9×10^6 .

may not easily enter 0.75% agarose, resulting in an area of bright fluorescence which was often observed at the origin of the gel.

The position on 0.75% agarose of linear plasmid DNA relative to the CCC and OC form depended on the molecular size of the plasmid and is illustrated in Fig. 4 for ColE1 (4.7×10^6) and RP1 (37.8×10^6). Linear ColE1 molecules (tracks 4 and 6) banded between CCC and OC plasmid DNA (tracks 5, 7 and 8), an observation similar to that of Johnson & Grossman (1977) for molecules up to 11×10^6 in size. However, the linear form of RP1 (track 2) banded ahead of CCC DNA (track 3), in the same position on the gel as chromosomal DNA fragments.

Electrophoresis of plasmid DNA isolated from different bacterial hosts

The molecular nature of some resistance plasmids depends on the bacterial host which harbours them. F-like R factors, such as R1, R6 and R100, are isolated predominantly in a single CCC form from *E. coli* K12. In *Proteus mirabilis*, the DNA of these plasmids consists of three molecular species of different sizes. These correspond to the complete R factor, which dissociates to give the transfer factor region of the molecule and a region, termed the r-determinant, containing some or all of the drug resistance genes (for review, see Rownd *et al.*, 1975). The plasmid R100, and other R factors coding for resistance to chloramphenicol, streptomycin and sulphonamides, also dissociate into three DNA species in *S. typhimurium*, but usually exist as a single molecular species in *E. coli* K12 (Sheehy *et al.*, 1973; Humphreys *et al.*, 1974; Humphreys, 1977).

Dissociation of plasmid DNA in *S. typhimurium* could be demonstrated by agarose gel

Table 2. *Properties of Salmonella typhimurium strains*

Strain no.	Origin	Phage type	Resistance type	Resistance plasmids carried		
				F _{1me} auto-transferring	F _{1me} non-auto-transferring	Other non-auto-transferring
14M6407	Britain, 1974*	208	ACKSSuT	—	ACSSuT	A, SSu
15M1708	Iran, 1975	208	ACKSSuT	—	ACSSuT	A, SSu
15M3557	Iran, 1975	208	ACKSSuT	ACKSSuT	—	AK, SSu
14M398	Israel, 1974	208	ACKSSuT	—	ACSSuT	AK, SSu
16M5937	Turkey, 1976	Untypable†	ACKSSuT	—	ACSSuT	AK, SSu

See Table 1 for definitions of drug resistance symbols.

* This strain was isolated from a patient who was infected in Iran (Anderson *et al.*, 1977).

† The untypable strain carries a small-plaque temperate phage that converts type 208 into the untypable strain (Anderson *et al.*, 1977).

Table 3. *Plasmids present in wild Salmonella typhimurium strains*

Original <i>S. typhimurium</i> strain	10 ⁻⁶ × Molecular weight of plasmid					
	F _{1me}	A	AK	SSu	Other	
14M6407 Britain	83	4.3	—	5.7	—	—
15M1708 Iran	90	4.5	—	5.8	3.2	3.9
15M3557 Iran	90	—	5.7	5.7	3.2	—
14M398 Israel	92	—	5.8	5.8	—	4.0
16M5937 Turkey	81	—	5.9	5.8	3.0	4.2

See Table 1 for definitions of drug resistance symbols.

electrophoresis. Cultures of *E. coli* K12 or *S. typhimurium* phage type 36 carrying the F_{II} plasmid R1-19K⁻ (Table 1) were lysed with SDS; partially purified plasmid DNA was prepared and subjected to electrophoresis on a 0.85% agarose gel (Fig. 5). DNA of R1-19K⁻ from *E. coli* K12 yielded a single plasmid band corresponding to a molecular weight of 57×10^6 (track 3). When the host strain was *S. typhimurium*, three plasmid DNA bands were visible, with molecular sizes of 57×10^6 , 41×10^6 and 16×10^6 (tracks 5 and 6; track 6 also contained a standard plasmid of molecular weight 26×10^6). The largest species present in *S. typhimurium* was the complete R1-19K⁻ molecule, as observed in *E. coli* K12. The band of molecular weight 41×10^6 represented the transfer factor of R1-19K⁻; this was isolated as a separate plasmid in *S. typhimurium* after loss of the drug resistance markers of the R factor and migrated as a single band of 41×10^6 (track 4). The resistance determinant region of R1-19K⁻, with a molecular weight of 16×10^6 , was clearly resolved from chromosomal DNA fragments on 0.85% agarose (tracks 5 and 6). In the examination of the plasmid content of uncharacterized strains by gel electrophoresis, the ability of some plasmids to exist in multiple species of different sizes in some hosts may complicate interpretation of the results.

Application of agarose gel electrophoresis to studies of drug-resistant salmonellae

A large number of drug-resistant *S. typhimurium* strains of predominantly Middle Eastern origin have been studied recently in this laboratory (Threlfall *et al.*, 1976; Anderson *et al.*, 1977). The majority of cultures belong to phage type 208 and they probably represent a

Fig. 6. Identification of plasmids in strains of *S. typhimurium* phage type 208 of Middle Eastern origin. Plasmid DNA was partially purified from the wild *S. typhimurium* strains and from plasmid-carrying *E. coli* K12 lines derived by transfer or transformation experiments. Samples were subjected to electrophoresis for 3.5 h on 0.75% agarose. Tracks 1 to 3 demonstrate small non-autotransferring plasmids in strain 14M6407: 1, wild *S. typhimurium*; 2, *E. coli* K12 transconjugant containing A and SSu resistance plasmids – the faint band just ahead of the chromosomal DNA region corresponds to the open circular form of the A plasmid; 3, *E. coli* K12 transconjugant with SSu resistance plasmid only. Tracks 6 to 9 show small non-autotransferring plasmids in strain 14M398: 6, wild *S. typhimurium*; 7, *E. coli* K12 transconjugant resistant to ampicillin and kanamycin, and containing two plasmid species; 8, *E. coli* K12 transformant with SSu plasmid only; 9, *E. coli* K12 transformant with AK plasmid only. DNA samples subjected to electrophoresis for demonstration of plasmids of less than 10×10^6 were too small for detection on the gel of the $F_{I}me$ plasmids present in the strains. The molecular sizes of these plasmids were determined separately and are shown in Table 3. Preparations of standard plasmid DNAs were run in tracks 4 and 5; the molecular sizes of some of these reference plasmids are indicated on the figure.

clone of wide geographical distribution. Most strains carry a resistance plasmid belonging to the $F_{I}me$ compatibility group which contains both autotransferring and non-autotransferring members. Molecular studies of $F_{I}me$ plasmids demonstrated that they have molecular weights, determined by electron microscopy, of between 88×10^6 and 103×10^6 and show a high degree of DNA homology (Willshaw *et al.*, 1978). In addition to the $F_{I}me$ plasmid, these *S. typhimurium* strains carry A or AK and SSu non-autotransferring resistance plasmids.

The properties of five wild *S. typhimurium* strains from different countries are shown in Table 2. Partially purified plasmid DNA preparations from these strains were examined by gel electrophoresis. All five strains each carried only one large plasmid, which belonged to group $F_{I}me$; the approximate molecular weights determined by electrophoresis on 0.75% agarose were between 81×10^6 and 92×10^6 (Table 3). In addition to $F_{I}me$, at least two other plasmid DNA species were present in each of the strains; the sizes of these plasmids are shown in Table 3. *Escherichia coli* K12 derivatives were then examined to identify the DNA bands corresponding to the A, AK and SSu plasmids listed in Table 2.

The A, AK or SSu plasmids were mobilized into *E. coli* K12 with the I-like R factor T- Δ in triparental crosses (Anderson, 1965). Plasmids present in recipient lines were examined by electrophoresis and compared with those detected in the five wild strains. With all five strains, *E. coli* K12 derivatives containing more than one species of small plasmid were obtained, and the identities of the different plasmids could not be determined with certainty. Partially purified plasmid DNA preparations from these strains were used

to transform *E. coli* K12 C600 selecting for resistance to ampicillin, kanamycin or streptomycin. The transformants were analysed by electrophoresis, and usually only one plasmid which coded for drug resistance was present in each strain. Electrophoresis of preparations from two strains and *E. coli* K12 derivatives is shown in Fig. 6 and the plasmid content of all five *S. typhimurium* strains is summarized in Table 3. Four of the strains carried plasmids which code for no known function and were of at least two sizes. These 'cryptic' plasmids were, in some cases, mobilized into *E. coli* K12 together with the A, AK or SSu plasmids but were not usually present in transformants selected for drug resistance.

The similarities in plasmid content of the strains from the different countries support the view that a single clone of *S. typhimurium* phage type 208 has been widely distributed (Anderson *et al.*, 1977). However, agarose gel electrophoresis has demonstrated differences in the number and size of the small plasmids present, and these changes have probably occurred during spread of the strain. These investigations emphasize the value of gel electrophoresis, in conjunction with genetic studies, for the identification of the plasmid content of enterobacteria. The techniques add to the range of methods now available for the epidemiological study of infection caused by these organisms.

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