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R124, an *fi*+ R Factor of a New Compatibility Class

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The plasmids of Gram-negative bacteria can be classified as *fi*+ or *fi*− (Watanabe *et al.* 1964). The former are capable of repressing the fertility functions, notably the production of sex pili, determined by the F factor (Nishimura, Ishibashi, Meynell & Hirota, 1967). Most *fi*+ plasmids carry genes determining the production of sex pili similar to those determined by the F factor (F-like pili) in antigenic specificity and phage adsorption (Meynell & Datta, 1966; Lawn & Meynell, 1970; Dennison & Hedges, 1972). Among the *fi*− plasmids a number of compatibility classes has been described (Watanabe, 1968; Datta & Hedges, 1971; Datta *et al.* 1971; Hedges & Datta, 1971). Two plasmids belonging to the same compatibility class cannot stably co-exist in a single cell. Among the *fi*+ plasmids four compatibility classes have been described, three of which we propose to name as follows:

FI, the class including F, ColV2, ColV3 (MacFarren & Clowes, 1967) and R386 (Dennison, 1972);

FII, the class including R1 and many other *fi*+ R factors (Meynell, Meynell & Datta, 1968);

FIII, the class including ColB-K98 and ColB-K166 (Frydman & Meynell, 1969);

fourthly, the class including R62 (R62, though *fi*+, determines I-like pili and has the compatibility of a typical I-like plasmid) (Lawn, Meynell, Meynell & Datta, 1967; N. Datta & R. W. Hedges, unpublished).

R124 is an *fi*+ R factor carrying tetracycline resistance and specifying F-like pili (Meynell & Datta, 1966). It is the only *fi*+ R factor so far tested to determine restriction and modification of a number of DNA phages including λ. The specificity of this restriction, which is unique, is termed *hsp* I (Bannister & Glover, 1968). Among the *fi*− R factors, all those capable of restriction or modification fall into a single compatibility group N (Hedges, 1972) and it seemed interesting to determine the compatibility specificity of R124. In this paper we show that this plasmid co-exists stably with members of all the compatibility groups listed above and is thus the first example of a new compatibility group, FIV.

METHODS AND RESULTS

R124 carried by a strain of *Escherichia coli* J53 (a derivative of K12, requiring proline and methionine) was used as the initial donor in all crosses. The recipient strain in each case was a derivative of K12 carrying genetic markers permitting selection after crosses with J53. All strains are described in Clowes & Hayes (1968). Recipients were selected as having acquired resistance to tetracycline (10 μg/ml medium was used).

R124 was transferred to J62 *his, pro, try, lac* carrying R1, an *fi*+ R factor carrying resistance to ampicillin, streptomycin, chloramphenicol, kanamycin and sulphonamides. All the recipients (20 of 20) retained R1 although no selection had been imposed. One of the

62J(R1)(R124) 'doubles' was chosen for further study. After growth in broth it retained both R factors in all tested clones (22 of 22). When used as a donor both plasmids were transferred to J53. The two plasmids were transferred independently: 20 of 20 clones selected as chloramphenicol resistant acquired R1 but not R124 while 17 of 17 clones selected as tetracycline resistant carried R124 but not R1. Thus, in the 'doubles', the two plasmids existed as separate replicons.

Transfer to C600 *thr, leu, thi, lac* carrying an F*lac*$^+$ was accomplished without eliminating the resident episome in 19 of 20 clones. The C600(F*lac*$^+$)(R124) 'double' selected for study was stable in replication in broth (354 *lac*$^+$ colonies among 382 colonies counted: 13 out of 13 tet^R). Transfer of R124 to *Escherichia coli* J62 was accompanied by transfer of F*lac*$^+$ in 5 cases of 20 studied. Transfer of F*lac*$^+$ to the same strain was accompanied by transfer of R124 in 16 of 20 cases. Strains of C600 and J62 carrying F*lac*$^+$ and R124 were tested for sensitivity to phage MS2. This phage adsorbs to F-like pili and produces visible lysis on strains producing such pili constitutively. Strains carrying the F factor produce pili constitutively unless repressed by a compatible *fi*$^+$ plasmid. Since our 'doubles' were not visibly lysed by MS2 we confirm that R124 is indeed *fi*$^+$.

Transfer of R124 to J53-1 (a mutant of J53 resistant to nalidixic acid) carrying a ColB-K98 plasmid was accomplished without eliminating the Col factor from any of the 20 tested recombinants. The 'double' chosen for testing was stably col$^+$ and tetracycline resistant (20 of 20). When it was used as a donor it was found that the unselected plasmid (ColB-K-98) usually but not invariably accompanied the R124 factor (18 col$^+$ among 20 tetracycline-resistant recombinants). Thus, the two plasmids, though frequently transferred together, were not inseparable and presumably replicate independently in the 'double'.

R62 (Lawn *et al.* 1967; N. Datta & R. W. Hedges, unpublished) carries resistance to ampicillin, streptomycin, tetracycline and sulphonamides. In order to test compatibility with R124, a segregant of R62 which no longer conferred resistance to tetracycline was isolated. This spontaneous variant, which was non-reverting, retained all other properties of R62. R124 was transferred into J62(R62) and in 19 of 20 tested clones the resident plasmid was retained. The 'double' chosen for testing was stable during growth in broth (24 of 24 colonies carried the resistance markers of both plasmids). Transfer of R124 (selected by tetracycline resistance) and R62 (selected by streptomycin resistance) to J53 was independent: 19 of 20 tetracycline-resistant colonies were streptomycin sensitive whilst 23 of 23 streptomycin-resistant colonies were sensitive to tetracycline.

The *hsp*$^+$II plasmids of group N are the only other R factors known to confer host specificity (Hedges, 1972) albeit with a specificity distinct from that of R124. Bannister & Glover (1970) showed that R124 co-existed stably with R313-T, an *fi*$^-$ R factor determining *hsp*II specificity, subsequently shown to be an N plasmid (Hedges, 1972). R15 is another N plasmid carrying resistance to streptomycin and sulphonamides and *hsp*II determinants (Watanabe *et al.* 1964: Bannister & Glover, 1968; Datta & Hedges, 1971). We mated J53(R15) with J62(R124) and selected for J62 resistant to streptomycin; 20 of 20 such clones were also resistant to tetracycline, i.e. carried R124 as well as R15. The clones tested inherited both plasmids stably (24 of 24 colonies were resistant to both streptomycin and tetracycline). When this strain was allowed to conjugate with J53 the two plasmids were transferred independently: after selection for tetracycline, resistant recombinants all (20 of 20) were streptomycin sensitive whilst, after selection for streptomycin resistance, all recombinants (18 of 18) remained sensitive to tetracycline.

DISCUSSION

We conclude that R124, already known to differ from other *fi*+ R factors so far described in carrying the *hsp*I determinants, belongs to a new compatibility group, the fifth to be described among *fi*+ plasmids. From strains carrying R124 and another plasmid, transfer of the two plasmids was independent when the second plasmid was R1, R62 or R15. Joint transfer was frequently observed from the strains carrying R124 and either F*lac* or ColB-K98. Co-transfer of plasmids replicating independently is not understood. It may depend on topography of sites of plasmid replication in the donor. Alternatively, it may be that a particular plasmid is better adapted for transfer in matings mediated by its own, or a closely related pilus. Salzman (1971) has shown that an *fi*+ R factor, R538-1, is not transferred efficiently in conjugation mediated by I-like sex pili.

It may be that, in the experiments described above, only closely related plasmids are co-transferable. Thus, R62, whose self-transfer is mediated by I-like pili, may be poorly adapted to transfer mediated by F-like pili. Similarly, R15 (an *fi*− R factor), is apparently quite unrelated to R124. The failure of R1 to show co-transfer with R124 is more surprising, but the pili produced by R1 are clearly separated structurally from other F-type pili, suggesting that this plasmid has evolved separately for a considerable period (Lawn & Maynell, 1970; Dennison & Hedges, 1972).

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