Two Compatibility Groups among ColI Plasmids

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INTRODUCTION

Several investigations have shown that f_i^- R factors fall into a number of compatibility groups (summarized by Datta & Hedges, 1973). Also Grindley, Grindley & Anderson (1972) and Hedges & Datta (1973) have described compatibility between certain pairs of R factors that determine I pili. Since Stocker (1966) first reported that the presence of the colicin I plasmid ColIb-P₉ in bacteria prevented them accepting ColIa-CA53 it has generally been assumed that ColI plasmids are incompatible. We therefore wish to report that our investigation of a collection of wild colI plasmids shows that some are compatible and that they can be grouped on this basis.

Incompatibility occurs between plasmids which are isogenic or closely similar and their inability to coexist in the same organism is explained on the basis of the maintenance site model; that is, by proposing that they have the same attachment site specificity and compete for a single maintenance site. Compatible plasmids, on the other hand, would have different specificities and would be matched to different maintenance sites (Novick, 1969). Incompatible plasmids are therefore inferred to be more closely related genetically than compatible ones.

With the exception of the now well-known ColIb-P₉ (Ozeki, Stocker & Smith, 1962), the other 24 ColI plasmids came from wild strains of *Salmonella typhimurium* and *Escherichia coli*. They have been described elsewhere with respect to their u.v.-protective effect and transferability (Howarth-Thompson, 1966; Howarth-Thompson, Meikle & Cave, 1971). Coexistence, or compatibility, has been tested only between Ia and Ib pairs and the outcome of superinfection inferred from the type, or types, of I-colicin produced by the progeny; that is, Ia or Ib, or both Ia and Ib. The superinfection procedure was simplified by the use of transfer derepressed (Drd) mutants of the superinfecting plasmids.

METHODS

ColI plasmids. ColIb-P₉ came originally from *Shigella sonnei* P₉ (Ozeki *et al.* 1962). The wild colicinogenic strains in which the other plasmids originated came from the Enteric Reference Laboratory, Colindale. The strains were kindly provided by Professor B. A. D. Stocker after being screened for the production of colicins of the Ia or Ib type (Stocker, 1966) and we transferred their ColI plasmids to laboratory strains for further investigations. Colindale reference numbers are used to identify them.

Evidence that they determine pili of the I-type is very good for two, Ia-N12 and Ib-1M1420, since we have obtained Drd mutants which confer on the host bacteria conspicuous sensitivity to the I sex phage Ifl (Lawn, Meynell, Meynell & Datta, 1967). HFCT cultures

(Stocker, Smith & Ozeki, 1963) of the other 22 show a small degree of sensitivity but the results are less conclusive (Howarth-Thompson, unpublished). Their fi^+ or fi^- status is unknown. Screening with low concentrations of tetracycline, chloramphenicol, streptomycin, kanamycin and ampicillin showed none carried resistance determinants (M. D. Cooke, personal communication).

Bacteria. Mutants of *Salmonella typhimurium* LT2, *cysD36* and *ath-5 str-r*, came originally from the Cold Spring Harbor collection and are maintained in this laboratory. *Escherichia coli* K12 strains W3110 *thy* and CR34 *thr leu thy lac str-s* F^- were kindly provided by Dr M. G. Smith and Dr D. Lane respectively. *E. coli* K12-Row (CL142), and indicator strains CL232 (which is sensitive to Ia colicin but immune to Ib) and CL223 (which is sensitive to Ib and immune to Ia) came from Professor B. A. D. Stocker.

Phage. Stock Ifl was obtained from Dr M. D. Cooke and was propagated on *S. typhimurium* LT2 M533, provided by Dr G. G. Meynell.

Superinfection procedure. All transfers were intraspecific with respect to the donor and recipient strains. Donor and recipient cultures were grown overnight in loosely capped 25 ml bottles holding 10 ml Difco brain-heart infusion broth (BH-broth), incubated without aeration or shaking. *E. coli* K12 and *S. typhimurium* LT2 grown in this way had viable counts of about 6.5×10^8 and 1.5×10^9 bacteria/ml respectively. A growing culture of the donor strain was prepared by subculturing 0.1 ml of the overnight culture into 10 ml of fresh BH-broth and growing without shaking until it contained about 10^8 bacteria/ml, as determined by viable counts on BH-agar (Difco). The culture was then blended on an MSE homogenizer at full speed for 1 min, to break up clumps of bacteria, and 2.0 ml was immediately mixed with 2.0 ml of the overnight recipient culture at 37 °C. The mixture was slowly rotated on an inclined turntable for 15 min at 37 °C, blended again for 2 min, then immediately diluted and plated by the sandwich plate technique (Ozeki *et al.* 1962). The donor was contra-selected by using BH-agar containing streptomycin (1000 µg/ml). After incubation for 60 h the plates were overlaid with soft agar inoculated with the appropriate indicator bacteria. Inhibition zones in the indicator lawn showed up those recipient colonies which had received the donor plasmid. Approximately 1.7×10^4 recipient colonies were screened in each experiment and transfer frequencies were calculated as the percentage of donor bacteria which had succeeded in transferring a plasmid to a recipient. Five of these colonies, inferred from their I-colicin type to have received the donor plasmid, were sampled by stabbing through the overlay, then resuspended in liquid medium and streaked on BH-agar. From each of the five streaks, five progeny colonies were tested for the production of colicins Ia or Ib, or both, using replica stab-plates and indicator strain CL232 or CL223. The process of streaking and stabbing was repeated two or three times, in series, with clones that were doubly colicinogenic, to establish whether or not they were stable.

RESULTS AND DISCUSSION

In the first series of transfers (Table 1) Ia-N12*drd-1* was transferred to colicinogenic strains each carrying one of the eighteen wild-type (w.t.) Ib plasmids. We have used only strains already in our stocks so there has been very little repetition of tests of pairwise combinations in both *S. typhimurium* and *E. coli*, except for Ia-N12*drd-1* with Ib-1M1420 or Ib-1M1240. Since their behaviour was substantially the same in both species the results presented in Table 1 are discussed as a whole.

Taking stable coexistence as the criterion of compatibility it appears that Ib-1M1168 and Ib-1M1420 fall into a different compatibility group, which we designate group 2, from the

Table 1. *A derepressed ColIa plasmid, Ia-N12drd-1, transferred to strains colicinogenic for various wild-type ColIb plasmids*

Ib w.t. plasmid of recipient	Ia transfer frequency (% of donor)	Inferred colicinogenic types in superinfected recipient colonies*		Predominant plasmid in singly colicinogenic progeny
		Doubly colicinogenic (Ia; Ib)	Singly colicinogenic	
<i>S. typhimurium</i> LT2 × <i>S. typhimurium</i> LT2				
1M1168	58.00	Stable	—	N.A.
1M1420†	104.00	Stable	Ib	N.A.
2M3841	1.90	Unstable	Ia or Ib	Ib
1M646	52.00	Unstable	Ia or Ib	Ib
2M607	36.20	Unstable	Ia or Ib	Ib
1M1679	30.70	Unstable	Ia or Ib	Ib
1M304	36.60	N.D.	Ia or Ib	Ib
1M1240	28.00	N.D.	Ia or Ib	Ib
2M1905	11.10	N.D.	Ib	Ib
2M3070	0.08	N.D.	Ib	Ib
<i>E. coli</i> w3110 × <i>E. coli</i> K12-Row				
1M1420†	16.00	Stable	Ia	N.A.
2M2035	12.90	Unstable	Ia or Ib	Either
2M2373	8.60	Unstable	Ia or Ib	Ia
1M1140	10.30	Unstable	Ia or Ib	Ib
1M1843	43.00	Unstable	Ia or Ib	Ib
P9	31.00	N.D.	Ib	Ib
2M59	51.00	N.D.	Ib	Ib
1M1240	16.00	N.D.	Ia or Ib	Ib
1M1992	6.70	N.D.	Ia or Ib	Ia
1M1995	6.90	N.D.	Ia	Ia

N.D., not detected; N.A., not applicable.

* All superinfected recipient colonies were initially detected by production of the donor colicin, Ia. However, when sampled, some yielded only Ib progeny, implying that Ia producers constituted only a minority of the colony population.

† Singly colicinogenic progeny rarely occur.

other sixteen Ib plasmids and Ia-N12 itself (group 1). The latter group may be further classified by their interactions with N12drd-1, according to whether there is either unstable coexistence or no detectable coexistence. Unstable coexistence means that doubly colicinogenic progeny persisted through two or three rounds of restreaking of such clones but that each time singly colicinogenic progeny appeared. There was, however, variation in the degree of instability, e.g. in the case of Ib-1M2035, Ib-1M2373 and Ib-2M3841 doubly colicinogenic progeny were usually in the majority, whereas for the rest of the plasmids in this category doubly colicinogenic progeny were in the minority. In Table 1 they have been arranged in descending order of stability. Even further classification on the basis of which of the two plasmids appeared most frequently in the singly colicinogenic progeny may be possible although, in general, it seemed to be the resident one (Table 1). However, the fact that one of the plasmids is w.t. and the other a Drd mutant may have influenced this outcome since a bacterium carrying a Drd mutant will be at a slight growth disadvantage compared with one carrying a w.t. plasmid. This was first reported by Dowman & Meynell (1970) and has also been observed by us. Nevertheless this growth disparity cannot always be effective, judging from the success of Ia-N12drd-1 following superinfection of strains carrying w.t. Ib-1M1992 or Ib-1M1995.

The reasons for the quite wide variation in transfer frequency to the various recipients is unknown. It seems unrelated to the outcome of the superinfection, i.e. whether there was coexistence or not. In general they were somewhat lower than the frequencies to non-colicinogenic recipients which were about 150% for *S. typhimurium* and about 30% for *E. coli*.

We also tested the group 2 Ib plasmid 1M1420 in *S. typhimurium* crosses with recipients each carrying a w.t. Ia plasmid, the group 1 plasmid N12 or one of three others not yet allocated: K29, 1M2037 or M6825. All showed stable coexistence with Ib-1M1420*drd-2*, indicating that they belong to compatibility group 1. These results also demonstrated that Ia-N12 and Ib-1M1420 coexist irrespective of which of them is *Drd* and which is w.t. In the case of this particular pair coexistence of the two w.t. plasmids was subsequently tested for and confirmed. Further support for this grouping was obtained by using the same four recipients and superinfecting with group 1 Ib-P9*drd-1*. No coexistence was found.

Finally, three additional Ia plasmids, N160, N14 and 2M70, were tested for coexistence with the group 1 Ib-P9*drd-1* in *E. coli*. One, N160, showed unstable coexistence and the other two showed none. All three have therefore been put in group 1.

The constitution of the two groups is as follows: group 1 comprises Ia plasmids, N12, K29, 1M2037, M6825, N14, 2M70 [N160] and presumably CA53 (Stocker, 1966), and Ib plasmids 1M304, 1M1240, 1M1905, 2M3070, P9, 2M59, 1M1992, 1M1995, [2M3841, 1M646, 2M607, 1M1679, 2M2035, 1M2373, 1M1140 and 1M1843]; and group 2 comprises Ib plasmids 1M1168 and 1M1420. Those whose inclusion is uncertain because they show unstable coexistence with another member of the same group are in brackets.

The relationship between these two groups and those recently described (Hedges & Datta, 1973) for R factors that determine I pili and the *ColI* plasmids Ia-CA53 and Ib-P9 has not been tested. It seems likely, however, that our group 1 corresponds to their group 1 α since Ia-CA53 and Ib-P9 are common to both.

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