Properties of an R Factor from *Bordetella bronchiseptica*

By R. W. HEDGES AND A. E. JACOB

Bacteriology Department, Royal Postgraduate Medical School, London W12 0HS

AND J. T. SMITH

*Microbiology Section, Department of Pharmaceutics, School of Pharmacy,
University of London, London WC1N 1AX*

(Received 11 April 1974)

SUMMARY

R906, a plasmid derived from a wild strain of *Bordetella bronchiseptica*, was transferred to *Escherichia coli* K12. In this host it is stably heritable, replicating as a DNA molecule with a molecular weight of 34.6 megadaltons. There are approximately three copies of the plasmid per chromosome. It is a member of compatibility group P. In *E. coli*, R906 confers resistance to ampicillin, streptomycin, sulphonamides and mercury salts. Resistance to ampicillin is determined by an oxacillin-hydrolysing β -lactamase whereas the resistance to streptomycin is not determined by either of the previously described streptomycin-inactivating enzymes but by some other mechanism.

INTRODUCTION

Terakado, Azechi, Ninomiya & Shimizu (1973) reported that several strains of *Bordetella bronchiseptica* (isolated from the nasal cavities of young pigs) carried R factors, transmissible to *Escherichia coli* K12, determining resistance to ampicillin, streptomycin and sulphonamides. Since these are the only plasmids reported in naturally occurring strains of this genus we decided to investigate the properties of one of these R factors.

METHODS

Bacterial strains: *B. bronchiseptica* strain B016R⁺; *E. coli* K12 strains J53 (F⁻, *pro*, *met*), J53.2 (a rifampicin-resistant mutant of J53), J62 (F⁻, *pro*, *his*, *trp*, *lac*), HfrC (*met*) and W3110T (*thy*) (Bachmann, 1972).

Plasmids are listed in Table 1.

Bacteriophages were MS2 (Davis, Strauss & Sinsheimer, 1961) and PRR1 (Olsen & Shipley, 1973).

Measurement of efficiency of plasmid transfer and determination of compatibility and fertility inhibition. Techniques were as described by Datta *et al.* (1971), Coetzee, Datta & Hedges (1972) and Dennison (1972).

β -Lactamase assay. The β -lactamase mediated by R906 in *E. coli* K12 J53.2 was prepared and assayed as previously described (Hedges, Datta, Kontomichalou & Smith, 1974).

Radiolabelling and lysis of strain W3110T(R906), isolation of R plasmid DNA by caesium chloride-ethidium bromide density gradient centrifugation, neutral sucrose gradient analysis and calculation of plasmid molecular weight were as described by Jacob & Hobbs (1974),

Table 1. *Plasmids, representative of the set of compatibility groups, tested with R906*

Plasmid	Compatibility group	Resistances*	Reference
RA1	A	T, Su	Datta & Hedges (1973 <i>a</i>)
R57b	C	A, C, Gk, Su	Datta & Hedges (1972 <i>a</i>)
R386	FI	T	Dennison (1972)
R1 <i>drd</i> 16	FII	K	Hedges & Datta (1972)
ColB.K98	FIII		Hedges & Datta (1972)
R124	FIV	T	Hedges & Datta (1972)
Folac	FV		N. Datta (pers. comm.)
R811	G	A	Hedges (1974)
R27	H	T	Grindley, Humphreys & Anderson (1973)
R144	I α	T, K	Hedges & Datta (1973)
R483	I β	S, Tp	Hedges & Datta (1973)
R621a	I γ	T	Hedges & Datta (1973)
JR66a	I ω	S, K	Datta & Hedges (1973 <i>b</i>)
R391	J	K	Coetzee, Datta & Hedges (1972)
R387	K	S, C	Hedges & Datta (1971)
R446b	M	S, T	Hedges, Datta, Coetzee & Dennison (1973)
N3T	N	T	Hedges (1972)
R724	O	S, T, C, Su	Datta & Olarte (1974)
RP4	P	A, T, K	Datta <i>et al.</i> (1971)
R751	P	Tp	Jobanputra & Datta (1974)
Rts1	T	K	Coetzee <i>et al.</i> (1972)
R388	W	Su, Tp	Datta & Hedges (1972 <i>b</i>)
R6K	X	A, S	Hedges <i>et al.</i> (1973)

* A, ampicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; K, kanamycin; Su, sulphonamides; Tp, trimethoprim; Gk, gentamicin and kanamycin.

except that protoplasts were prepared by incubation with lysozyme for 10 min at 37 °C before lysis with detergent.

RESULTS

Phenotypic characteristics of R906⁺ strains of E. coli K12

Bordetella bronchiseptica, strain B016R⁺, transferred a plasmid, designated R906, to *E. coli* 153.2. R 906 is presumably identical with Rte16 (Terakado *et al.* 1973). In the latter host, R906 conferred resistance to ampicillin, streptomycin, sulphonamides and mercury salts. Strain Hfrc(R906) was visibly lysed by MS2 showing that R906 was f⁻. R906⁺ strains were visibly lysed by phage PRR1, a sensitivity so far limited to strains carrying plasmids of group P (Olsen & Shipley, 1973; Jobanputra & Datta, 1974; Hedges & Jacob, 1974).

R906 was transferred between strains of *E. coli* K12 with an efficiency of about 2×10^{-3} /donor/h (Table 2).

Molecular properties of R906

The covalently closed circular (c.c.c.) DNA tertiary form of plasmid R906 was isolated by using dye-buoyant density centrifugation, and its molecular weight was determined by comparing its sedimentation rate through a neutral sucrose gradient with bacteriophage λ DNA. Figure 1(*a*) shows the sedimentation of ³H-labelled R906 DNA together with ¹⁴C-labelled λ DNA. Sedimentation was from right to left. It was proved that the ³H-DNA bands peaking at fractions 14 and 25 were c.c.c. DNA and its open circular (o.c.) form respectively, by treating the R906 DNA with a very low concentration of deoxyribonuclease I. This enzyme converts c.c.c. DNA to the o.c. form, and the result of sedimenting the

Fig. 1. Neutral sucrose gradient analysis of plasmid R906 DNA. DNA samples were sedimented through a 5 to 20 % sucrose gradient at 100000 g and 20 °C, for 115 min. (a) ^3H -R906 DNA plus ^{14}C - λ monomer DNA. (b) DNase I-treated ^3H -R906 DNA. The DNA sample was treated with an equal volume of DNase I (5×10^{-10} g/ml) in 25 mM- MgSO_4 for 7 min at 25 °C. DNase I activity was stopped by the addition of one volume of 60 mM- Na_2EDTA . Fractions (0.1 ml) were collected directly onto glass-fibre discs and, after drying, washing and re-drying, were assayed for radioactivity. ●, ^3H ; ○, ^{14}C .

treated DNA is shown in Fig. 1(b). All the DNA previously peaking at fraction 14 (Fig. 1a) now peaked at fraction 25. A ^3H -DNA band peaking at fraction 2 (Fig. 1a) was an artifact since no DNA band corresponding to this band was observed in Fig. 1(b). It was probably formed by concentration of the leading edge of the c.c.c. DNA band at the tube bottom. Only one plasmid species was therefore present in strain W3110T(R906). The molecular weight of R906 DNA was calculated to be 34.6 megadaltons, and 3.1 plasmid copies per *E. coli* chromosome were isolated as c.c.c. DNA.

Table 2. *Interactions of R906 with other plasmids in E. coli K12*

Donor	Recipient	Drug used for selection*	Rate of transfer	Characteristics of transconjugants
J53.2(R906)	J62	S	2×10^{-3}	—
J53.2(R906)	J62(RP4)	S	1×10^{-6}	10/10 S ^R K ^S (i.e. RP4 eliminated)
J62(RP4)	J53.2	K	1×10^{-3}	—
J62(RP4)	J53.2(R906)	K	2×10^{-6}	10/10 S ^S K ^R (i.e. R906 eliminated)
J53.2(R906)	J62(R751)	S	NM	10/10 S ^R Tp ^S (i.e. R751 eliminated)
J62(R751)	J53.2(R906)	Tp	NM	10/10 S ^S Tp ^R (i.e. R906 eliminated)
J53(R906) (R144)	J62	K	1×10^{-3}	—
		S	6×10^{-4}	—

NM, Not measured.

* For abbreviations, see Table 1.

Table 3. *Properties of the β -lactamase mediated by R906*

	Rate of hydrolysis as a percentage of that of benzylpenicillin
Cephaloridine	83.3
Oxacillin	558.9
Methicillin	38.3
Ampicillin	140.7

The absolute rate of hydrolysis of benzylpenicillin was $1.9 \text{ nmol/min}/10^9 \text{ R}^+ \text{ bacteria}$.*Interactions of R906 with other plasmids*

R906 was tested for compatibility with the representative plasmids of the compatibility groups listed in Table 1. It was compatible with all except the representatives of group P, RP4 and R751. The evidence for incompatibility is presented in Table 2. Also shown in this Table is the evidence for exclusion of transfer of RP4 by R906 and of the reciprocal transfer.

The frequency of transfer of several P group plasmids has been shown to be inhibited by the presence of an R factor of group I α in the donor (Datta *et al.* 1971; Hedges & Jacob 1974). The rate of transfer of R906 is not significantly reduced by the presence of R144 in the donor (Table 2).

Ampicillin resistance of R906

In *E. coli* K12, R906 conferred resistance to low levels of β -lactam antibiotics (up to $5 \mu\text{g/ml}$ with ampicillin). Cultures of J53(R906) produced low levels of an oxacillin-hydrolysing β -lactamase of a type not previously reported (Table 3). The substrate specificity profile of this enzyme resembled that determined by R⁷ and R¹⁶, two plasmids of compatibility group O (Hedges *et al.* 1974), but the R906 β -lactamase hydrolysed cephaloridine more efficiently. The R906 enzyme was completely resistant to inhibition by 0.5 mM -*p*-chloromercuribenzoate, but its activity could be inhibited by chloride ions since its activity was reduced to 50 % by a 50 mM solution of sodium chloride.

Streptomycin resistance of R906

Although the presence of R906 increased the resistance of the strain towards both streptomycin and spectinomycin no enzymic activity modifying either of these antibiotics could be detected (J. Davies, personal communication).

DISCUSSION

Plasmids of group P have been shown to be transferable to and capable of stable inheritance in a remarkable range of genera (Datta *et al.* 1971; Olsen & Shipley, 1973). The finding of a P group R factor in a strain of *B. bronchiseptica* adds another genus to this list. P plasmids have been identified in bacterial strains isolated in the U.K. (Lowbury *et al.* 1969; Black & Girdwood, 1969; Jobanputra & Datta, 1974; Hedges, 1974), the U.S.A. (Hedges & Jacob, 1974) and the Republic of South Africa (A. van Rensburg, personal communication). Thus, the finding of a P plasmid in a strain isolated in Japan is further evidence for the worldwide distribution of plasmids of this group. The existence of phages, such as PRR1, which adsorb to pili produced only by bacteria carrying P plasmids (R. H. Olsen, personal communication) is also evidence for the abundance of these plasmids.

The antibiotic resistance mechanisms of R906 were also studied. The plasmid determined very low levels of an oxacillin hydrolysing β -lactamase, which presumably explains its ability to confer resistance to penicillins. Resistance to streptomycin and spectinomycin is apparently conferred by a mechanism different from that previously described (Davies, Brzezinska & Benveniste, 1971) since cultures of J53.2(R906) showed no ability to alter the structure of either antibiotic. Perhaps the presence of R906 reduces the rate of uptake of these drugs.

We are grateful to Dr N. Terakado for the *B. bronchiseptica* isolate and to Dr J. Davies for investigating the resistance to aminoglycosides conferred by R906. A.E.J. was supported by a grant to Naomi Datta from the Medical Research Council.

REFERENCES

- BACHMANN, B. J. (1972). Pedigrees of some mutant strains of *Escherichia coli* K12. *Bacteriological Reviews* **36**, 525-557.
- BLACK, W. A. & GIRDWOOD, R. W. A. (1969). Carbenicillin resistance in *Pseudomonas aeruginosa*. *British Medical Journal* **4**, 234.
- COETZEE, J. N., DATTA, N. & HEDGES, R. W. (1972). R factors from *Proteus rettgeri*. *Journal of General Microbiology* **72**, 543-552.
- DATTA, N. & HEDGES, R. W. (1972*a*). R factors identified in Paris, some conferring gentamicin resistance, constitute a new compatibility group. *Annales de l'Institut Pasteur* **123**, 849-852.
- DATTA, N. & HEDGES, R. W. (1972*b*). Trimethoprim resistance conferred by W plasmids in Enterobacteriaceae. *Journal of General Microbiology* **72**, 349-355.
- DATTA, N. & HEDGES, R. W. (1973*a*). R factors of compatibility group A. *Journal of General Microbiology* **74**, 335-336.
- DATTA, N. & HEDGES, R. W. (1973*b*). An I pilus-determining R factor with anomalous compatibility properties, mobilizing a gentamicin-resistance plasmid. *Journal of General Microbiology* **77**, 11-17.
- DATTA, N., HEDGES, R. W., SHAW, E. J., SYKES, R. B. & RICHMOND, M. H. (1971). Properties of an R factor from *Pseudomonas aeruginosa*. *Journal of Bacteriology* **108**, 1244-1249.
- DATTA, N. & OLARTE, J. (1974). R factors in strains of *Salmonella typhi* and *Shigella dysenteriae* 1 isolated during epidemics in Mexico: classification by compatibility. *Antimicrobial Agents and Chemotherapy* **5**, 310-317.
- DAVIES, J., BRZEZINSKA, M. & BENVENISTE, R. (1971). R factors: biochemical mechanisms of resistance to aminoglycoside antibiotics. *Annals of the New York Academy of Sciences* **182**, 226-233.
- DAVIS, J. E., STRAUSS, J. H. & SINSHEIMER, R. L. (1961). Bacteriophage MS2: another RNA phage. *Science, New York* **134**, 1427.
- DENNISON, S. (1972). Naturally occurring R factor, derepressed for pilus synthesis, belonging to the same compatibility group as the sex factor F of *Escherichia coli* K12. *Journal of Bacteriology* **109**, 416-422.

- GRINDLEY, N. D. F., HUMPHREYS, G. O. & ANDERSON, E. S. (1973). Molecular studies on R factor compatibility groups. *Journal of Bacteriology* **115**, 387-398.
- HEDGES, R. W. (1972). Phenotypic characterization of *fi*⁻ R factors determining the restriction and modification *hsp* II specificity. *Molecular and General Genetics* **115**, 225-233.
- HEDGES, R. W. (1974). R factors from Providence. *Journal of General Microbiology* **81**, 171-181.
- HEDGES, R. W. & DATTA, N. (1971). *fi*⁻ R factors giving chloramphenicol resistance. *Nature, London* **234**, 220-221.
- HEDGES, R. W. & DATTA, N. (1972). R124, an *fi*⁺ R factor of a new compatibility class. *Journal of General Microbiology* **71**, 403-405.
- HEDGES, R. W. & DATTA, N. (1973). Plasmids determining I pili constitute a compatibility complex. *Journal of General Microbiology* **77**, 19-25.
- HEDGES, R. W., DATTA, N., COETZEE, J. N. & DENNISON, S. (1973). R factors from *Proteus morganii*. *Journal of General Microbiology* **77**, 249-259.
- HEDGES, R. W., DATTA, N., KONTOMICHALOU, P. & SMITH, J. T. (1974). Molecular specificities of R factor determined beta-lactamases: correlation with plasmid compatibility. *Journal of Bacteriology* **117**, 56-62.
- HEDGES, R. W. & JACOB, A. E. (1974). *Molecular and General Genetics* **7**, 169-177.
- JACOB, A. E. & HOBBS, S. J. (1974). Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. *Journal of Bacteriology* **117**, 360-372.
- JOBANPUTRA, R. S. & DATTA, N. (1974). Trimethoprim resistance factors in Enterobacteria from clinical specimens. *Journal of Medical Microbiology* **7**, 169-177.
- LOWBURY, E. J. L., KIDSON, A., LILLY, H. A., AYLIFFE, G. A. J. & JONES, R. J. (1969). Sensitivity of *Pseudomonas aeruginosa* to antibiotics: emergence of strains highly resistant to carbenicillin. *Lancet* *ii*, 448-452.
- OLSEN, R. H. & SHIPLEY, P. (1973). Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. *Journal of Bacteriology* **113**, 772-780.
- TERAKADO, N., AZECHI, H., NINOMIYA, K. & SHIMIZU, T. (1973). Demonstration of R factors in *Bordetella bronchiseptica* isolated from pigs. *Antimicrobial Agents and Chemotherapy* **3**, 555-558.