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SHORT PAPER

R-Factors used for genetic studies in strains of *Pseudomonas aeruginosa* and their origin

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SUMMARY

The origin of a number of R-factors used for genetic studies in *Pseudomonas aeruginosa* is described.

A number of R-factors have recently been used for genetic studies in strains of *Pseudomonas aeruginosa*, and in some cases there is already some confusion as to their origin and nomenclature. This note seeks to clarify this situation.

All the R-factors used so far (Table 1) were originally detected in strains of *P. aeruginosa* isolated either in the M.R.C. Industrial Injuries and Burns Unit at the Birmingham Accident Hospital (Lowbury *et al.* 1969; Roe, Jones & Lowbury, 1971) or in the Bacteriology Department of the Glasgow Royal Infirmary (Black & Girdwood, 1969). The Birmingham strains were widely distributed soon after the nature of their R-factor mediated resistance to carbenicillin was published (Sykes & Richmond, 1970; Fullbrook, Elson & Slocombe, 1970) and some R-factors, although ultimately derived from the same strain, have subsequently been maintained in different laboratories and may, by now, differ in some respects. For example, RP9 and R9169 both originated in *P. aeruginosa* strain 9169, and RP1 and R1822 are each derived from strain 1822 (Table 1). It is very likely that the original strains isolated by Lowbury *et al.* were heterogeneous with respect to the R-factors that can be isolated from each of them. It is known that the R-factors characterized as RP1 and RP1–1 can be isolated from the Lowbury strain 1822 depending upon the selection procedure used.

Evidence is accumulating that these plasmids can undergo variation when transferred to various bacterial strains and in at least one case (Stanisich, 1972) this appears to be due to genetic recombination between the R-factor and other components of the *P. aeruginosa* genome. At the moment it does not appear possible to set up criteria of identity or stability, but workers with these plasmids should be aware of the possibility of genetic change in them during laboratory passage in bacterial strains.

A particular problem of identity concerns the plasmid RP4. This R-factor was originally defined as the plasmid present in *P. aeruginosa* S8 (Black & Girdwood, 1969) and its properties have been described in detail (Sykes *et al.* 1972; Saunders & Grinsted, 1972). The version of RP4 whose compatibility properties were studied by Datta and her colleagues (Datta *et al.* 1971) is certainly different from the RP4 plasmid described elsewhere and seems to be very similar in its molecular properties to a plasmid of the RP1 type (J. R. Saunders, unpublished work) (Table 2). It seems very likely that a labelling error, in either the Department of Bacteriology, University of Bristol, or the Department of Bacteriology, Royal Postgraduate Medical School, occurred at some stage and that a strain carrying RP1, or a similar plasmid, has become identified as RP4.

It follows that the RP4 used by Heumann and his colleagues in their work on transfer of R-factors to *Rhizobium* spp. (Pühler *et al.* 1972) is likely to be an R-factor of the RP1 type. Indeed the only papers that describe the original RP4 are those by Saunders & Grinsted (1972) and Sykes *et al.* (1972). Henceforward the original RP4 will be renamed RP8, and 'RP4' will be retained for the plasmid distributed by Dr Datta's laboratory at the Royal Post-graduate Medical School (Table 1).

Table 1. *Origin and properties of R-factors used in studies with* Pseudomonas aeruginosa

R-factor	Initial carrier strain	Antibiotic resistance pattern	Alternative designation	Transfer of chromosomal genes	Refs.
RP1	1822*	P.N/K.T	—	+	3, 5, 8
RP1–1	1822*	P	?R18–1(R18)	?+	5
RP2	3425*	P.N/K.T	None	N.D.	—
RP4	?1822*, 3425* or a related strain	P.N/K.T	None	N.D.	2
RP8	S8§	P.N/K.T	(RP4)	N.D.	1, 6, 7
RP9	9169*	P.N/K.T	?R91	N.D.	—
R18–1	1822*	P	?RP1–1(R18)	?+	4
R30	3098*	P.N/K.T	None	+	9
R68	6886*	P.N/K.T	R6886	+	4
R91	9169*	P.N/K.T	R9169 ?RP9	+	4

Abbreviations: N.D., not demonstrated; +, certainly occurs; ?+, transfer occurs but at very low frequency. *Antibiotic resistance markers:* P, Type IIIa β-lactamase production; N/K, neomycin and kanamycin phosphorylating enzyme; T, tetracycline resistance (mechanism unknown). * These numbers refer to isolate numbers assigned by Lowbury *et al.* 1969. § Isolate numbers assigned by Black & Girdwood, 1969. Bracketed entries indicate designations that have been used in the past but are now discontinued

References: 1, Black & Girdwood (1969); 2, Datta *et al.* (1971); 3, Grinsted *et al.* (1972); 4, Stanisich & Holloway (1971); 5, Ingram *et al.* (1972); 6, Saunders & Grinsted (1972); 7, Sykes *et al.* (1972); 8, Lowbury *et al.* (1969); 9, Stanisich (1972).

Table 2. *A comparison of the properties of two versions of 'RP4' DNA with a standard preparation of RP1 DNA*

Plasmid designation	Marker pattern	% G+C	Buoyant density (g/c.c)	M.W. ($\times 10^{-6}$)	Hybridization with RP1 DNA (%)
RP1	P.N/K.T	60	1·719	40	> 85
RP4 (Saunders & Grinsted, 1972)	P.N/K.T	60	1.719	62	65
RP4 (Datta *et al.* 1971)	P.N/K.T	60	1·719	40	> 85

Abbreviations as in Table 1. Data from Grinsted *et al.* (1972), Saunders & Grinsted (1972) and from J. R. Saunders (unpublished experiments). Hybridization with RP1 was carried out with the purified RP1 DNA immobilized on filters (Ingram *et al.* 1972).

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