Naturally Occurring R.ColBM Plasmids Belonging to the IncFIII Incompatibility Group

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(Received 15 January 1980)

Two *Escherichia coli* strains isolated from urinary tract infections were resistant to streptomycin, kanamycin, neomycin, tetracycline and sulphonamides. The strains also produced colicins B and M. The resistance to streptomycin, kanamycin and neomycin and the ability to produce colicins B and M could be transferred to an *E. coli* K12 recipient. Resistance and colicinogeny markers were transferred together by conjugation, and did not segregate even after interrupted mating or phage P1-mediated transduction. Hence, the drug-resistance and colicinogeny markers were carried by the same plasmid, designated as R.ColBM plasmid. The two R.ColBM plasmids were Fi^+ and produced an F-like pilus. They belonged to the IncFIII incompatibility group, being thus the first R plasmids identified in this group. The two plasmids were isolated and their molecular sizes were determined by electrophoresis in agarose gels and by contour length measurements. Both methods showed that both plasmids were about 52 megadaltons.

INTRODUCTION

F-like plasmids of the *Enterobacteriaceae* (Meynell *et al.*, 1968) are classified into five incompatibility groups (Datta, 1975; Novick *et al.*, 1976). A sixth group was proposed recently by Monti-Bragadin *et al.* (1975). The distribution of the F-like plasmids among these groups is not uniform; most of the plasmids belong to the IncFII and IncFI groups (Dennison, 1972; Hedges *et al.*, 1973; Datta, 1975). The IncFIII, IncFIV and IncFV groups are each represented by only a few plasmids (Hedges & Datta, 1972; Datta, 1975; De La Cruz *et al.*, 1979). Until recently, only three plasmids were known in the IncFIII incompatibility group, namely ColBM-K98 (Frydman & Meynell, 1969), Hly-20-72 and MIP240 (Le Minor & Le Coueffic, 1975; Le Minor *et al.*, 1976), and none of these carried drug-resistance markers.

During a search for R plasmids in *Escherichia coli* strains isolated from urinary tract infections, several R.ColBM plasmids were identified. Incompatibility studies showed that these plasmids belonged to the IncFIII group. As they were the first R plasmids identified in this incompatibility group, the biological and molecular properties of two of them were studied extensively. The results are reported here.

METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids are listed in Table 1. Plasmids were designated as recommended by Novick *et al.* (1976).

Bacteriophages. Phage P1 kc and the F-specific RNA phage f2 were obtained from P. Fredericq.

Media. The basic medium used for the growth of bacterial strains was brain-heart infusion (BHI, Difco). Simmons citrate agar supplemented with glucose (0.4 %) and the required growth factors (40 $\mu\text{g ml}^{-1}$, except for thiamin, 4 $\mu\text{g ml}^{-1}$) was the synthetic medium used for genetic analysis. Media for other purposes are indicated with the corresponding techniques.

Chemicals. Chemicals were reagent grade or USP. Antibiotics were purchased from Sigma.

Antibiotic susceptibility testing. Antibiotic susceptibility of bacteria was tested by the single disk method of Kirby-Bauer (Bauer *et al.*, 1966). The medium employed was Mueller-Hinton agar (Difco).

Colicinogeny. Colicinogeny was studied as described by Fredericq & Smarda (1970). The group of the colicin(s) produced by bacteria was identified by use of the corresponding resistant mutants from Professor Fredericq's collection.

Haemolysin production. Haemolysin production was tested on BHI-blood agar prepared with sheep erythrocytes (4 %).

Transfer of resistance. For the transfer of resistance, 0.1 ml of an exponential phase culture of the donor and 0.1 ml of an overnight culture of the recipient were mixed with 5 ml BHI broth and incubated for 48 h at 37 °C. Simmons citrate agar containing the required growth factors (40 $\mu\text{g ml}^{-1}$) and antibiotics (25 $\mu\text{g ml}^{-1}$) was used for selection. When resistance was transferred from wild-type donors, the counter-selected marker was nalidixic acid sensitivity. Transconjugants were re-isolated twice on the selection medium.

Transduction. Phage P1-mediated transduction was performed as described by Lennox (1955).

Fi character. Fi character was determined as previously described (Sonea *et al.*, 1971). Plasmids were in an Hfr host (SAS241) and the recipient strain for the transfer of chromosomal markers was W677-Azi r Sm r . Selection was for Thr $^+$ Leu $^+$.

Type of pilus. The type of donor pilus was determined as described by Meynell & Datta (1966). Plasmids were in *E. coli* C600 and the second host used to obtain an HFT (high frequency of transfer; Meynell & Datta, 1966) population was strain J53. The donor-specific phage was the RNA phage f2.

Plasmid incompatibility. For incompatibility studies, 1 ml of an exponential phase culture of the donor and 1 ml of an overnight culture of the recipient were mixed with 2 ml BHI broth and incubated for 1 h at 37 °C. In the first series of experiments, selection was made for the donor plasmid only. The transconjugants were re-isolated twice on the selection medium. This technique produced a 100 % elimination of the recipient plasmid when incompatible control plasmids were used (see Table 3). In a second series of experiments, selection was made for both donor and recipient plasmid markers. To determine the stability of clones carrying both plasmids, 10 to 18 transconjugants from each transfer were subcultured ten times in antibiotic-free BHI (size of the inoculum 10 $^{-3}$). The incubation period was generally short in order to reduce the probability of re-infection. At the end of the experiment at least 25 colonies per transconjugant were analysed for the presence of markers from the two plasmids.

Plasmid DNA isolation. Plasmid DNA was isolated by the method of Humphreys *et al.* (1975) from cleared lysates obtained by the method of Clewell & Helinski (1969). The plasmid DNA was separated by ultracentrifugation (Beckman L2-65B preparative ultracentrifuge, Ti75 rotor, 36000 rev. min $^{-1}$ for 66 h at 20 °C) in a CsCl density gradient (initial density 1.625 g ml $^{-1}$) in the presence of ethidium bromide (500 $\mu\text{g ml}^{-1}$). DNA bands were visualized by a long-wave ultraviolet lamp. The polyallomer tubes were punctured just below the covalent closed circular DNA band and the latter was extracted with a syringe. Dialysis was performed as described by Palchaudhuri *et al.* (1975). DNA in the extract was precipitated by alcohol and collected by centrifugation at 10000 rev. min $^{-1}$ in the cold. It was redissolved in Tris/EDTA buffer (10 mM-Tris pH 7.9, 10 mM-NaCl and 0.1 mM-Na $_2$ EDTA) and kept refrigerated.

Agarose gel electrophoresis. Agarose gel electrophoresis of plasmid DNA was performed as described by Meyers *et al.* (1976) in a vertical slab electrophoresis apparatus (model 220, Bio-Rad) at room temperature. Electrophoresis was carried out at 120 V and 60 mA. Plasmids of known size were applied in the same gel as controls: R1, 62 megadaltons (Mdal); RP4, 34 Mdal; Sa, 23 Mdal; ColE1, 4.2 Mdal (Meyers *et al.*, 1976). For the coloration of the DNA bands the gel was immersed for 30 min in electrophoresis buffer (Meyers *et al.*, 1976) containing 0.5 μg ethidium bromide ml $^{-1}$.

Electron microscopy. Samples for electron microscopy were prepared by a modified Kleinschmidt technique (Zollinger *et al.*, 1977). The sample contained R.ColBM plasmid DNA at 0.2 $\mu\text{g ml}^{-1}$, internal standard DNA (ColE1, 4.2 Mdal; Meyers *et al.*, 1976) at 0.1 $\mu\text{g ml}^{-1}$, 0.16 M-ammonium acetate, 0.02 % cytochrome *c* (horse heart type VI, Sigma) and sodium laurylsarcosinate (Ciba-Geigy). The sample was spread on 0.2 M-ammonium acetate. The molecules were rotary shadowed with platinum. The contour length of the plasmid molecules was determined with a Keuffel & Esser map measurer.

Table 1. *Bacterial strains and plasmids*

Designation	Genotype	Source
<i>Derivatives of Escherichia coli</i> K12		
Hfr Cavalli	<i>metB1 rel-1</i> , Hfr POA2	W. Hayes
SAS241	Hfr Cavalli <i>nal</i>	This laboratory
J53	<i>pro-22 met-63</i> , F ⁻	N. Datta
C600	<i>thr-1 leu-6 thi-1 lac Y1 tonA21 supE44</i> λ ⁻ , F ⁻	F. Jacob
W677-Azi,Sm	<i>thr-1 leu-6 thi-1 lac Y1 xyl-7 ara-13 mtl-2 gal-3 tonA2</i> λ ^R <i>azi rpsL supE44</i> , F ⁻	F. Jacob
AB2463	<i>thr-1 leu-6 thi-1 argE3 his-4 proA2 mtl-1 xyl-5 ara-15 galK2 lac Y1 tsx-33 rpsL31 sup-37 recA13</i> λ ⁻ , F ⁻	B. Bachmann (CGSC)
185Nx	prototroph, colicin-sensitive	P. Fredericq
186/T1	185 resistant to colicin M	P. Fredericq
185/Ab 3a	185 resistant to colicin B	P. Fredericq
185/Ab 3a/T1	185 resistant to colicins B and M	P. Fredericq
J53/R1	R1 in J53	N. Datta
SAS313	R1 in C600	This laboratory
L-A633	<i>thr leu thi</i> , ColE1 ⁺ , F ⁻	S. Luria
SAS393	R1, RP4 and Sa in L-A633	This laboratory
SLM	MIP240 in C600, <i>nal rpsL</i>	L. Le Minor
SAS407	pSAS1205 in SAS241	This laboratory
SAS408	pSAS1205 in C600	This laboratory
SAS409	pSAS1201 in SAS241	This laboratory
SAS410	pSAS1201 in C600	This laboratory
SAS413	pSAS1203 in SAS241	This laboratory
SAS414	pSAS1203 in C600	This laboratory
SAS417	pSAS1205 in AB2463	This laboratory
SAS418	pSAS1201 in AB2463	This laboratory
SAS419	pSAS1203 in AB2463	This laboratory
<i>Plasmids</i>		
R1	<i>amp cml str kan sul</i> , Fi ⁺ , IncFII	N. Datta
RP4	<i>amp tet kan</i>	N. Datta
Sa	<i>str cml kan sul</i>	N. Datta
ColE1	ColE1-K30	S. Luria
MIP240	Hly ⁺ , Fi ⁺ , IncFIII	L. Le Minor
pSAS1201	<i>str kan neo</i> , ColB ⁺ , ColM ⁺ , Fi ⁺ , IncFIII	This laboratory
pSAS1203	<i>str kan neo</i> , ColB ⁺ , ColM ⁺ Fi ⁺ , IncFIII	This laboratory
pSAS1205	<i>tet</i> , Fi ⁺ , IncFII	This laboratory

RESULTS

Transferability by conjugation. The original plasmid-carrying *E. coli* strains were isolated from urinary tract infections. They were resistant to streptomycin (Sm), kanamycin (Km), neomycin (Nm), tetracycline (Tc) and sulphonamides (Su) and produced a B-type colicin. The resistance to Sm, Km and Nm, as well as the ability to produce the colicin, could be transferred to derivatives of *E. coli* K12 (SAS241 and C600) by conjugation. Resistance and colicinogeny markers were always transferred together, even after interrupted mating or P1-mediated transduction (results not shown).

Identification of the colicin. Preliminary identification of the colicin produced by the plasmid-carrying strains was based on the results obtained with a colicin B-resistant mutant. The zone of inhibition obtained with this mutant was barely visible compared with the medium-sized zone obtained with the colicin-sensitive indicator strains. Suspecting the presence of a colicin M, which often accompanies colicin B production (Fredericq & Smarda, 1970), a double mutant, resistant to colicins B and M (185/Ab 3a/T1), was used. As expected, the double mutant was totally insensitive to the colicins produced by the two plasmids. Hence, the two R plasmids carry determinants for colicinogeny B and M. They will be designated collectively as R.ColBM plasmids.

Fi character. The Fi character of the two plasmids was determined in an Hfr donor (SAS241). Both plasmids inhibited the transfer of chromosomal markers to an F⁻ recipient

Table 2. *Increase in plaque-forming units of phage f2 in the presence of various plasmid-carrying strains*

Strain	Plasmid	Second host*	Plaque-forming units ml ⁻¹		
			Initial (T ₀)	After 1.5 to 2 h incubation (T ₁)	Increment (T ₁ /T ₀)
SAS410	pSAS1201	—	1.3 × 10 ⁴	4.9 × 10 ⁶	377
SAS410	pSAS1201	J53	2.6 × 10 ⁴	6.0 × 10 ⁸	23077
SAS414	pSAS1203	—	2.1 × 10 ⁴	2.5 × 10 ⁷	1190
SAS414	pSAS1203	J53	5.2 × 10 ⁴	1.8 × 10 ⁹	34615
Hfr Cavalli	F (integrated)	—	4.4 × 10 ⁶	1.5 × 10 ¹¹	34091
C600	—	—	1.4 × 10 ³	7.0 × 10 ²	—
J53	—	—	9.3 × 10 ³	9.0 × 10 ³	—

* Used in the case of an HFT population. The plasmid-carrying strain and strain J53 were inoculated in TYECa (Oxoid Tryptone, 10 g l⁻¹; yeast extract, 1 g l⁻¹; NaCl, 8 g l⁻¹; glucose, 0.15 %; CaCl₂, 2 mM) and incubated overnight. The following day the culture was diluted 1/20 in the same medium and re-incubated for 2 h at 37 °C. Bacteria were then infected with phage f2 (multiplicity of infection, about 20) and further incubated for a short period to allow phage adsorption. Excess phage was neutralized with f2 antiserum and the mixture was filtered (Millipore membrane, 0.45 µm pore size). The filter was transferred to TYECa and incubated for 1.5 to 2 h at 37 °C. The phage titre was determined in samples taken at the beginning (T₀) and at the end (T₁) of the incubation period. The increment (I) of the phage titre is given by the formula $I = T_1/T_0$. When I was determined in a non-HFT population the same method was used except that the second host (strain J53) was omitted.

Table 3. *Incompatibility reactions of R. ColBM plasmids*

Donor	Plasmid in donor	Recipient	Plasmid in recipient	Selected marker*	No. of trans-conjugants analysed	Elimination of recipient plasmid (%)
SAS409	pSAS1201	SLM	MIP240 (IncFIII)	Km	100	100
SAS413	pSAS1203	SLM	MIP240 (IncFIII)	Km	100	100
SAS409	pSAS1201	SAS408	pSAS1205 (IncFII)	Km	100	25
SAS407	pSAS1205 (IncFII)	SAS410	pSAS1201	Tc	140	17.8
SAS413	pSAS1203	SAS408	pSAS1205 (IncFII)	Km	100	23
SAS407	pSAS1205 (IncFII)	SAS414	pSAS1203	Tc	140	14.2
SAS407	pSAS1205 (IncFII)	SAS313	R1 (IncFII)	Tc	100	100
SAS407	pSAS1205 (IncFII)	SLM	MIP240	Tc	128	2.3

* In this series of experiments selection was made for the donor plasmid only. Transconjugants were re-isolated twice on the selection medium.

Table 4. *Effect of the recA mutation of the host on partial incompatibility*

Donor	Plasmid in donor	<i>recA</i> recipient	Plasmid in recipient	Selected marker*	No. of trans-conjugants analysed	Elimination of recipient plasmid (%)
SAS409	pSAS1201	SAS417	pSAS1205 (IncFII)	Km	200	74.5
SAS407	pSAS1205 (IncFII)	SAS418	pSAS1201	Tc	100	2
SAS413	pSAS1203	SAS417	pSAS1205 (IncFII)	Km	100	5
SAS407	pSAS1205 (IncFII)	SAS419	pSAS1203	Tc	100	3
J53/R1	R1 (IncFII)	SAS417	pSAS1205 (IncFII)	Km	100	65†

* In this series of experiments selection was made for the donor plasmid only. Transconjugants were re-isolated twice on the selection medium.

† After repeated inoculations of the remaining 35 transconjugants in antibiotic-free medium (ten subcultures in BHI at short intervals; size of the inoculum 10⁻⁸), only 23.4 % of the resulting colonies still carried both plasmids.

Table 5. *Stability of transconjugants carrying both plasmids*

Donor	Recipient	Selected markers*	No. of transconjugants analysed	No. of generations in antibiotic-free media	Total no. of colonies analysed	Percentage of colonies carrying:		
						Both plasmids	Donor plasmid only	Recipient plasmid only
SAS407 (pSAS1205, <i>tet</i> , IncFII)	SAS410 (pSAS1201, <i>str kan neo</i> , ColBM, IncFIII)	Tc Km	10	~100	482	90.3	3.5	6.2
SAS407 (pSAS1205, <i>tet</i> , IncFII)	SAS414 (pSAS1203, <i>str kan neo</i> , ColBM, IncFIII)	Tc Km	16	~100	658	91.3	3.5	5.2
SAS407 (pSAS1205, <i>tet</i> , IncFII)	SAS418† (pSAS1201, <i>str kan neo</i> , ColBM, IncFIII)	Tc Km	18	~100	1258	67.0	0.2	32.8
SAS407 (pSAS1205, <i>tet</i> , IncFII)	SAS419† (pSAS1203, <i>str kan neo</i> , ColBM, IncFIII)	Tc Km	12	~100	870	95.8	0.3	3.9

* In this series of experiments selection was made for both plasmids. A number of transconjugants from each transfer were analysed for the presence of both plasmids after repeated subcultures in antibiotic-free medium (ten successive re-inoculations at short intervals in BHI; size of the inoculum 10^{-5}). At the end of the experiments at least 25 colonies were analysed for each transconjugant.

† Plasmid in a *recA* host.

(W677). Hence they are Fi^+ plasmids like the majority of R plasmids isolated from *E. coli* (Meynell *et al.*, 1968).

Pilus production. The results obtained in studies on the type of pilus produced are presented in Table 2. The production of an F-type pilus by the two R.ColBM plasmids is clearly shown by the net increase in plaque-forming units, particularly when HFT populations were used. Hence, the two plasmids are F-like plasmids, as defined by Meynell *et al.* (1968).

Incompatibility studies. The results of the incompatibility studies with R.ColBM plasmids are presented in Table 3. The 100% elimination of the MIP240 (IncFIII) plasmid by the R.ColBM plasmids clearly indicates that the latter belong to the IncFIII incompatibility group.

The results obtained with an IncFII plasmid are more difficult to interpret (Table 3) since both R.ColBM plasmids give a partial incompatibility reaction with pSAS1205 (IncFII). This partial incompatibility is not due to a delayed elimination of one of the plasmids, since even after ten subcultures in antibiotic-free medium more than 90% of the clones originally selected for the presence of the two plasmids were still carrying both of them (Table 5, lines 1 and 2).

The study of the partial incompatibility between R.ColBM and IncFII plasmids in a *recA* host (Table 4) allows easy differentiation between the two R.ColBM plasmids (pSAS1201 and pSAS1203). In the case of the former, 74.5% of the transconjugants lost the IncFII plasmid when selection was made for pSAS1201, whereas the reciprocal cross gave only 2% elimination of pSAS1201 (Table 4). This asymmetrical loss of the IncFII plasmid was also evident when *recA* transconjugants carrying both plasmids were subcultured in an antibiotic-free medium (Table 5, line 3).

In the case of plasmid pSAS1203, partial incompatibility between pSAS1203 and the IncFII plasmid (pSAS1205) in a *recA* host was diminished in both directions (Table 4, lines 3 and 4) when selection was made for the donor plasmid. However, when transconjugants carrying both plasmids were subcultured in antibiotic-free medium, an asymmetric loss of pSAS1205 was obtained as with pSAS1201 (Table 5, line 4). Nonetheless, the elimination of IncFII by pSAS1203 was only 3.9%, whereas the elimination of the same plasmid by pSAS1201 was 32.8% (Table 5, lines 3 and 4).

Molecular studies. The molecular size of the two R.ColBM plasmids was determined by agarose gel electrophoresis and by contour length measurements in the electron microscope. The agarose gel electrophoresis results indicated that both R.ColBM plasmids were about 51 Mdal. Contour length measurements of ten molecules of each R.ColBM plasmid, using plasmid ColE1 as internal standard (Fig. 1), showed that both were about 52 Mdal.

DISCUSSION

There are only a few reports of naturally occurring plasmids which carry resistance and colicinogenic markers on the same molecule (Siccardi, 1966; Fredericq & Delhalle, 1972; Chabbert *et al.*, 1972) and I-like R-plasmids probably represent the exception to the rule (Falkow, 1975). This is puzzling since the association of the two types of markers might offer a selective advantage to the host. Moreover, it is difficult to understand how Col plasmids can avoid 'contamination' with resistance genes, especially with those carried by transposons (Cohen & Kopecko, 1976; Starlinger & Saedler, 1976). The same observation is also true for Ent plasmids which only rarely carry resistance genes (Gyles *et al.*, 1977). It would therefore seem that recombination between different types of plasmids is less frequent in nature than in laboratory conditions (Fredericq & Delhalle, 1972).

Study of the incompatibility of the R.ColBM plasmids showed that they belong to the IncFIII incompatibility group. Plasmids belonging to this group were found to give partial incompatibility reactions either with IncFII plasmids (Finnegan & Willetts, 1972; this paper)

Fig. 1. Electron micrograph of pSAS1201 (*R. ColBM*) and ColE1 plasmids. Bar marker represents 1 μ m.

or with an IncFIV plasmid (Le Minor & Le Coueffic, 1975; De La Cruz *et al.*, 1979). However, these partial reactions cannot be compared to the strong incompatibility reactions between IncFIII plasmids as observed in the present study (see Table 3). Therefore, we consider that IncFIII should be maintained as an independent group rather than as a member of an IncFIII–IncFIV incompatibility complex (De La Cruz *et al.*, 1979), at least until cloning experiments (Cabello *et al.*, 1976; Timmis *et al.*, 1978; Manis & Kline, 1978) clearly establish the nature of its incompatibility relations.

The mechanism of partial incompatibility is not well understood and the repressor model of incompatibility (Pritchard *et al.*, 1969; Novick, 1969; Uhlin & Nordström, 1975; Cabello *et al.*, 1976; Timmis *et al.*, 1978) allows several different interpretations of this phenomenon. Firstly, partial incompatibility might reflect the presence of a less specific effector of incompatibility (i.e. repressor) which reacts with plasmids of different specificities. This also supposes that the operator of an IncFIII plasmid might be recognized by repressors of various specificities. We do not have experimental evidence which could favour such a mechanism.

A second hypothesis may consider partial incompatibility as a particular form of a double incompatibility (Grant & Pittard, 1974; Cabello *et al.*, 1976; Timmis *et al.*, 1978) with one of the repressors being partially defective. The partial defectiveness of one of the repressors could be the direct result of the recombination which originated the composite plasmid. If this hypothesis is correct, and if the conditions which protect lambda repressor from inactivation, such as a *recA* mutation (Roberts & Roberts, 1975; Roberts *et al.*, 1978), could also protect a partial defective incompatibility repressor, we should observe the appearance of an asymmetrical loss of one of the plasmids in a *recA* host. This is because protection of a partially defective repressor might re-establish the conditions of a double incompatibility, characterized by the asymmetrical loss of one of the plasmids (Cabello *et al.*, 1976; Timmis *et al.*, 1978). Such an asymmetrical loss was indeed observed when incompatibility between *R. ColBM* and IncFII plasmids was studied in a *recA* host (see Tables 4 and 5). This would suggest that partial incompatibility of *R. ColBM* and IncFII plasmids might be due to the production of a defective IncFII incompatibility repressor by the *R. ColBM* plasmids. However, we must be cautious with this interpretation until the protective effect of a *recA* mutation on incompatibility repressors is clearly established.

Other interpretations of the partial incompatibility are also possible, such as a divergent evolution of two identical incompatibility determinants (Manis & Kline, 1978). Finally, it might be possible that several different mechanisms can lead to a partial incompatibility and only cloning experiments will establish the real mechanism in each case.

The two R.ColBM plasmids isolated in the present study are the first R plasmids belonging to the IncFIII incompatibility group described so far. The uniqueness of the R.ColBM plasmids makes them ideal epidemiological markers for studying the spread of drug-resistant strains in a hospital environment. It is known that the appearance of a drug-resistant strain in a patient is more often the result of the spread of hospital strains rather than of an *in vivo* transfer of an R plasmid (Richmond, 1975; Richmond & Petrocheilou, 1978). Such a mechanism seems also to be confirmed in the present case, since *E. coli* strains carrying R.ColBM plasmids were isolated from two other patients in the same hospital (unpublished results).

The isolation of the first IncFIII plasmids carrying resistance genes is also important for incompatibility studies. The other known plasmids in this group (Frydman & Meynell, 1969; Le Minor & Le Coueffic, 1975; Le Minor *et al.*, 1976) cannot be selected for easily and can be used mainly as recipient plasmids. This makes the study of incompatibility difficult, particularly with other plasmids that also cannot be selected for. The newly isolated R.ColBM IncFIII plasmids do not have this disadvantage and can be used in incompatibility studies as either donor or recipient plasmids.

We are indebted to Claude Vézina and Talat El Kersh for helping us with their experience in plasmid isolation. The contribution of Michel Truffy and Pierre Saint-Michel is gratefully acknowledged. Part of this investigation was supported by grant MA4997 from the Medical Research Council of Canada and by grant CAFIR 113 from the University of Montreal to A. S. B. M. is a recipient of a studentship from the Medical Research Council of Canada.

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