Characterization of Plasmids from Antibiotic-resistant *Shigella* Isolates by Agarose Gel Electrophoresis

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Gel electrophoresis of DNA from 95 clinical isolates of *Shigella sonnei* and *Shigella flexneri* resistant to antibiotics revealed a heterogeneous plasmid population. Most of the plasmids were smaller than 6 megadaltons (Mdal). Six *S. sonnei* isolates with the most common antibiotic resistance pattern were characterized. They had two plasmids in common: one was a self-transmissible Fi^+ plasmid of 46 Mdal encoding tetracycline resistance, while the other was a 5.5 Mdal non-conjugative plasmid encoding resistance to streptomycin and sulphafurazole. In addition, several cryptic plasmids ranging in size from 1.0 to 24.5 Mdal were present. Mobilization of the 5.5 Mdal SuSm plasmid and a 1.0 Mdal cryptic plasmid was demonstrated with all six *S. sonnei* isolates during conjugation. This mobilization was mediated by the 46 Mdal self-transmissible Fi^+ R plasmid and also by a 24.5 Mdal Fi^- plasmid carrying no known drug resistance determinants.

INTRODUCTION

Recent studies (see, for example, Møller *et al.*, 1978) have shown that most naturally occurring strains of the Enterobacteriaceae contain small circular non-conjugative plasmids ranging in size from 0.8 to 10 megadaltons (Mdal). The molecular weight and base composition of these plasmids vary even within a single bacterial species (Rush *et al.*, 1969; Møller *et al.*, 1978). The effects of some of these plasmids have been determined, for example, drug resistance (Smith *et al.*, 1974), colicin production and immunity (Porter *et al.*, 1973) and antigen production (Bak *et al.*, 1972). The role of the remainder is not established although some may contribute to pathogenicity (Christiansen *et al.*, 1973; Falkow, 1975).

The widespread occurrence of these small molecules could be explained by efficient transfer systems responsible for their dissemination. Considerable genetic information is required to code for a complete conjugation system. For example, the transfer operon of the sex factor F encompasses 15 Mdal of DNA (Achtman & Helmuth, 1975). Thus plasmids considerably smaller than 15 Mdal might be unable to transfer their DNA by conjugation unless they were mobilized by a self-transmissible plasmid. Mobilization of some of these small plasmids encoding drug resistance or colicin production by large self-transmissible plasmids has been observed (Smith & Linggood, 1970).

The use of gel electrophoresis techniques (Meyers *et al.*, 1976) has simplified the detection of small plasmids conferring no observable phenotype. In this paper, we describe the use of these techniques to examine the varied plasmid population which exists in *Shigella* isolated from clinical material, and to characterize systems responsible for the transfer of small non-conjugative plasmids among these bacteria.

METHODS

Shigella sonnei and *Shigella flexneri* isolates were obtained from the New Zealand National Health Institute, Wellington, or isolated from patients at Auckland Hospital. These isolates were resistant to one or more of the following agents: sulphafurazole (minimum inhibitory concentration $> 500 \mu\text{g ml}^{-1}$), ampicillin, tetracycline, chloramphenicol, streptomycin and cephaloridine (minimum inhibitory concentration for each $> 32 \mu\text{g ml}^{-1}$).

Transfer of plasmids from *S. sonnei* to *Escherichia coli* K12 was attempted by conjugation with nalidixic acid-resistant derivatives of J5-3 (F^- *met-63 pro-22*) or PB1395 (F^- *met supE supF hsdR*) as described by Bremner (1978). Transformation of plasmids to C600 (F^- *thi-1 thr-1 leu-6 lac Y1 supE44 tonA21*) or PB1395 was done essentially as described by Mandel & Higa (1970). PB501 (F^- *galT12*) was used as the recipient in K12 $\times$ K12 crosses.

The fertility-inhibiting (Fi) character of conjugative *Shigella* plasmids was assessed by examining ex-conjugant strains containing the conjugative plasmid and F' -*gal*⁺ (F8) for susceptibility to lysis by the F-specific phage R17.

Plasmid DNA was prepared by the method of Meyers *et al.* (1976). Care was taken to prevent shearing of chromosomal DNA before its removal by differential salt precipitation. This precaution significantly reduced the concentration of chromosomal DNA present in cleared lysates, allowing detection of 6 to 10 Mdal plasmids which can be obscured by chromosomal DNA. Open circular and linear DNA were removed by the method of Zasloff *et al.* (1978).

Digestion of plasmid DNA by restriction endonuclease *Eco*R1 was carried out at 37°C for 1 h in a reaction mixture containing 20 μl DNA (dissolved in 10 mM-Tris/HCl, pH 8.0, 1 mM-EDTA), 2 μl concentrated enzyme buffer (0.9 M-Tris/HCl, pH 7.4, 0.1 M-MgCl₂) and 2 μl *Eco*R1 (1 unit μl^{-1} , prepared as described by Tanaka & Weisblum, 1975). Agarose gel electrophoresis was carried out in a horizontal gel apparatus similar to that described by McDonnell *et al.* (1977) for 3 h at 135 V in 0.7 or 0.85 % (w/v) agarose (Sigma A6877) in borate buffer (Meyers *et al.*, 1976). After electrophoresis, gels were placed on a Blak-Ray UV transilluminator screen (Ultraviolet Products, San Gabriel, Calif., U.S.A.) and photographed with Agfapan 400 film, which was developed using a reversal process.

Radioactive labelling of RSF1010 (R300b) DNA from PB1379 (RSF1010/ F^- *thyA36 deoC2*) with [³²P]dATP was by nick translation as described by Jeppesen *et al.* (1976). Hybridization of ³²P-labelled DNA to plasmid DNA separated in agarose gels and transferred directly to nitrocellulose filters was carried out using a modification of the method of Southern (1975). Hybridization was in 50 % formamide/2 $\times$ SSC buffer (SSC is 0.15 M-NaCl/0.015 M-trisodium citrate).

RESULTS

Demonstration of a heterogeneous plasmid pool in clinical isolates of Shigella

Antibiotic-resistant isolates of *Shigella sonnei* and *Shigella flexneri* obtained from geographically distinct areas of New Zealand between 1972 and 1976 were screened for the presence of plasmid DNA. Cleared lysates from 95 isolates were electrophoresed in agarose gels. Figure 1 shows the distribution of the molecular weights of plasmid molecules from these isolates. All isolates examined contained at least two plasmids of different molecular weights; some contained as many as ten. Although sizes ranged from 1 to 70 Mdal, the majority of plasmids were smaller than 6 Mdal.

Open circular (OC) as well as covalently closed circular (CCC) forms of the plasmid molecules were present in cleared lysates prepared as described by Meyers *et al.* (1976). Open circular DNA was distinguished from CCC DNA by removal of OC plasmid (and linear chromosomal) DNA by extraction with acid phenol (Zasloff *et al.*, 1978) as shown in Fig. 2.

Molecular weight determination of the plasmids from six antibiotic-resistant S. sonnei isolates

To investigate the extent of mobilization of small plasmids from *Shigella*, we examined six isolates each containing plasmids ranging in size from 1 to 45 Mdal. These isolates were representatives of the predominant class of antibiotic-resistant *S. sonnei* detected between

Fig. 1. Histogram of the distribution of molecular weights of CCC plasmid molecules from 95 *S. sonnei* and *S. flexneri* isolates resistant to one or more antibiotics. Molecular weights were calculated from relative electrophoretic mobilities as described in Fig. 3.

Table 1. *Molecular weights of plasmids present in six antibiotic-resistant S. sonnei isolates*

Molecular weights were determined as described in Fig. 3. The antibiotic resistance pattern of all isolates was ApTcSuSm.

Isolate	Plasmids present (Mdal)									
	46	5.5	4.8	4.2	3.7	2.0	1.9	1.6	1.2	1.0
SH1	46	5.5			3.7		1.9	1.6	1.2	1.0
SH7	46	5.5	4.8	4.2	3.7	2.0	1.9	1.6	1.2	1.0
SH16	46	5.5	4.8				1.9	1.6		1.0
SH20	46	5.5					1.9	1.6	1.2	1.0
SH28	46	24.5	5.5		3.7			1.6	1.2	1.0
SH34	46	24.5	5.5				1.9	1.6	1.2	1.0

1972 and 1974 and were resistant to ampicillin (Ap), tetracycline (Tc), sulphafurazole (Su) and streptomycin (Sm). Table 1 lists the molecular weights of the plasmids present in the isolates as determined by gel electrophoresis. Wells B and C of Fig. 3 show DNA from two isolates: strain SH34 (well C), for example, contains seven plasmids (46, 24.5, 5.5, 1.9, 1.6, 1.2 and 1.0 Mdal).

Identification of plasmids specifying antibiotic resistance

R plasmids were identified following transformation of antibiotic resistance to *Escherichia coli* K12. Tc and SuSm resistance could be transferred independently to *E. coli* by transformation of C600 with DNA from each of the six *S. sonnei* isolates. Gel electrophoresis of DNA from the Tc^r and SuSm^r transformants revealed that the plasmids present in the transformed cells were of 46 and 5.5 Mdal, respectively. Transformation of ampicillin resistance to either C600 or PB1395, a restriction-negative (*hsdR*) mutant of *E. coli*, was not observed.

Characterization of R plasmids

Comparison of the numbers and sizes of the DNA fragments produced by digestion of any two plasmids by a sequence-specific nuclease can be used as a measure of the relatedness of the plasmids (Thompson *et al.*, 1974). The 46 Mdal Tc R plasmids from each of the six

Fig. 2

Fig. 3

Fig. 2. Electrophoresis in 0.7% agarose of DNA from a cleared lysate of *S. sonnei* isolate SH34 before and after extraction with acid phenol. Arrows indicate the slower running OC DNA. The CCC DNA of the fourth smallest plasmid obscures the OC DNA of the second smallest plasmid. Chr indicates the banding position of chromosomal DNA. A: SH34 DNA before acid phenol extraction. B: One acid phenol extraction of the SH34 cleared lysate in well A resulted in removal of > 90% of OC and linear DNA. The efficiency of recovery of plasmid DNA decreased with increasing size for plasmids greater than 25 Mdal (see 46 Mdal plasmid).

Fig. 3. Electrophoresis in 0.7% agarose of DNA from cleared lysates of *S. sonnei* isolates resistant to Ap, Tc, Su and Sm. Arrows indicate OC DNA not obscured by CCC DNA. Chr indicates the banding position of chromosomal DNA. Molecular weights were calculated from the mobilities of plasmids relative to the mobilities of the plasmids of known molecular weights in well A. Additional estimates of the sizes of the 46, 5.5 and 1.0 Mdal plasmids in wells B and C were obtained by comparison of the mobilities of their linear fragments produced on digestion by *Eco*R1 with the markers derived from *Eco*R1 digestion of R100-1 and bacteriophage λ (see, for example, Figs 4 and 5). A: DNA standards; R100-1 (60 Mdal), R6K (26 Mdal), pMB9 (3.8 Mdal) and pBR322 (2.5 Mdal). B: DNA from *S. sonnei* isolate SH1; 46, 5.5, 3.7, 1.9, 1.6, 1.2 and 1.0 Mdal plasmids present. C: DNA from *S. sonnei* isolate SH34; 46, 24.5, 5.5, 1.9, 1.6, 1.2 and 1.0 Mdal plasmids present.

S. sonnei isolates gave identical sets of fragments on digestion with *Eco*R1 (Fig. 4). The patterns are similar to the *Eco*R1 pattern for the RTF portion of R100-1 obtained by Chandler *et al.* (1977). Similarly, the 5.5 Mdal SuSm R plasmids present in each of the six isolates were all cut once with *Eco*R1 to give a single fragment (Fig. 5). In addition, 32 P-labelled DNA from the RSF1010 (R300b) plasmid isolated by Grinter & Barth (1976) hybridized to each of the 5.5 Mdal SuSm plasmids from the six *S. sonnei* isolates, demonstrating a high degree of homology between these plasmids (Fig. 6).

The 46 Mdal plasmid was shown to be Fi^+ and could be transferred to a second strain of *E. coli* K12 (PB501) by conjugation. The 5.5 Mdal plasmid was not self-transmissible and could not be transferred to PB501 by conjugation from a strain containing this plasmid alone.

Fig. 4. Electrophoresis in 0.85 % agarose of *Eco*R1 digests of 46 Mdal Tc R plasmids from *S. sonnei* isolates. A: *Eco*R1-digested λ DNA. B: *Eco*R1-digested R100-1 DNA. C: *Eco*R1-digested DNA of the 46 Mdal R plasmid present in a tetracycline-resistant *E. coli* constructed by transformation of C600 with SH1 DNA. D: *Eco*R1-digested DNA of the 46 Mdal plasmid from SH34. The 46 Mdal plasmids from all six *S. sonnei* strains examined gave identical sets of linear fragments on digestion with *Eco*R1.

Fig. 5. Electrophoresis in 0.85 % agarose of *Eco*R1 digests of 5.5 Mdal plasmids from *S. sonnei* isolates SH1 and SH34. A, B: Undigested DNA of 5.5 Mdal SuSm plasmids from *E. coli* C600 transformed with DNA from cleared lysates of SH1 (well A) and SH34 (well B). C: *Eco*R1-digested λ DNA. D, E: *Eco*R1-digested DNA of the plasmids shown in wells A and B. Both the OC and CCC forms were cleaved once to give a 5.5 Mdal fragment.

Fig. 6. Hybridization of ³²P-labelled RSF1010 DNA to SuSm plasmids from *S. sonnei* isolates. (a) Bands present after electrophoresis of the SuSm plasmids through a 0.85% agarose gel before transfer to a nitrocellulose filter. OC is 5.5 Mdal open circular DNA, CCC is 5.5 Mdal covalently closed circular DNA, Chr marks the banding position of chromosomal linear DNA and Ori marks the origin. (b) Autoradiograph of the nitrocellulose filter from the gel in (a) after hybridization with ³²P-RSF1010 DNA. A: RSF1010. B to G: SuSm plasmids from SH1, SH7, SH16, SH20, SH28 and SH34, respectively.

Mobilization of non-conjugative Shigella plasmids

Conjugation of the six ApTcSuSm *S. sonnei* isolates with a nalidixic acid-resistant derivative of the *hsdR* strain PB1395 gave rise to Tc^R, SuSm^R and TcSuSm^R exconjugants. The restriction-negative recipient was employed to assess the extent to which transfer of plasmids was reduced as a result of degradation by recipient restriction endonucleases. Ampicillin-resistant exconjugants were not obtained ($< 10^{-8}$ per donor cell). The simplest explanation for the inability of ampicillin resistance to be transferred by conjugation or transformation is that ampicillin resistance is specified by chromosomal genes. DNA from Tc^R, TcSuSm^R and SuSm^R exconjugants was electrophoresed in agarose gels. Tc^R cells contained a 46 Mdal plasmid, SuSm^R cells contained a 5.5 Mdal plasmid and TcSuSm^R exconjugant cells contained both, indicating that the 46 Mdal plasmid was able to mediate the transfer of the 5.5 Mdal SuSm plasmid.

Transfer to *E. coli* of plasmids not encoding any known drug resistance markers was detected by electrophoresis of DNA from exconjugant clones. For example, Table 2 illustrates the inheritance of two unselected plasmids (24.5 and 1.0 Mdal) by SuSm^R exconjugants from the cross SH34 × PB1395. Table 2 also shows that the use of an HsdR⁺ strain (a nalidixic acid-resistant derivative of J5-3) as a recipient resulted in a reduction in the proportion of SuSm^R exconjugants carrying these unselected plasmids. Plasmid complements of representative exconjugants are shown in Fig. 7. In summary, all six *S. sonnei* isolates were able to mobilize the 1.0 Mdal plasmid present in each. The two isolates containing a cryptic 24.5 Mdal plasmid transferred this molecular species also. However, none of the remaining cryptic plasmids present in the six *S. sonnei* isolates were detected in any exconjugant resulting from crosses of these isolates with *E. coli*. For example, the 1.9, 1.6 and 1.2 Mdal plasmids were not transferred to any of 30 TcSuSm^R and SuSm^R exconjugants derived from the cross SH34 × PB1395.

Exconjugants containing the 46 Mdal Tc plasmid, the 5.5 Mdal SuSm plasmid and the 1.0 Mdal plasmid were able to transfer Tc and SuSm resistance to a second strain of *E. coli* (PB501). Electrophoresis of cleared lysates from these exconjugants showed that all three plasmids had been transferred to PB501, confirming that the 46 Mdal self-transmissible plasmid is able to mediate the mobilization of both smaller molecular species. The two *S. sonnei* isolates containing the 24.5 Mdal plasmid gave rise to SuSm^R exconjugants at

Table 2. *Plasmid complements of SuSm^R exconjugants selected after mating S. sonnei SH34 with E. coli PB1395 (hsdR) or E. coli J5-3 (HsdR⁺)*

Cross	SH34 × PB1395 (<i>hsdR</i>)	SH34 × J5-3 (HsdR ⁺)
SuSm ^R exconjugants per donor cell	7.5×10^{-6}	6.0×10^{-6}
Unselected plasmids in each exconjugant class (Mdal)	No. of exconjugants containing each unselected plasmid class	
46 24.5 1.0	11	7
24.5 1.0	14	7
24.5	0	12
1.0	5	0
None	0	4
Total screened	30	30

Fig. 7. Electrophoresis in 0.7% agarose of DNA from SuSm^R and TcSuSm^R exconjugants obtained on mating *S. sonnei* SH34 with *E. coli* J5-3. A: DNA from a cleared lysate of a TcSuSm^R exconjugant containing the 46, 24.5, 5.5 and 1.0 Mdal plasmids. B to D: DNA from SuSm^R exconjugants containing 24.5, 5.5 and 1.0 Mdal plasmids (well B), 24.5 and 5.5 Mdal plasmids (well C) or 5.5 Mdal plasmid (well D). The small differences in the mobilities of the 24.5 and 5.5 Mdal plasmids were caused by variations in the concentration of DNA in the cleared lysates (Meyers *et al.*, 1976).

frequencies 20- to 50-fold higher than those obtained when any of the four *S. sonnei* isolates lacking this plasmid were used as donors. For example, SuSm^R exconjugants arose at a frequency of 7.5×10^{-6} per donor cell from the cross SH34 × J5-3, but were produced at a frequency of only 3×10^{-7} per donor cell from the cross SH1 × J5-3. Since 24.5 Mdal of DNA is more than sufficient to encode an entire conjugational transfer system, exconjugants carrying the 24.5, 5.5 and 1.0 Mdal plasmids were mated with a second *E. coli* strain (PB501). All transferred SuSm resistance to PB501 at high frequencies (10^{-3} to 10^{-4} per donor cell). Electrophoresis of plasmid DNA from 10 SuSm^R exconjugants resulting from one of these crosses showed that the three plasmids were transferred to each exconjugant.

Hence both the 24.5 and 46 Mdal plasmids are able to mediate mobilization of the 5.5 and 1.0 Mdal plasmids. The 24.5 Mdal plasmid was shown to be Fi^- .

DISCUSSION

Isolates of the largest single class of resistant organisms among the 95 antibiotic-resistant *Shigella* isolates examined were resistant to tetracycline, sulphafurazole and streptomycin. Many other isolates were resistant to tetracycline or to sulphafurazole and streptomycin. The presence of a high proportion of plasmids with sizes in the range of 5 to 6 Mdal and 40 to 50 Mdal in these isolates suggests that the 5.5 and 46 Mdal plasmids present in the six well-characterized strains are the major molecular species which mediate SuSm and Tc resistance in these bacteria. SuSm plasmids of 5.5 Mdal occur frequently in other species of bacteria isolated in New Zealand. For example, all 10 of a representative sampling of SuSm-resistant *Salmonella typhimurium* isolates referred to the National Health Institute between 1972 and 1976 carried 5.5 Mdal non-conjugative plasmids which were cleaved once by *EcoR*I, hybridized to RSF1010 and conferred SuSm resistance on transformed *E. coli* cells (A. F. Jamieson & P. L. Bergquist, unpublished results). Hence both the *Salmonella* and *Shigella* SuSm plasmids have similar properties to those described by Grinter & Barth (1976).

The *S. sonnei* isolates also contained small non-conjugative plasmids which did not encode drug resistance and whose function is unknown. Similar small plasmids are commonly found in diverse bacterial taxa, for example, in *Bacillus* (Stahly *et al.*, 1978), *Neisseria* (Mayer *et al.*, 1974) and *Pseudomonas* (Pemberton & Clark, 1973), as well as in species of Enterobacteriaceae (Møller *et al.*, 1978) such as *Shigella dysenteriae* (Rush *et al.*, 1969). Mobilization of one such plasmid, as well as the 5.5 Mdal SuSm plasmid, was demonstrated from six *S. sonnei* isolates. This mobilization was mediated by the Fi^+ 46 Mdal Tc plasmid present in all six isolates, and by a 24.5 Mdal Fi^- cryptic plasmid present in two of the six. The small plasmids could be transferred to *E. coli* independently from the self-transmissible molecules, suggesting that co-integration is not required for transfer, and that these two transfer systems are therefore members of the class 2 system of Anderson & Natkin (1972).

Mobilization of the small non-conjugative plasmids ColE1 and CloDF13 by self-transmissible plasmids has been shown to require both an origin of transfer and the presence of transfer-promoting proteins encoded by the non-conjugative and self-transmissible plasmids (Warren *et al.*, 1978; Van de Pol *et al.*, 1978). Our inability to demonstrate mobilization of seven of the nine non-conjugative plasmids present in six *S. sonnei* isolates may reflect the absence of transfer origins recognizable by the gene products of the self-transmissible plasmids present. Alternatively, the non-mobilized plasmids may not specify the particular proteins required to interact with those encoded by the conjugative plasmids present to achieve mobilization. The size of the 1.0 Mdal plasmid is sufficient to code for only one or two average-sized proteins. If each non-conjugative plasmid must code for proteins involved in its mobilization, then most or all of the information encoded by this plasmid would be required to facilitate its transfer.

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REFERENCES

- ACHTMAN, M. & HELMUTH, R. (1975). The F factor carries an operon of more than 15×10^6 daltons coding for deoxyribonucleic acid transfer and surface exclusion. In *Microbiology—1974*, pp. 95–103. Edited by D. Schlessinger. Washington: American Society for Microbiology.
- ANDERSON, E. S. & NATKIN, E. (1972). Transduction of resistance determinants and R factors of the Δ transfer systems by phage P1kc. *Molecular and General Genetics* **114**, 261–265.
- BAK, A. L., CHRISTIANSEN, G., CHRISTIANSEN, C., STENDERUP, A., ØRSKOV, I. & ØRSKOV, F. (1972). Circular DNA molecules controlling synthesis and transfer of the surface antigen K88 in *Escherichia coli*. *Journal of General Microbiology* **73**, 373–385.
- BREMNER, D. A. (1978). The transfer of antibiotic resistance by R-plasmids in *Shigella*. *New Zealand Medical Journal* **88**, 9–10.
- CHANDLER, M., SILVER, L., FREY, J. & CARO, L. (1977). Suppression of an *Escherichia coli* *dnaA* mutation by the integrated R factor R100-1: generation of small plasmids after integration. *Journal of Bacteriology* **130**, 303–311.
- CHRISTIANSEN, C., CHRISTIANSEN, G., BAK, A. L. & STENDERUP, A. (1973). Extra-chromosomal deoxyribonucleic acid in different Enterobacteria. *Journal of Bacteriology* **114**, 367–377.
- FALKOW, S. (1975). *Infectious Multiple Drug Resistance*, pp. 253–271. London: Pion Ltd.
- GRINTER, N. J. & BARTH, P. T. (1976). Characterisation of SmSu plasmids by restriction endonuclease cleavage and compatibility testing. *Journal of Bacteriology* **128**, 394–400.
- JEPPESEN, P. G. N., SANDERS, L. & SLOCOMBE, M. (1976). A restriction cleavage map of ϕ X174 DNA by pulse-chase labelling using *E. coli* DNA polymerase. *Nucleic Acids Research* **3**, 1323–1339.
- MCDONELL, M. W., SIMON, M. N. & STUDIER, F. W. (1977). Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. *Journal of Molecular Biology* **110**, 119–146.
- MANDEL, M. & HIGA, A. (1970). Calcium-dependent bacteriophage DNA infection. *Journal of Molecular Biology* **53**, 159–162.
- MAYER, L. W., HOLMES, K. K. & FALKOW, S. (1974). Characterisation of plasmid deoxyribonucleic acid from *Neisseria gonorrhoeae*. *Infection and Immunity* **10**, 712–717.
- MEYERS, J. A., SANCHEZ, D., ELWELL, L. P. & FALKOW, S. (1976). Simple agarose gel electrophoretic method for the identification and characterisation of plasmid deoxyribonucleic acid. *Journal of Bacteriology* **127**, 1529–1537.
- MØLLER, J. K., JØRGENSEN, H. F., CHRISTIANSEN, C., CHRISTIANSEN, G., BAK, A. L. & STENDERUP, A. (1978). Characterisation of plasmids from wild-type Enterobacteriaceae. In *Microbiology—1978*, pp. 257–261. Edited by D. Schlessinger. Washington: American Society for Microbiology.
- PEMBERTON, J. M. & CLARK, A. J. (1973). Detection and characterisation of plasmids in *Pseudomonas aeruginosa* strain P.A.O. *Journal of Bacteriology* **114**, 422–433.
- PORTER, B. W., KOLODNER, R. & WARNER, R. C. (1973). Plasmids of *Shigella dysenteriae* Y6R: a defective Col factor. *Journal of Bacteriology* **116**, 163–174.
- RUSH, M. G., GORDON, C. N. & WARNER, R. C. (1969). Circular deoxyribonucleic acid from *Shigella dysenteriae* Y6R. *Journal of Bacteriology* **100**, 803–808.
- SMITH, H. R. & LINGGOOD, M. A. (1970). Transfer factors in *Escherichia coli* with particular regard to their incidence in enteropathogenic strains. *Journal of General Microbiology* **62**, 287–299.
- SMITH, H. R., HUMPHREYS, G. O. & ANDERSON, E. S. (1974). Genetic and molecular characterisation of some non-transferring plasmids. *Molecular and General Genetics* **129**, 229–242.
- SOUTHERN, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503–517.
- STAHLY, D. P., DINGMAN, D. W., IRGENS, R. L., FIELD, C. C., FEISS, M. G. & SMITH, G. L. (1978). Multiple extrachromosomal deoxyribonucleic acid molecules in *Bacillus thuringiensis*. *FEMS Microbiology Letters* **3**, 139–141.
- TANAKA, T. & WEISBLUM, B. (1975). Construction of a colicin E1-R factor composite plasmid *in vitro*: means for amplification of deoxyribonucleic acid. *Journal of Bacteriology* **121**, 354–362.
- THOMPSON, R., HUGHES, S. G. & BRODA, P. (1974). Plasmid identification using specific endonucleases. *Molecular and General Genetics* **133**, 141–149.
- VAN DE POL, H., VELTKAMP, E. & NIJKAMP, H. J. J. (1978). Genetic analysis of the mobilisation of the non-conjugative plasmid CloDF13. *Molecular and General Genetics* **160**, 139–149.
- WARREN, G. J., TWIGG, A. J. & SHERRATT, D. J. (1978). ColE1 plasmid mobility and relaxation complexes. *Nature, London* **274**, 259–261.
- ZASLOFF, M., GINDER, G. D. & FELSENFELD, G. (1978). A new method for the purification and identification of covalently closed circular DNA molecules. *Nucleic Acids Research* **5**, 1139–1152.