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# The characterization of plasmids in the enterobacteria

By E. S. ANDERSON and E. J. THRELFALL
*Enteric Reference Laboratory, Public Health Laboratory Service, Colindale Avenue, London, NW9 5HT, England*

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SUMMARY

The routine methods used in the Enteric Reference Laboratory for the study of enterobacterial plasmids are described. The results of their application to plasmids of diverse origin, and their value for the categorization of those plasmids, are presented and discussed.

## INTRODUCTION

Plasmids in the enterobacteria mediate the transfer of a variety of genetic determinants, including those for drug resistance, haemolysin and enterotoxin synthesis, colicinogeny, heavy metal tolerance, resistance to ultra-violet irradiation, carbohydrate fermentation, $H_2S$ synthesis and other metabolic characters. The drug resistances transferred include those to ampicillin, carbenicillin, cephalosporins, chloramphenicol, neomycin-kanamycin, streptomycin, sulphonamides, tetracyclines, gentamicin and trimethoprim.

Transfer systems have been characterized and classified by various methods in different laboratories. However, standard methods of plasmid characterization are needed to compare transfer systems from different sources. Such comparisons may ultimately furnish information about the phylogenetic relationships and origins of the plasmids concerned, and about their host bacteria.

In the Enteric Reference Laboratory (ERL), we have studied many transfer systems of enterobacteria from human and animal sources. These have been routinely characterized by the examination of the following properties:

1. The range of resistances in the wild host strain.
2. The range of resistances transferred from that strain to 'standard' recipient strains.
3. The possible multiplicity of plasmids present in a strain.
4. The presence and identity of colicinogeny.
5. The class of resistance factor (R factor) involved, that is, whether it belongs to Class 1, in which the resistance determinant and transfer factor are covalently bonded to form a single plasmid; or whether it is a Class 2 system, in which the resistance determinant (R determinant) and transfer factor form distinct plasmids, possibly associated only during transfer (Anderson, 1968, 1969; Anderson & Threlfall, 1970; Anderson & Natkin, 1972; Humphreys, Grindley & Anderson, 1972).

6. The transfer kinetics of the plasmid(s).

7. The mobilization of non auto-transferring (= non-transferring) resistance determinants in the strains by 'standard' transfer factors (Anderson, 1965*a*, *b*).

8. The determination of whether the plasmids enable their hosts to support multiplication of known donor-specific phages (Grindley & Anderson, 1971).

9. The compatibility group(s) of the plasmid(s) present in the wild strain (Hedges & Datta, 1971; Datta & Hedges, 1971; Chabbert *et al.* 1972; Grindley, Grindley & Anderson, 1972; Grindley, Humphreys & Anderson, 1973).

10. The fertility inhibition (*fi*) character (Egawa & Hirota, 1962; Watanabe & Fukasawa, 1962; Watanabe, Fukasawa & Takano, 1962; Watanabe, 1963; Watanabe *et al.* 1964).

11. Phage restriction in *Escherichia coli* K12 (= K12) and salmonellas (Anderson, 1966; Anderson, Threlfall, Carr & Savoy, 1973).

12. The degrees of drug resistance conferred by R factors or R determinants.

13. The molecular characteristics of the plasmid DNA.

In this article we shall describe the application of these methods of characterization to nine transfer systems.

## MATERIALS AND METHODS

### *Media*

Liquid media for the growth of bacterial cultures contained 20 g. Bacto dehydrated nutrient broth (Difco Laboratories) and 8·5 g. NaCl/l.; for solid media, 13 g./l. of Davis New Zealand powdered agar was added. Lactose and non-lactose fermenting cultures were differentiated by plating on Oxoid MacConkey agar.

### *Bacterial strains*

The laboratory strains used in these studies are listed in Table 1.

### *Bacteriophages and phage-typing*

The donor-specific phages for F- and I-specificity determination were $\mu 2$ (Dettori, Maccacaro & Piccinin, 1961) and If1 (Meynell & Lawn, 1968) respectively. The receptors for these phages are the F and I sex fimbriae respectively.

Phage restriction in K12 was tested with the 'female-specific' phage $\phi 2$ of Cuzin (1965) (Pitton & Anderson, 1970).

Phage restriction in salmonellas was investigated by phage-typing the respective strains. *S. typhimurium* was phage-typed by the methods of Callow (1959) and Anderson (1964, and in preparation); *S. typhi* carrying transfer systems was phage-typed according to Craigie & Yen (1938*a*, *b*) and Craigie & Felix (1947); and *S. paratyphi B* by the method of Felix & Callow (1943, 1951) and Anderson, (1964). The relevant techniques were summarized by Anderson & Williams (1956).

### *Drug resistance*

Resistance to ampicillin (A), chloramphenicol (C), gentamicin (G), neomycin-kanamycin (K), streptomycin (S) and tetracyclines (T) was routinely tested for by a diffusion method on nutrient agar plates, using strips of blotting paper (Ford's

Table 1. *Laboratory strains used as donors and recipients*

| ERL No. | Genotype | Plasmid carried | Designation |
|---|---|---|---|
| 14R525 | *E. coli* K12F$^-$*lac*$^+$Nx$^r$ | — | K12 |
| 19R689 | *S. typhimurium* phage type 36 Nx$^r$ | — | Type 36 |
| 26R862 | *S. typhi* Vi-phage type A Nx$^r$ | — | *S. typhi* type A |
| B1363 | *S. paratyphi B* phage type 1 var. 2 | — | *S. paratyphi* B type 1 var. 2 |
| RT641 | *S. typhimurium* phage type 6 Δ$^+$ | Δ | Type 6 Δ$^+$ |
| 1R380 | *E. coli* K12F$^-$*lac*$^+$Δ$^+$ | Δ | K12 Δ$^+$ |
| 22R149 | *S. typhimurium* phage type 125 Str$^r$ColI$^+$ | ColI | Type 125 ColI$^+$ |
| 22R81 | *E. coli* K12F$^-$*lac*$^+$Str$^r$ColI$^+$ | ColI | K12 ColI$^+$ |
| 18R951 | *E. coli* K12F$^-$*lac*$^-$Str$^r$X$^+$ | X | K12 X$^+$ |
| 27R207 | *E. coli* K12HfrH Nx$^r$ | F | K12HfrH |
| 40R880 | *E coli* K12 F$^+$ | F | K12 F$^+$ |
| 20R770 | *E. coli* K12 F$^-$*lac*$^+$T-Δ*drp*1$^+$ | T-Δ*drp*1$^+$ | K12 T-Δ*drp*1 |
| 38R778 | *E. coli* K12 F$^-$*lac*$^+$ R-144-3$^+$ | R144-3 | K12 R144-3$^+$ |

*Symbols*

Nx$^r$ = chromosomal resistance to nalidixic acid.
Str$^r$ = chromosomal resistance to streptomycin.

*Plasmid designation*

Δ = Δ transfer factor (Anderson & Lewis, 1965*b*).
T-Δ = tetracycline R factor (Anderson & Lewis, 1965*b*).
ColI = ColI-P9 factor (Fredericq, 1956).
X = *fi*$^+$ F-like transfer factor from *S. typhimurium* 5M4136 (Anderson *et al.* 1968).
F = F factor.
T-Δ*drp*1 = derepressed mutant of T-Δ (Grindley & Anderson, 1971).
R144-3 = derepressed mutant of the I-like R factor R144 (Meynell & Datta, 1967). R144 was isolated from *S. typhimurium* 4M91, characterized in the Enteric Reference Laboratory in 1964 (Anderson & Datta, unpublished).

428 Mill) 80 mm × 7 mm impregnated with the respective drugs and freeze-dried. These strips were prepared in the Enteric Reference Laboratory. The cultures were streaked at right angles to the antibiotic strips, and control sensitive and resistant cultures were included in each test. This method not only detects resistance, but enables its magnitude to be roughly compared with that of other cultures on the same plate (see Plate 1). Resistance to sulphonamides and trimethoprim was detected by spotting 0·01 ml. drops of a $10^{-4}$ dilution of a late exponential phase broth culture of the test strain on nutrient agar containing 5% v/v of lysed horse blood, and either 100 μg./ml. of sulphathiazole or 0·5 μg./ml. of trimethoprim (see Plate 2, Figs. 1 and 2). Sensitive and resistant controls were always included. Nalidixic acid resistance was also detected by this method, the nutrient agar containing 40 μg./ml. of the drug (see Plate 2, Figs. 3 and 4). Screening for furazolidone resistance was effected by spotting 0·01 ml. of undiluted culture of the strain on nutrient agar containing 100 μg./ml. of the drug.

*Colicinogeny*

Colicinogeny was detected by the agar-overlay method of Fredericq (1948), using K12 as the indicator strain. Colicins were identified on the basis of the

immunity of standard colicinogenic cultures to the lethal effects of their respective colicins, and of the resistance of known mutants of K12 to the action of specific colicins (Fredericq, 1948).

*Transfer of plasmids*

The conjugation techniques used were those of Anderson & Lewis (1965*a*, *b*). Resistance transfer was detected by plating mating mixtures, generally after overnight incubation, on agar plates containing the appropriate drugs. Drug-resistant donor strains of salmonellas in mating mixtures were suppressed with nalidixic acid (40 $\mu$g./ml.), when the recipient strains were nalidixic acid-resistant mutants, or by spreading salmonella phage O1 of Felix & Callow (1943) on the surface of the agar before plating the cross (Anderson & Lewis, 1965*a*, *b*). When the donor was K12 it was eliminated with colicin E2 (Anderson & Lewis, 1965*a*, *b*).

*R determinant mobilization*

The mobilizability of wild non-transferring R determinants was tested with the triparental cross for determinant mobilization (Anderson, 1965*a*, *b*). Three transfer factors were routinely used for this purpose in the primary donor strains: the *fi*$^-$ I-like $\Delta$ factor (Anderson & Lewis, 1965*b*); the *fi*$^-$ I-like ColI factor ColIb-P9; and the *fi*$^-$ F-like transfer factor X (Anderson, Pitton & Mayhew, 1968). As results with $\Delta$ and the ColI factor were identical, ColI is not shown in Table 4.

*Phage multiplication*

Plasmids were examined for their ability to enable host strains to propagate the donor-specific phages $\mu$2 and If1 by the method of Grindley & Anderson (1971).

*Determination of compatibility group*

Plasmids were assigned to compatibility groups by examination of their ability to coexist with R factors of the known compatibility groups (Grindley *et al.* 1972).

*Examination of* fi *character*

This character was routinely identified by examining the inhibition of visible lysis by the F-specific phage $\mu$2, which was spotted on surface cultures of K12F$^+$ and K12HfrH into which plasmids had been introduced (Pitton & Anderson, 1970). Factors that are *fi*$^+$ inhibit the synthesis of F fimbriae, which are the receptors for F-specific phages. Thus, when *fi*$^+$ factors are introduced into strains carrying the F factor, such as K12F$^+$ and K12Hfr, they reduce or abolish visible lysis by these phages. In contrast, *fi*$^-$ plasmids do not affect F-fimbrial synthesis, and therefore do not affect lysis of F$^+$ or Hfr strains by F-specific phages.

*Isolation and measurement of plasmid DNA*

Plasmid DNA was isolated, and the mean contour length (MCL) determined as described by Grindley *et al.* (1973). The molecular weight of the plasmids was calculated on the assumption that 1 $\mu$m. of DNA = $2{\cdot}07 \times 10^6$ daltons (Lang, 1970).

Table 2. *Examination of the properties of transfer systems*

| Property | Routine method of examination | Reference |
|---|---|---|
| Drug resistance | Strip diffusion tests for A, C, K, S, T and G | — |
| | Spot tests on solid media for Su, Tm, Fu and Nx | — |
| Colicinogeny: | | |
| Production | Sensitivity of K12 to colicins | Fredericq (1948) |
| Identification | Immunity and resistance of standard strains | Fredericq (1948) |
| Transferability and frequency of transfer | Conjugation | Anderson & Lewis (1965*a*, *b*) |
| Mobilization of non auto-transferring resistance determinants | Triparental cross for determinant mobilization | Anderson (1965*a*, *b*) |
| F and I fimbrial synthesis | Donor-specific phage multiplication | Grindley & Anderson (1971) |
| Compatibility group | Ability to coexist with plasmids of defined groups | Grindley *et al.* (1972) |
| *fi* character | Inhibition of lysis of K12HfrH and K12F$^+$ by phage $\mu 2$ | Pitton & Anderson (1970) |
| Phage restriction: | | |
| In K12 | Inhibition of lysis by phage $\phi 2$ | Pitton & Anderson (1970) |
| In *S. typhimurium* | Phage-typing | Callow (1959); Anderson (1964) |
| In *S. typhi* | Phage-typing | Craigie & Yen (1938*a*, *b*); Craigie & Felix (1947) |
| In *S. paratyphi B* | Phage-typing | Felix & Callow (1943, 1951); Anderson (1964) |
| Plasmid DNA characteristics | Isolation and measurement of plasmid DNA | Grindley *et al.* (1973) |
| Degrees of drug resistance | Estimation of MICs of host strains to the respective drugs in liquid and on solid media | — |

Drug resistance symbols: A, ampicillin; C, chloramphenicol; K, neomycin-kanamycin; S, streptomycin; Su, sulphonamides; G, gentamicin; T, tetracyclines; Tm, trimethoprim; Fu, furazolidone; Nx, nalidixic acid.

*Minimal inhibitory concentration (MIC)*

The MICs of strains carrying the ampicillin (A), chloramphenicol (C), streptomycin (S) and tetracycline (T) resistance determinants were estimated with doubling dilutions of the respective antibiotics in nutrient broth. A standard inoculum of approximately $10^2$ bacterial cells/ml. was used. The MIC was the lowest concentration of antibiotic that inhibited visible growth of the test strain in nutrient broth. Kanamycin MICs were similarly determined in Mueller–Hinton broth. Attempts were made to measure resistance to sulphonamides (sulpha-

thiazole) on nutrient agar containing concentrations of the drug up to its limit of solubility (2000 $\mu$g./ml.). The resistance always exceeded this concentration.

The methods for the examination of the properties of transfer systems are summarized in Table 2.

The identification of the spectrum of activity of plasmid-determined enzymes such as penicillinases and those for inactivation of streptomycin, and the serological specificity of such enzymes, are also methods of plasmid characterization that can be added when necessary. As a general rule, we have routinely used only the differentiation of streptomycin adenylylation from phosphorylation by inclusion of spectinomycin in tests of streptomycin resistance. Spectinomycin is inactivated only by adenylylation.

## RESULTS

The sources and designations of nine transfer systems used to exemplify the methods of characterization are shown in Table 3. Their properties are summarized in Table 4.

### *Drug resistance transfer*

In the Δ transfer systems, first identified in *S. typhimurium* type 29 ASSuTFu, strain RT1 (Anderson & Lewis, 1965*a*, *b*), the Δ factor mediates independently the transfer of resistances to ampicillin, streptomycin-sulphonamides and tetracyclines. Furazolidone resistance has not yet been transferred.

The R factors TP110 (Anderson & Smith, 1972*a*) and TP102 (Grindley & Anderson, 1971), isolated from wild *S. typhimurium* strains 8M5251 and 8M5654, and the R factor TP114 (Grindley *et al.* 1972), from *E. coli* EC593, all carry a K determinant only; this codes for resistance to kanamycin, neomycin and paromomycin.

Factor 334 was isolated from a spontaneous kanamycin-sensitive segregant of *S. paratyphi B* type 3a var. 4, 7R334. This strain was first characterized in the ERL in 1964. R factor 334 transfers resistance to ampicillin, chloramphenicol, streptomycin and sulphonamides and, with the exception of loss of kanamycin resistance, is probably identical with the R factor R1, isolated from this strain by Meynell & Datta (1966).

R factor TP123, isolated from *S. typhi* 1T4739 (Anderson & Smith, 1972*b*), transfers resistance to chloramphenicol, streptomycin-sulphonamides and tetracyclines. 1T4739 is representative of the strain responsible for a widespread outbreak of chloramphenicol-resistant typhoid fever, which started in Mexico early in 1972 and was still active in 1973.

TP118 (Anderson & Threlfall, 1970) is an R factor identified in *S. enteritidis* strain E3538, belonging to phage type 8. It confers resistance to ampicillin and streptomycin.

### *Colicinogeny*

The Ib colicinogeny determinant is covalently bonded to the transfer factor in TP110, and transfers with kanamycin resistance (= KColIb) (Anderson & Smith, 1972*a*). No identifiable Col determinants are associated with the remaining eight transfer systems.

Table 3. *Origin of transfer systems characterized*

| Host strain | | | | | |
|---|---|---|---|---|---|
| Serotype | Phage type | R-type* | ERL No. | Transfer system designation | Reference |
| *S. typhimurium* | 29 | ASSuTFu | RT1 | A, SSu, T } resistance determinants | |
| | | | | Δ transfer factor | Anderson & Lewis (1965*a*, *b*) |
| | | | | A,Δ; SSu,Δ; T-Δ } Δ-mediated transfer systems | |
| *S. typhimurium* | 104 | K | 8M5251 | TP110 | Anderson & Smith (1972*a*) |
| *S. typhimurium* | Untypable | K | 8M5654 | TP102 | Grindley & Anderson (1971) |
| *E. coli* | . | K | EC593 | TP114 | Grindley *et al.* (1972) |
| *S. paratyphi B* | 3a var. 4 | ACSSu | 7R334 | 334** | Pitton & Anderson (1970) |
| *S. typhi* | Degraded Vi-strain | CSSuT | 1T4739 | TP123 | Anderson & Smith (1972*b*) Grindley *et al.* (1973) |
| *S. enteritidis* | 8 | AS | E3538 | TP118 | Anderson & Threlfall (1970) |

Drug resistance symbols: see Table 2.

* R-type = spectrum of drug resistance.

** Isolated from a spontaneous kanamycin-sensitive segregant of strain 7R334, characterized in the Enteric Reference Laboratory in 1964. The transfer system is probably identical with that of R1, isolated from this strain by Meynell & Datta (1966).

Table 4. *Properties of transfer systems*

| Properties | Resistance determinants: A | Resistance determinants: SSu | Transfer systems: Δ-mediated transfer systems: Δ (transfer factor) | Transfer systems: Δ-mediated transfer systems: A, SSu, Δ | Transfer systems: Δ-mediated transfer systems: T-Δ | Transfer systems: TP110 | Transfer systems: TP102 | Transfer systems: TP114 | Transfer systems: 334 | Transfer systems: TP123 | Transfer systems: TP118 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Drug resistances transferred | . | . | . | A, SSu | T | K | K | K | ACSSu | CSSuT | AS |
| Colicinogeny | − | − | − | − | − | Ib | − | − | − | − | − |
| Class of R factor | . | . | . | 2 | 1 | 1(KColIb) | 1 | 1 | 1 | 1 | 1 |
| Transfer frequency in overnight crosses* | | | | | | | | | | | |
| From original host to K12 | 0 | 0 | $5 \times 10^{-1}$ | A $10^{-2}$ SSu $10^{-2}$ Δ $5 \times 10^{-1}$ | $10^{-6}$ | $5 \times 10^{-1}$ | $10^{0}$ | $5 \times 10^{-1}$ | $5 \times 10^{-1}$ | $10^{-4}$ | $10^{-3}$ |
| From K12 to type 36 | 0 | 0 | $5 \times 10^{-1}$ | A $10^{-3}$ SSu $10^{-3}$ Δ $5 \times 10^{-1}$ | $5 \times 10^{-1}$ | $5 \times 10^{-1}$ | $10^{0}$ | $10^{-2}$ | $10^{-4}$ | $10^{-6}$ | $10^{-4}$** |
| From type 36 to K12 | 0 | 0 | $5 \times 10^{-1}$ | A $10^{-2}$ SSu $10^{-2}$ Δ $5 \times 10^{-1}$ | $5 \times 10^{-1}$ | $5 \times 10^{-1}$ | $10^{0}$ | $5 \times 10^{-1}$ | $5 \times 10^{-1}$ | $10^{-4}$ | $10^{-3}$*** |
| Mobilization of non auto-transferring resistance by: | | | | | | | | | | | |
| *fi*⁻ I-like factor Δ | + | + | . | . | . | . | . | . | . | . | . |
| *fi*⁺ F-like factor X | − | − | . | . | . | . | . | . | . | . | . |
| Supports multiplication of 'male' phages: | | | | | | | | | | | |
| If I | . | . | + | + | + | + | + | + | − | − | − |
| $\mu 2$ | . | . | − | − | − | − | − | − | + | − | − |
| Compatibility group | . | . | $I_1$ | $I_1$ | $I_1$ | $I_1$ | $I_1$ | $I_2$ | $F_{II}$ | H | N |
| *fi* character | . | . | *fi*⁻ | *fi*⁻ | *fi*⁻ | *fi*⁻ | *fi*⁺ | *fi*⁻ | *fi*⁺ | *fi*⁻ | *fi*⁻ |
| Phage restriction: | | | | | | | | | | | |
| in K12 (phage $\phi 2$) | − | − | + | + | + | + | − | − | − | − | − |
| in salmonellae (typing phages) | + | − | + | + | + | + | − | − | − | − | − |

Table 4 (*cont.*)

| Properties | Resistance determinants: A | Resistance determinants: SSu | Transfer system: Δ-mediated transfer systems: Δ (transfer factor) | Δ-mediated transfer systems: A, SSu, Δ | Δ-mediated transfer systems: T-Δ | TP110 | TP102 | TP114 | 334 | TP123 | TP118 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Resultant phage type in: | | | | | | | | | | | |
| *S. typhimurium* type 36 | 125 | NM | 6 | Δ+6 | 6 | 125 | NM | NM | NM | NM | NM |
| *S. typhi* Vi-type A | ND | ND | Resistant to all Vi-phages | Δ+ resistant to all Vi-phages | Vi-type 29 Vi-phages I, IV, V, VI, VII restricted | Restricts Vi-phages III, V, VI, VII | NM | NM | NM | NM | NM |
| *S. paratyphi B* type 1 var. 2 | ND | ND | Beccles var. 2 | Δ+ Beccles var. 2 | Beccles var. 2 | NM | NM | NM | NM | NM | NM |
| MIC (μg/ml) in: | | | | | | | | | | | |
| Original host | A 3000 | S 2000 | . | A 3000 S 2000 | T 250 | K > 10,000 | K > 10,000 | K 1250 | A 1000 C 500 S 125 | C 150 S 32 T 125 | A 500 S 125 |
| K12 | A 3000 | S 250 | . | A 3000 S 250 | T 125 | K > 10,000 | K > 10,000 | K 1250 | A 1000 C 500 S 62·5 | C 150 S 32 T 125 | A 500 S 125 |
| Type 36 | A 3000 | S 1000 | . | A 3000 S 1000 | T 250 | K > 10,000 | K > 10,000 | K 2500 | A 1000 C 500 S 125 | C 150 S 32 T 125 | A 500 S 125 |
| DNA characteristics: MCL of plasmid (μm) | 2·70 | 2·74 | 28·7 | A 2·70 SSu 2·74 Δ 29·5 | 32·3 | 31·3 | 26·3 | 19·7 | 26·2 | 59·5 | 13·2 |
| Molecular weight (daltons × $10^{-6}$) | 5·6 | 5·7 | 59 | A 5·6 SSu 5·7 Δ 61 | 67 | 64·8 | 54·4 | 40·8 | 54·2 | 123·2 | 27·3 |

Drug resistance symbols, see Table 2. Plasmid designation, see Table 3. Phage designations: If1, I-specific phage; μ2, F-specific phage.
* All frequencies are approximate. ** Transfer frequency to *S. enteritidis* type 1 (see text). *** Transfer frequency from *S. enteritidis* type 1 to K12.
MCL, mean contour length; NM, unchanged; ND, not determined.

*Class of R factor*

The tetracycline resistance determinant and Δ factor of the Δ-mediated transfer system are covalently bonded to form a single plasmid which is transmitted as an intact linkage group, the Class 1 R factor T-Δ. A,Δ and SSu,Δ are Class 2 resistance transfer systems in which the A and SSu resistance determinants and the Δ transfer factor are independent of each other in the host cell. The plasmids of a Class 2 system may be transmitted simultaneously or separately (Anderson & Lewis, 1965*a*, *b*; Anderson, 1968, 1969). The Δ factor is necessary for the transfer of the A and SSu determinants.

TP110, TP102, TP114, 334, TP123 and TP118 are Class 1 R factors. The R determinants and transfer factors are transmitted together in these transfer systems. The respective R determinants have not been found separately in exconjugants, although a low percentage of recipient cells may acquire only the transfer factor (Anderson & Lewis, 1965*b*; Anderson, 1966).

*Transfer kinetics*

Since overnight crosses are routinely used in these investigations, transfer frequencies are estimated as a fraction of the total recipient population at the termination of the cross. All frequencies quoted are near approximations. The plasmids Δ, T-Δ, TP110 and TP102, all transfer at high frequencies, up to $10^0$, in overnight crosses. The A determinant of A,Δ transfers at lower frequency ($10^{-2}$) than that of Δ alone (up to $10^0$) (Anderson & Lewis, 1965*b*). The SSu determinant of RT1 behaves similarly to A. TP114 transfers at $5 \times 10^{-1}$ from *E. coli* EC593 to K12, and from K12 to type 36 at $10^{-2}$ or less. Type 36 transfers TP114 to K12 at $5 \times 10^{-1}$.

Factor 334 transfers at $5 \times 10^{-1}$ in crosses from *S. paratyphi B* 7R334 and from type 36 to K12, but at only $10^{-4}$ from K12 to salmonella hosts.

TP123 transfers to K12 at $10^{-4}$ in crosses from *S. typhi* 1T4739 and type 36 respectively, and at $10^{-6}$ or less from K12 to *S. typhi* and type 36.

TP118 is relatively host specific: without modification this R factor will transfer only from *S. enteritidis*, in which it was first identified, to *S. enteritidis* ($10^{-4}$) or to K12 ($10^{-3}$) and from K12 to *S. enteritidis* ($10^{-4}$) (Anderson & Threlfall, 1970). Transfer of N group plasmids from K12 to *S. typhimurium* often occurs at very low frequencies. For example, TP120 (Grindley *et al.* 1972, 1973) which has the resistance spectrum ASSuT, transfers to *S. typhimurium* 36 at about $10^{-6}$ in overnight crosses. TP120, and other group N plasmids isolated during the same period in 1962 (Anderson & Datta, 1965) may lose resistance markers during transfer to *S. typhimurium*. Thus, TP120 may lose T or S during such transfer (Anderson & Janet White, unpublished observations).

*Mobilization of non-transferring resistance determinants*

The A and SSu determinants of the Δ transfer systems are plasmids that can be mobilized by I-like factors such as the Δ transfer factor and the ColI factor, but not by the F-like transfer factor X. Thus, there is some specificity in determinant-transfer factor associations (Anderson, 1968).

Host specificity may be an important character of some plasmids. For example, we have so far been unable to demonstrate transfer of $I_1$ plasmids to *Proteus mirabilis* PM1 (Anderson and Deniset, unpublished observations), although a number of F-like factors can enter that host.

*Donor-specific phage multiplication*

The transfer systems tested were grouped under three headings, as shown below:

1. F-like factors, which code for F sex fimbriae, thereby enabling their hosts to propagate the F-specific phages. R factor 334 belongs to this category.

2. I-like factors, which code for I sex fimbrial synthesis, enable their hosts to support multiplication of the I-specific phage If1. Examples are the transfer factor Δ and the R factors T-Δ, TP110, TP102 and TP114.

3. Transfer systems that do not confer on their hosts the ability to propagate either F-specific or I-specific phages; for example, TP123 and TP118. This category is heterogeneous.

*Compatibility groups*

Transfer systems have been divided into compatibility groups (Romero, 1970; Khatoon & Iyer, 1971; Hedges & Datta, 1971; Datta & Hedges, 1971; Chabbert *et al.* 1972; Grindley *et al.* 1972, 1973). In general, the members of each group are compatible with those of other groups, but are incompatible with each other.

As Table 4 shows, the I-like factors have been divided into the $I_1$ and $I_2$ compatibility groups. Δ, TP110 and TP102 are incompatible with other I-like plasmids such as R144-3, and belong to the group designated $I_1$ by Grindley *et al.* (1972). However, TP114, which codes for the synthesis of I fimbriae, is compatible with the $I_1$ factor T-Δ, and is the prototype of the $I_2$ group (Grindley *et al.* 1972).

The F-like R factor 334 is an $F_{II}$ plasmid which is compatible with $F_I$ factors such as the original F transfer factor (Hedges & Datta, 1972). TP123 belongs to the H group (Anderson & Smith, 1972*b*; Grindley *et al.* 1972, 1973), and TP118 to the N group (Grindley *et al.* 1972, 1973).

*The fertility inhibition* (fi) *character*

The $I_1$ plasmids Δ, T-Δ and TP110, and the $I_2$ plasmid TP114, are *fi*$^-$: they do not inhibit visible lysis of strains of K12F$^+$ or K12HfrH by the F-specific phage $\mu 2$. However, the $I_1$ R factor TP102 is *fi*$^+$; it reduces lysis by phage $\mu 2$ and transfer of chromosomal characters by K12HfrH (Grindley & Anderson, 1971).

The $F_{II}$ R factor 334 is *fi*$^+$ (Pitton & Anderson, 1970). Most wild F-like factors show this character.

The H group plasmid TP123 and the N group plasmid TP118 are *fi*$^-$.

*Phage restriction*

*In K*12.

Of the $I_1$ factors examined, the *fi*$^-$ plasmids Δ, T-Δ and TP110 all reduce visible lysis of K12 by phage $\phi 2$. When the K12 strain carrying these factors is also F$^+$ or Hfr, visible lysis by phage $\phi 2$ is abolished. In contrast, the *fi*$^+$ I-like

R factor TP102 is non-restricting for this phage. TP114, the *fi*$^-$ plasmid of the $I_2$ group, is also non-restricting for $\phi 2$ in K12, as are the *fi*$^+$ $F_{II}$ R factor 334 and the H and N plasmids TP123 and TP118 respectively.

*In* S. typhimurium *type* 36, S. typhi *Vi-type A and* S. paratyphi B 1 *var.* 2.

Δ *transfer systems.* A is one of the rare non auto-transferring resistance determinants so far observed to cause phage restriction (Anderson *et al.* 1968). It restricts phages 12 and 13 of the *S. typhimurium* typing scheme, thereby converting type 36 into type 125 (Anderson, 1966; Anderson *et al.* 1968). The SSu determinant does not cause phage restriction.

The Δ transfer factor and the T-Δ R factor restrict the lysis of type 36 by 24 of the 30 *S. typhimurium* typing phages, to produce type 6 (Anderson & Lewis, 1965*b*). They convert *S. paratyphi B* 1 var. 2, which is sensitive to all 11 of the *S. paratyphi B* typing phages, into phage type Beccles var. 2, sensitive to only 3 of the phages (Anderson, 1966). However, Δ differs from T-Δ in that it inhibits lysis of *S. typhi* type A by all 96 Vi-typing adaptations of Vi-phage II, whereas T-Δ converts type A into type 29, which is sensitive to 13 of the adaptations (Anderson, 1966). Moreover, Δ also restricts lysis of *S. typhi* by the unadapted Vi-phages I, III, IV, V, VI and VII, whereas *S. typhi* type A carrying T-Δ remains sensitive to Vi-phage III (Anderson, 1966).

*TP*110 *KColIb.* TP110 converts type 36 of *S. typhimurium* into type 125, and is a representative of the Γ group of transfer systems (Anderson *et al.* 1973). This R factor does not alter the Vi-type of *S. typhi* type A, but restricts the unadapted Vi-phages III, V, VI and VII of that host. It does not affect the phage type of *S. paratyphi B* 1 var. 2.

*Other transfer systems.* The I-like factors TP102 and TP114, the $F_{II}$ R factor 334, the H plasmid TP123 and the N plasmid TP118, are all non-restricting in *S. typhimurium* type 36, *S. typhi* type A and *S. paratyphi B* 1 var. 2.

*Degrees of drug resistance*

Δ *systems.* The penicillin MIC of the wild host strain of *S. typhimurium* type 29, RT1, and of both type 36 and K12 carrying the A determinant, is 3000 μg./ml. (Anderson & Lewis, 1965*a*; Anderson *et al.* 1968). The streptomycin MIC is 2000 μg./ml. in RT1, 1000 μg./ml. in type 36, and 250 μg./ml. in K12. The tetracycline MIC of strains carrying T-Δ is 250 μg./ml. in the original host and in type 36, and 125 μg./ml. in K12. Plasmid-borne tetracycline resistance is commonly of this magnitude.

*TP*110, *TP*102 *and TP*114. *Kanamycin resistance.* The kanamycin resistance in strains of *S. typhimurium* and K12 carrying TP110 and TP102 is greater than 10,000 μg./ml. In contrast, the MIC of kanamycin in strains carrying TP114 is 1,250 μg./ml. in both the original *E. coli* host and K12, and about 2,500 μg./ml. in type 36.

*R factor* 334 *ACSSu.* The ampicillin MIC of the original strain of *S. paratyphi B* carrying 334 is 1000 μg./ml.; it is about the same in K12 and type 36. The chloramphenicol MIC of these strains is about 500 μg./ml. The streptomycin MIC is

125 μg./ml. in *S. paratyphi B* and *S. typhimurium*, and 62·5 μg./ml. in K12. All host strains are resistant to at least 2000 μg./ml. of sulphathiazole, the limit of solubility of the drug.

*TP*123 *CSSuT*. TP123 confers a chloramphenicol MIC of 150 μg./ml. on its original host strain of *S. typhi* (Anderson & Smith, 1972*b*), and on K12 and type 36. Its host strains also have MICs of 32 μg./ml. to streptomycin, and 125 μg./ml. to tetracycline. The sulphonamide MIC exceeds 2000 μg./ml.

*TP*118 *AS*. The ampicillin MIC of *S. enteritidis*, *S. typhimurium* and K12 carrying TP118 is 500 μg./ml., and the streptomycin MIC, 125 μg./ml.

*DNA characteristics*

The DNA molecules of factors of a given compatibility group are of similar size, and usually of similar composition (Grindley *et al.* 1973). The group $I_1$ factors, Δ, T-Δ, TP110 and TP102 have mean contour lengths (MCLs) of 28·7, 32·3,* 31·3 and 26·3 μm. respectively, corresponding to molecular weights of 59, 67, 65 and $54 \times 10^6$ daltons. The prototype of the $I_2$ group is smaller, with a MCL of 19·7 μm. and a molecular weight of $41 \times 10^6$ daltons. Although it codes for I fimbriae, it is distinct from plasmids of the $I_1$ group.

The $F_{II}$ R factor 334 has a contour length of 26·3 μm., corresponding to a molecular weight of $54 \times 10^6$ daltons. The contour length of the H group factor TP123 is 59·5 μm. and its molecular weight $123 \times 10^6$ daltons. The N group R factor TP118 is 13·2 μm. long, with a molecular weight of $27 \times 10^6$ daltons.

The non auto-transferring resistance determinants A and SSu are plasmids which are distinct from each other and from the compatibility groups described above. The mean contour length of A is 2·70 μm., and that of SSu 2·74 μm., corresponding to molecular weights of $5{\cdot}6 \times 10^6$ and $5{\cdot}7 \times 10^6$ daltons respectively. Such determinants are present in multiple copies per chromosone, whereas only one copy of transferable plasmids is found.

## DISCUSSION

We have described the properties of nine transfer systems in salmonellas and *E. coli* carrying resistance to various drugs. These systems have been divided into two classes according to the relationships between the R determinants and the transfer factors (Anderson, 1968, 1969; Anderson & Threlfall, 1970; Anderson & Natkin, 1972; Humphreys *et al.* 1972). Seven of the transfer systems, T-Δ, TP110, TP102, TP114, 334, TP123 and TP118, are Class 1 R factors, that is, they are single covalently bonded plasmids which occupy the cellular attachment site of the respective transfer factor, and are transferred as intact linkage groups. Two, the A,Δ and SSu,Δ systems, belong to Class 2, in which the R determinants and the Δ transfer factor are distinct molecules, independent of each other and occupying separate attachment sites in the host cell: they are possibly associated only during transfer to a new host.

The R factor TP110 is a Class 1 system in which the kanamycin resistance

* The difference between the contour lengths of Δ and T-Δ, 3·6 μm., gives an approximate length for the T resistance determinant.

determinant, the ColIb determinant and the I-like transfer factor are covalently bonded to form a single plasmid. ColI determinants are usually found in wild strains recombined with I-like transfer factors, and are common in *S. typhimurium*. Other Col determinants, such as E1 and E2, occur as independent non auto-transferring plasmids, analogous to A and SSu (Anderson & Lewis, 1965*b*).

Transferable plasmids can also be categorized according to the nature of the sex fimbrial synthesis they encode and by compatibility studies. Thus Δ, T-Δ, TP110 and TP102 are plasmids of the $I_1$ compatibility group, while TP114, although it supports multiplication of phage If1, is compatible with $I_1$ plasmids and is the prototype of the $I_2$ group (Grindley *et al.* 1972). R factor 334 is an $F_{II}$ plasmid, TP123 belongs to group H (Anderson & Smith, 1972*b*; Grindley *et al.* 1972) and TP118 to group N.

Molecular characterization of plasmid DNA confirms the groupings obtained by genetic analysis. The contour lengths of plasmids of the $I_1$ group are similar (average contour length 29·7 μm.) and differ substantially from that of the $I_2$ plasmid TP114 (contour length 19·7 μm.). The contour lengths of TP118, the N group plasmid (13·2 μm.), and TP123, the H plasmid (59·5 μm.), are also quite distinct from each other and from $I_1$ and $I_2$ plasmids (Grindley *et al.* 1973).

DNA reassociation of high degree usually occurs between plasmids of a single compatibility group, but not between those of different groups (Grindley *et al.* 1973). For example, reassociation readily takes place between the DNA of different $I_1$ plasmids, but not between plasmids of this group and the DNA of the $I_2$ plasmid TP114, or that of plasmids of other compatibility groups. However, some plasmids may be atypical. Thus, no reassociation could be demonstrated between the DNA of TP116, an H group R factor, and that of other H group plasmids (Grindley *et al.* 1973). Incompatibility, therefore, may not necessarily indicate molecular similarity.

Plasmids of a given compatibility group have similar transfer kinetics, which often differ from those of other groups. For example, the $I_1$ plasmids we have studied invariably transfer at high frequencies (up to 10°) in overnight crosses, whereas the H plasmid TP123 transfers at low frequencies, about $10^{-4}$ into K12, and as low as $10^{-6}$ into *S. typhimurium* and *S. typhi*. These low transfer frequencies are a feature of all H group plasmids so far examined.

It is worth noting that members of the same compatibility group may carry different resistance determinants in a Class 1 association, and, conversely, that members of different groups may carry determinants coding for similar resistances. Resistance markers are therefore of limited value in the identification of R factors. Thus, the Class 1 R factor in the strain of *Shigella dysenteriae* 1, which caused the huge dysentery outbreak in Central America from 1968 onwards, codes for resistance to chloramphenicol, streptomycin, sulphonamides and tetracyclines, and belongs to compatibility group B. The strain of *S. typhi* that caused the widespread typhoid outbreak in Mexico in 1972 is resistant to the same drugs, but belongs to group H (Grindley *et al.* 1972). These two R factors are thus quite distinct from each other, despite the similarity of their resistance markers.

The *fi*$^+$ character (Egawa & Hirota, 1962; Watanabe & Fukasawa, 1962; Watanabe, 1963; Watanabe *et al.* 1962, 1964) has now been identified in several

groups of plasmids. Although most $I_1$ and N factors are *fi*$^-$, *fi*$^+$ factors of these groups have been identified (Grindley & Anderson, 1971; Grindley *et al.* 1973). However, all wild $F_{II}$ R factors so far examined are *fi*$^+$.

Plasmids within a single compatibility group can be subdivided by their phage-restrictive effects in K12 and in salmonellas. *fi*$^-$ I-like R factors and transfer factors have been divided into eleven types by their typing phage restriction in *S. typhimurium* (Anderson *et al.* 1973). Further subdivision may be possible by the determination of phage restriction in *S. typhi* and *S. paratyphi B* (Anderson, 1966). The F factor of K12 restricts one *S. typhimurium* typing phage (Anderson *et al.* 1973) and unadapted Vi-phages III, V, VI and VII of the *S. typhi* Vi-typing scheme.

Non-transferring resistance determinants can be characterized by the specificity of their mobilization by transfer factors: for example, SSu determinants are in general most easily mobilized by I-like factors. The degrees of resistance they confer on their host strains may also be characteristic. Only resistance determinants from Class 2 systems can be characterized by their mobilization specificity, since they can be isolated without transfer factors in the host cell.

The foregoing description outlines the methods currently used in the Enteric Reference Laboratory for the characterization of transfer systems in the enterobacteria. These methods are useful for classifying the systems on the basis of their genetic properties and molecular structure, and may ultimately expose their origins and host relationships in man and animals.

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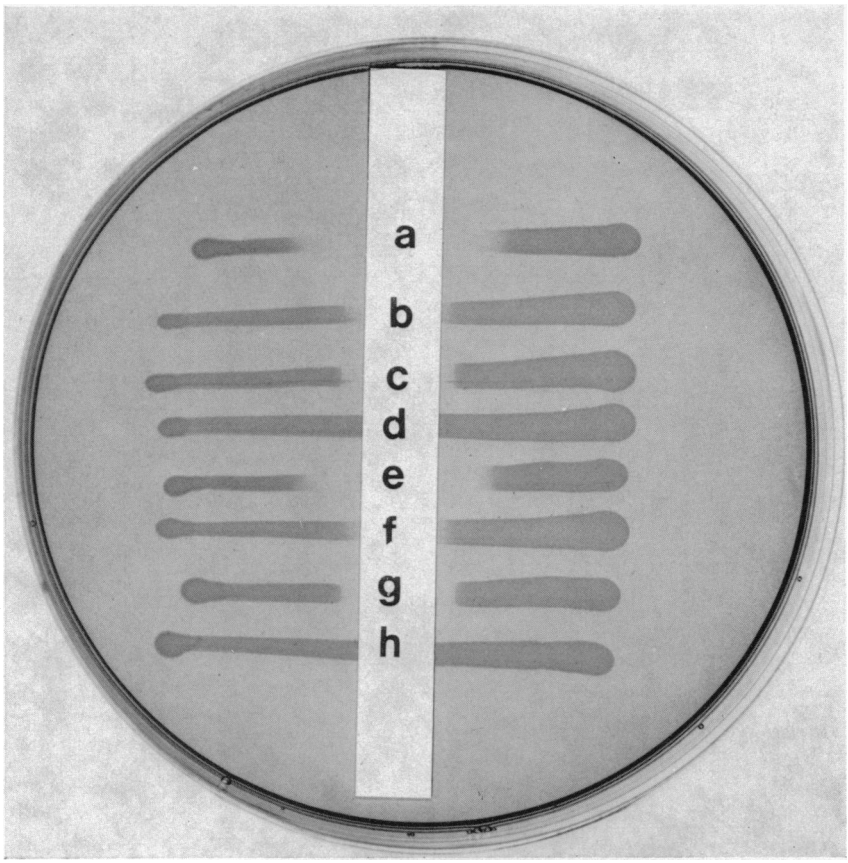




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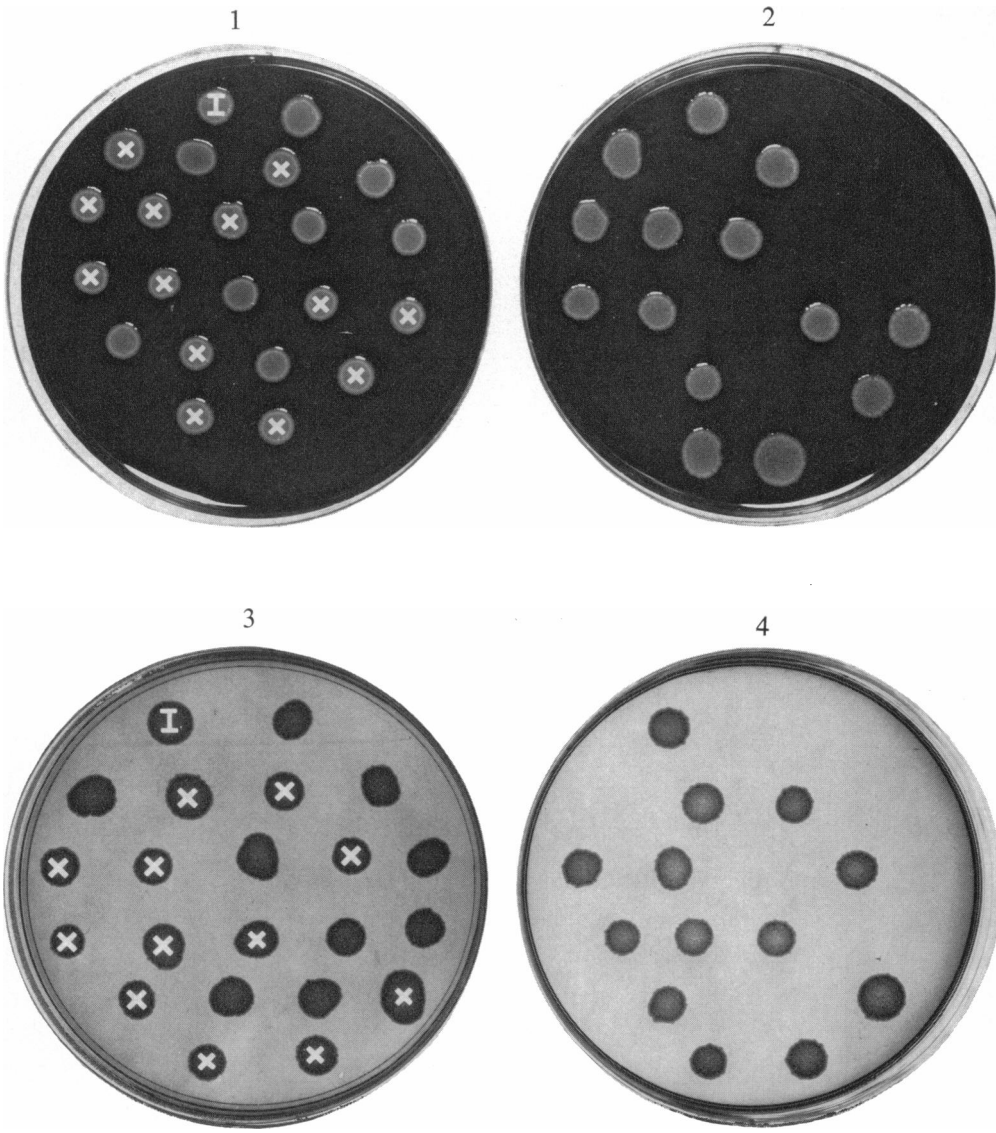
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## EXPLANATION OF PLATES

### PLATE 1

Resistance typing by strip-diffusion method. The central blotting-paper strip is impregnated with ampicillin Cultures (*a*)–(*d*) have the following MICs (*μg.*/ml.): (*a*) 4 (sensitive control); (*b*) 32; (*c*) 16; (*d*) 500. (*e*) (*f*) (*g*) and (*h*) are duplicates of (*a*), (*b*), (*c*) and (*d*).

### PLATE 2

Figs. 1 and 2. Sulphonamide resistance testing. In Fig. 1 the plate contains nutrient agar with 5% v/v lysed horse blood. In Fig. 2 the plate contains the same medium + 100 *μg.*/ml. sulphathiazole. Cultures marked with crosses in Fig. 1 are sulphonamide-resistant. I = resistant control.

Figs. 3 and 4. Nalidixic acid resistance testing. In Fig. 3 the plate contains nutrient agar, and in Fig. 4 nutrient agar with 40 *μg.*/ml. nalidixic acid. Cultures marked with crosses in Fig. 3 are nalidixic acid resistant. I = resistant control.