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# R Plasmids from Yersinia

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## INTRODUCTION

The recipient ability of *Yersinia pestis* and *Yersinia pseudotuberculosis* for R plasmids was demonstrated in the primary period of R plasmid research (Ginoza & Matney, 1963). Afterwards, transfer of R plasmids to *Y. enterocolitica* was confirmed by several workers (Knapp & Lebek, 1967; Rusu, Baron & Lazaroae, 1970; Kimura *et al.*, 1975*a*) and transfer to *Y. pseudotuberculosis* was shown by Zaremba (1972). However, there are few reports of detection of R plasmids in naturally occurring *Y. enterocolitica* and *Y. pseudotuberculosis* (Cornelis, Wauters & Bruynoghe, 1973; Kimura *et al.*, 1975*b*; Ikeda, Eda & Kimura, 1975).

Classification of R plasmids by incompatibility was initiated by several workers (Watanabe *et al.*, 1964; Meynell, Meynell & Datta, 1968; Chabbert *et al.*, 1972). The distribution of incompatibility groups was examined, not only in wild-type strains of Shigella, Escherichia and Salmonella, but also in *Proteus rettgeri* (Coetzee, Datta & Hedges, 1972), *Proteus morganii* (Hedges *et al.*, 1973), *Pseudomonas aeruginosa* (Datta *et al.*, 1971; Datta & Hedges, 1972), *Aeromonas liquefaciens* (Aoki *et al.*, 1971), *Providence* (Hedges, 1974) and *Serratia marcescens* (Hedges, Rodriguez-Lemoine & Datta, 1975). The epidemiology and classification of these plasmids were summarized by Datta (1975*a*, *b*).

In this paper, we describe patterns of drug resistance, *fi* types and incompatibility grouping of plasmids from six strains of *Y. enterocolitica* and *Y. pseudotuberculosis* obtained in Japan.

## METHODS

*Yersinia strains.* All 42 strains examined were isolated from the intestinal contents of swine, except one *Y. pseudotuberculosis* strain that came from a human patient with gastroenteritis. Of the 37 strains of *Y. enterocolitica*, of three different serotypes (O-3, O-5 and O-9), 29 were drug-sensitive, four were resistant to streptomycin alone, three to streptomycin and tetracycline, and one to ampicillin, streptomycin and tetracycline. All five *Y. pseudotuberculosis* strains were resistant to streptomycin alone.

*Escherichia coli* K12 *strains.* These were: J53, F$^-$ *lac*$^+$ *met pro*; J53 *nal*, a nalidixic-acid-resistant mutant of J53; W3104, F$^-$ *gal* $\lambda^-$; W3104 *rif*, a rifampicin-resistant mutant of W3104; W1895, Hfr *met*.

*Plasmids.* Standard plasmids, one of each of 16 incompatibility groups (Hedges, 1974), were kindly supplied by Drs Yoshikawa and Arai (who had received them from Dr N. Datta). The standard N group plasmid, N3 (Datta & Hedges, 1971), determined resistance to streptomycin (Sm), tetracycline (Tc) and sulphonamides (Su). R plasmids from Yersinia strains are shown in Table 1.

Table 1. *Nature of R plasmids from Yersinia strains*

| Plasmid designation | Strain of origin | Resistance markers* | *fi* type | Exclusion of N3 | *hspII* | Incompatibility group |
|---|---|---|---|---|---|---|
| pTE101 | *Y. pseudotuberculosis* Tateishi | Sm | − | − | − | N |
| pTE102 | *Y. pseudotuberculosis* TP1004 | Sm | − | + | + | N |
| pTE201 | *Y. enterocolitica* Y-42 | Sm | − | + | + | N |
| pTE211 | *Y. enterocolitica* TP83 | Sm,Tc | − | NT | + | Undesignated |
| pTE211-1† | *Y. enterocolitica* TP83 | Sm | − | + | + | N |
| pTE212 | *Y. enterocolitica* TP83 | Tc | − | − | − | Undesignated |
| pTE213 | *Y. enterocolitica* TP247 | Sm,Tc | − | + | + | N |
| pTE214 | *Y. enterocolitica* TP248 | Sm,Tc | − | + | + | N |

* Sm, Streptomycin; Tc, tetracycline.
† Segregant obtained after ultraviolet irradiation.
NT, Not tested.

*Phages.* P1kc and λ were used for the *hspII* restriction test and *f2* for the *fi* determination test.

*Media.* Liquid medium was Bacto-Penassay broth (Difco). Plating medium was Heart Infusion nutrient agar (Eiken).

*Transfer experiments.* Donor culture (0·1 ml) and recipient culture (0·9 ml) were added to 4·0 ml Penassay broth. After 3 or 24 h incubation at 37 °C, 0·1 ml portions of the mixture were inoculated on agar plates containing streptomycin (25 μg ml$^{-1}$) or tetracycline (12·5 μg ml$^{-1}$) and nalidixic acid (25 μg ml$^{-1}$).

*Plasmid incompatibility and entry exclusion.* Methods were as described by Coetzee *et al.* (1972).

*fi determination.* The method of Watanabe, Fukasawa & Takano (1962) was used. Hfr strains of *E. coli* K12 W1895 (NR84) and W1895 (NR45) were used as *fi*$^+$ and *fi*$^-$ controls respectively.

*Restriction and modification of phages.* The *hspII* restriction by R plasmids was determined as described by Bannister & Glover (1968). Phages P1kc and λ were each propagated on W3104 R$^-$ and on W3104 (N3). R plasmid N3 determines *hspII* restriction and modification specificity (Hedges, 1972). Each phage preparation was titrated on W3104 R$^-$ and on the same strain carrying N3 and each of the Yersinia R plasmids; efficiencies of plating (e.o.p.) were compared.

## RESULTS AND DISCUSSION

*Transfer of drug resistance to Escherichia coli* K12. The 13 resistant strains were examined for transfer of R plasmids to *E. coli* K12. Such plasmids were detected in six strains (Table 1). These R plasmids could also re-transfer to *E. coli* K12 W3104 *rif.* One strain (TP83) of *Y. enterocolitica* transferred Tc resistance and Sm,Tc resistance separately to the recipients (Table 1).

*fi character of Yersinia plasmids.* All R plasmids derived from *Yersinia* were found to be *fi*$^-$.

*Compatibility of R plasmids from Yersinia among the standard plasmids.* Because the Yersinia plasmids were all *fi*$^-$ they were not tested for compatibility with F-like plasmids. In the first tests, Yersinia strains were used as donors, the recipients being *E. coli* K12 strains carrying standard plasmids of known compatibility groups with distinguishable resistance markers. By this means it was shown that the Yersinia R plasmids all co-existed stably with plasmids of groups Iα, Iγ, P, A, C, J, H, L and S, i.e. they belonged to none of these groups. However, the compatibility of the Yersinia plasmids with plasmids of groups N, W and M

could not be tested in this way, since we had no standard plasmids of those groups that lacked Sm or Tc resistance. We decided to test for phage restriction and modification (r–m) because it has been reported that all R plasmids with r–m specificity *hspII* fall into compatibility group N (Hedges, 1972) and many of the *fi*$^-$ plasmids previously identified in Japan belonged to group N (Datta & Hedges, 1971).

*Restriction and modification of R plasmids from Yersinia.* Plasmid N3 and six of the Yersinia plasmids, when present in the indicator strain W3104, reduced the e.o.p. of P1kc or $\lambda$ at least 100-fold. No such restriction was seen when the phage had been propagated on an N3$^+$ strain. No greater than threefold differences in e.o.p. were found between titrations where there was no restriction. This result indicated that these six Yersinia plasmids determined *hspII* (Table 1) and suggested that they belonged to incompatibility group N.

*Compatibility of R plasmids from Yersinia with N3 (Su,Sm,Tc).* The frequency of transfer of N3 (per donor bacterium in 24 h mating) was measured using, as recipients, W3104 *rif* R$^-$ and W3104 *rif* carrying each of the Yersinia plasmids. With five of the plasmids so tested, the frequency of transfer was reduced by at least 200-fold compared with that of the R$^-$ control, i.e. entry exclusion of N3 was demonstrated (Table 1).

The W3104 *rif* R$^+$ transconjugants from the above experiments were then used as donors, the recipient being J53 *nal* and selection being for Sm and Tc resistance separately. Only the whole N3 resistance pattern was observed to be transferred, never the resistance pattern of the Yersinia plasmid without N3. This was taken as evidence that the Yersinia plasmids had been eliminated from the W3104 *rif* strains on the introduction of N3, i.e. that they were incompatible with N3.

The nature of the R plasmids derived from Yersinia is summarized in Table 1.

Strains of Escherichia (Otaya *et al.*, 1975) and Shigella (Tanaka, Tsunoda & Mitsuhashi, 1975) isolated in Japan during the past 4 to 7 years have most frequently been resistant to four drugs (streptomycin, tetracycline, chloramphenicol and sulphonamide) and most plasmids from those strains belonged to incompatibility group FII (Datta, 1975*b*). However, drug-resistance patterns of strains from Salmonella were mostly Sm,Tc and plasmids from them were mostly *fi*$^-$ (Nakaya, Yoshida & Terawaki, 1975) and belonged to incompatibility group N (Datta, 1975*a*). It seems that plasmids from Yersinia isolated in Japan resemble those from Salmonella.

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