JOURNAL OF BACTERIOLOGY, Nov. 1978, p. 808-811
0021-9193/78/0136-0808$02.00/0

Vol. 136, No. 2
Printed in U.S.A.

Plasmids of Incompatibility Group P Code for the Capacity to Propagate Bacteriophage IKe

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Received for publication 11 August 1978

Seven of eight plasmids of incompatibility group P were found to code for the capacity to propagate bacteriophage IKe in *Escherichia coli*. Six of the seven plasmids allowed propagation of IKe by one bacterial host (RG172) but not by another (RG176); the other plasmid allowed IKe propagation by both hosts. IKe propagation by a number of *E. coli* K-12 strains was quite variable. IKe*h*, an extended host range mutant of IKe, was found to plaque specifically on N^+ and P^+ strains.

Plasmids of the P incompatibility group are distinguished by their broad host range, free transmissibility among species of enterobacteria, and transmissibility to and among many bacterial species outside of the family *Enterobacteriaceae* including *Pseudomonas aeruginosa* (5, 14, 27). Antibiotic resistance plasmids of incompatibility group N, on the other hand, are very common in many genera of *Enterobacteriaceae* (3) but have a more limited host range than P plasmids, being generally not transmissible from *Escherichia coli* to *P. aeruginosa* (5, 23). It has been reported that representative plasmids from the N and P groups have little DNA homology with each other (9), although a conflicting report maintains that as much as 50% homology exists between the two groups (21). The phenotypic or functional similarities between N and P plasmids have not been thoroughly investigated. Plasmids of both groups code for a constitutively synthesized receptor for phage PRD1, a phage which also infects R^+ bacteria containing certain W group plasmids (29). One N plasmid inhibits the fertility of the P plasmid RP1, but other N plasmids do not interact with RP1 in such a manner (28). In this communication we report an additional property shared by the two plasmid groups. We have found that most plasmids of the P incompatibility group code for the capacity to propagate bacteriophage IKe, a phage previously believed to be specific to N plasmids (8).

Bacterial strains are listed in Table 1. All except RG176 are *E. coli* K-12 derivatives. The N and P plasmids used in the present study are listed in Tables 2 and 3. The plasmid-dependent phages employed were IKe (25), received from V. N. Iyer, and PRD1 (29) and PRR1 (30), received from R. H. Olsen. IKe propagation experiments were carried out principally with R^+ derivatives of two bacterial strains, RG172 and RG176. Titrations of IKe plaque-forming units (PFU) were done with the indicator strain RG945, a lactose-nonfermenting derivative of RG176 which contains the N plasmid RM98 (24). LBC basal and top agars (31) were used for all phage assays.

To determine the extent of propagation of phage IKe by R^+ strains we used a modified version of a protocol developed by Grindley and Anderson (12). The bacteria were diluted from overnight cultures into 1-ml aliquots of Penassay broth (Difco antibiotic medium no. 3) to give 1 $\times 10^7$ to 2 $\times 10^7$ viable cells/ml. Freshly titered phage IKe was immediately added to each culture to a final concentration of 10^5 PFU/ml. The infected cultures were incubated statically overnight at 37°C, and then centrifuged; and the supernatants were assayed for IKe PFU. The phage propagation factor was determined for each culture as the final phage titer in the supernatant divided by the concentration of the original input phage.

The information in Table 2 illustrates the diversity in origin of the P plasmids used in this study. R^+ derivatives of RG172 and RG176, which we have prepared with each P plasmid, are all sensitive to the phages PRR1 and PRD1. None is sensitive by plaque assay to phage IKe. In Table 3 are presented the IKe propagation factors obtained for the P^+ strains compared with the results of similar experiments with two N plasmids. All R^- control experiments showed propagation factors less than 1. From these data one recognizes that P plasmids can mediate three phenotypes with respect to IKe propagation. Most of them allow propagation of phage IKe by their RG172 hosts but not by their

TABLE 1. *Bacterial strains used*

Strain	Other designation	Genotype or phenotype	Derivation or source (reference)
RG1	W3110	F prototrophic	L. A. MacHattie (1)
RG11	SA495	F *his rpsL* Δ(*chlD-blu*)	Lambda-cured derivative of SA320 (10) from S. Adyha via D. Hoar
RG12	UC17	F Δ*lac arg his rpsL malB*	D. Hoar (18)
RG13	AB1157	F multiply auxotrophic, *rpsL*	D. Hoar (1)
RG15	UC198	F multiply auxotrophic	D. Hoar
RG17	K140	HfrC *ura*	J. R. Scott (20)
RG27		HfrC *met*	Lambda-cured derivative of DH50 from D. Hoar
RG28		HfrH *met*	Lambda-cured derivative of W1895 (1) from D. Kessler
RG172		F *his rpsL* Δ(*chlD-blu*) Nal^r	Nal^r derivative of RG11
RG176		*E. coli* C, F Nal^r prototroph	(11)

TABLE 2. *Plasmids of incompatibility group P used in the present study*[a]

Plasmid	Original carrier strain	Reference
RP1	*Pseudomonas aeruginosa*	14, 27
RP4	*Pseudomonas aeruginosa*	4, 6
R26	*Serratia marcescens*	32
R527	*Pseudomonas aeruginosa*	32
R702	*Proteus mirabilis*	15
Rm16b	*Pseudomonas aeruginosa*	G. Jacoby, personal communication
R906	*Bordetella bronchiseptica*	17
RP638	*Pseudomonas aeruginosa*	34

[a] P plasmids were received from the following individuals: R. H. Olsen (RP1), N. Datta (RP4, R702), V. A. Stanisich (RP1, R26, R906), and G. A. Jacoby (Rm16b, R527, RP638). Although it is possible that RP1 and RP4 are the same plasmid (19), we use the conventional separate designations.

TABLE 3. *IKe propagation by bacteria containing N and P plasmids*[a]

Incompatibility group	Plasmid	IKe propagation factor with plasmid in bacterial host	
		RG172	RG176
N	RM98	2.8×10^4	1.7×10^4
	N3	7.9×10^4	1.4×10^5
P	RP1	1.0×10^3	0.30
	RP4	5.5×10^1	0.46
	R26	8.5×10^2	0.56
	R527	2.5×10^2	0.28
	R702	3.3×10^3	0.28
	RP638	4.5×10^2	0.65
	Rm16b	0.17	0.44
	R906	9.9×10^1	1.8×10^2

[a] The P plasmids are described in Table 2. The N plasmids RM98 and N3 were received from V. N. Iyer et al. (25) and N. Datta (3), respectively.

RG176 hosts. One plasmid, R906, allows propagation of IKe by both hosts, and one, Rm16b, does not allow propagation by either host.

The extent of IKe phage propagation among the positive assays is quite variable, a phenomenon which has been noted with other plasmid-specific phages (12). It appears likely that the range of values greater than 1 in Table 3 represents variability in the assay system itself rather than differences among the plasmids. The value of 55 for RP4 is the lowest value for a positive assay that we have obtained with a P$^+$ derivative of RG172. The nonpropagating strains always give propagation factors with values less than 1. Presumably there is either nonspecific adsorption of the phage or inactivation of some of the phage particles during growth of nonpropagating cultures. The values for RP1 in Table 3 are the averages obtained with two plasmids designated RP1 that we received from different sources. These plasmids were indistinguishable from each other in the IKe growth experiments. The RG172 derivative harboring plasmid Rm16b has been confirmed as a nonpropagating strain by repeated tests.

To estimate the distribution of the genetic capacity for IKe propagation among plasmids belonging to incompatibility groups other than N and P, we have constructed R$^+$ derivatives of RG172 and RG176 with 29 plasmids from 18 incompatibilitiy groups and tested them in the standard IKe propagation experiment. None of these strains propagated IKe. All the propagation factors were less than 1; the averages were 0.30 for the RG172 and 0.40 for the RG176 derivatives. The incompatibility groups (and plasmids) used in these experiments were A (RA1), C (R57b-1, R40a), FI (R455-2), FII (R100, R100*drd-97*, R1-19K), FIV (R124), H1 (pRG1251), H2 (pSD114), Iα (R144, R144-3, R64, R483), J (R391), K (R387), L (R831), M (R446b), O (R16), S (R477-1, R478), T (R401,

R394, Rts1), W (S-a, R388, R7K), X (R6K), and Y (phage P1CM). Most of the plasmids were received from N. Datta (3, 16); R100*drd-97* was obtained from D. Hoar (18); R1-19K and R144-3 came from E. S. Anderson (13). We have previously described the H plasmids pRG1251 and pSD114 (33), and P1CM (16) is from our laboratory stock phage collection.

The selection of bacterial strains RG172 and RG176 as R^+ hosts for IKe propagation tests was dictated in part by the strains' nalidixic acid resistance, which allows counterselection against the variety of R plasmid donors used in strain construction. The choice of RG172 proved to be particularly fortunate as a number of subsequent experiments have demonstrated that some *E. coli* K-12 strains, e.g., RG1 and RG13, will not allow IKe propagation. Also, with strains other than RG11 and RG172, there is considerable variability in the extent of IKe propagation from experiment to experiment.

Direct plating of 10^8 to 10^{10} PFU of phage IKe in lawns of P plasmid-containing RG172 strains did not reveal any host range mutants of the phage which could plaque on P^+ strains. However a host range mutant eventually was selected by repeated propagation of IKe on RG172(RP4). After six consecutive propagation cycles, each cycle using a dilution of the phage yield from the previous growth cycle as the seed phage, plaques were detected in a lawn of the propagating host. One of these was purified, made into a stock by confluent lysis plate growth on RG172(RP4), and designated IK*eh*.

Phage IK*eh* is an extended host range mutant of IKe. It forms plaques identical in morphology to those of IKe when plated with N^+ *E. coli* and slightly smaller plaques on P^+ *E. coli*. It plaques on all the N^+ and P^+ strains of our collection including those which did not propagate IKe, except for the P^+ strains made with the plasmids Rm16b and R906. Phage IK*eh* does not plaque on RG172 and RG176 derivatives harboring either the R906 or Rm16b plasmid. It does not grow (by spot test of 10^7 to 10^8 PFU) on any other R^+ derivatives of RG172 and RG176 tested, including those made with the 29 plasmids from 18 incompatibility groups (listed above) which were found to be negative for IKe propagation. It is particularly noteworthy that, unlike phage PRD1, IK*eh* does not plaque on any of the W plasmid-containing strains. The host range of IK*eh* is specific to N^+ and P^+ strains.

That IK*eh* is not some kind of contaminating phage is supported by its inactivation by antiserum prepared against IKe. A high-titer preparation of IKe antiserum, kindly received from V. N. Iyer, which in our hands had an inactivation constant against IKe (K_{IKe}) of 3,090, was found to inactivate the plaque-forming capacity of IK*eh* such that a 250-fold dilution of the antiserum reduced the PFU of IK*eh* to a surviving fraction of 0.067 in 5 min ($K_{IKeh} = 135$). To control for possible nonspecific phage inactivation by the antiserum treatment, a preparation of the filamentous phage fd was treated for 15 min with a 50-fold dilution of the antiserum and was found not to be inactivated at all. It may also be noted that the protocol of consecutive propagation cycles of IKe used to obtain IK*eh* has been repeated, again resulting in the enrichment of IK*eh*.

The efficiency of plating (EOP) of IK*eh* varies from strain to strain, although repeated assays indicate that it is a constant feature of any given strain. In addition to the strains described in Table 1, we have constructed $RP4^+$ derivatives of 18 *E. coli* K-12 Hfr strains, most of them received as an "Hfr kit" from B. J. Bachmann; and we have found that these strains, like those of Table 1, fall into two groups, those with a low EOP (0.02 to 0.004), and those with a high EOP (0.10 to 0.86) for IK*eh*. RG1(RP4) is unique among the strains tested, having a very low EOP of 10^{-6}.

The selection and preliminary characterization of IK*eh* shed considerable light on the basis for IKe propagation by certain P^+ bacteria. It is probable that all or most of the cells of any P^+ *E. coli* culture contain an adsorption site which is inefficiently utilized by IKe but which is identified by comparatively efficient utilization by IK*eh*. This adsorption site for IK*eh* is constitutively synthesized by P^+ *E. coli* but appears to be greatly influenced (i.e., variable EOPs) by the genetic background of the host. Both N and P plasmids appear to be naturally derepressed for fertility (7), and there is some evidence that the IKe receptor is associated with the N plasmid's mating apparatus (8). Whether the IKe or IK*eh* receptors of P plasmids are involved in the conjugation process remains to be determined. In any event, there is variability among the P plasmids we have studied in the nature of the function (which we assume is a receptor) which allows IKe or IK*eh* growth. Most of the P plasmids, like RP4, allow IKe propagation and IK*eh* plaquing on RG172-derived strains. One plasmid, R906, is unique among the set because it allows IKe propagation by RG176 (confirmed by four separate experiments) yet does not allow plaquing or propagation of IK*eh*. Another unique plasmid, Rm16b, does not allow IKe propagation or IK*eh* plaquing by RG172 or RG176. However, IK*eh* propagates, although inefficiently (3- to 10-fold), on RG172(Rm16b). Thus, it appears that each of the P plasmids

codes for some kind of IKe or IKe*h* receptor.

The variation in IKe propagation by individual P^+ hosts remains to be explained. An analogous phenomenon has been described by Iyer and associates (22) who found that some transconjugants of *E. coli* B/r that received an N plasmid had greatly reduced levels of a particular outer membrane protein, whereas others from the same conjugation experiment had wild-type levels.

We acknowledge the following people for supplying bacterial strains, bacteriophages, and plasmids: E. E. Anderson, B. J. Bachmann, N. Datta, D. Hoar, V. N. Iyer, G. A. Jacoby, D. Kessler, L. A. MacHattie, R. H. Olsen, J. R. Scott, and V. A. Stanisich. We thank V. N. Iyer for his generous gift of IKe antiserum.

This work was supported by the Medical Research Council, Ottawa, Canada (grant MA-6091).

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