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A survey of Col plasmids in natural isolates of *Escherichia coli* and an investigation into the stability of Col-plasmid lineages

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(Received 30 September 1991; revised 17 March 1992; accepted 31 March 1992)

A survey of colicins in the ECOR reference collection of *Escherichia coli* is presented. Twenty-five of the 72 ECOR strains exhibited a phenotype consistent with colicin production and *E. coli* isolated from human hosts were more likely to be colicinogenic than those from animal hosts. Multiple representatives of two Col plasmids, low-molecular-mass ColE1 plasmids and high-molecular-mass, conjugative ColIa plasmids were isolated from the ECOR collection and were examined with a combination of restriction fragment and Southern analysis. These data suggested that ColE1 plasmids comprise a stable (cohesive) plasmid lineage, while ColIa plasmids represent a family of distinct plasmid lineages united by the presence of the colicin Ia operon.

Introduction

Colicins are bacteriocins produced by and active against *Escherichia coli* and related bacteria. Over 20 distinct colicins have been described which, although differing in their precise modes of killing, share a number of features in common. Bacteria are immune to the specific colicin they carry, colicin is generally released following cell lysis or quasi-lysis and there are usually three colicin-related genes: colicin, lysis and immunity genes (reviewed in Nomura, 1967; Hardy, 1974; Pugsley, 1984). In addition, colicins are almost always carried on plasmids, either small, high-copy-number plasmids, or large, conjugative plasmids (Pugsley, 1987).

The biochemistry and molecular biology of colicins have been widely studied (reviewed in Konisky, 1982; Luria & Suit, 1987), however, little is known about their ecological or evolutionary significance. There exists some data suggesting a role for colicins in bacterial competition, in the establishment of new bacterial strains in a bacterial community and as virulence determinants (Branche *et al.*, 1963; Ikari *et al.*, 1969; Kelstrup & Gibbons, 1969; Smith, 1974; Chao & Levin, 1981). However, these data are limited and often conflicting (Hardy, 1974; Pugsley, 1984). Indeed, as suggested by Pugsley (1984), the best evidence that colicins are of significance in bacterial population

Abbreviation: MLEE, multi-locus enzyme electrophoresis.

dynamics is the high frequency with which they are encountered in *E. coli*. There have been two large-scale colicin distribution surveys published. The first was restricted to K1 serotype isolates of human *E. coli* and the strains were further biased in favour of disease isolates (Achtman *et al.*, 1983). The second was restricted to clinical isolates from domestic animals (Singh *et al.*, 1989).

In this study, a survey of colicin frequencies from a more representative collection of E. coli isolates, the ECOR collection (Ochman & Selander, 1984), is presented. This collection is a set of 72 strains representing clinical and non-clinical isolates from man, domestic and zoo animals that have been characterized by a combination of serotyping, biotyping and multi-locus enzyme electrophoresis (MLEE). This survey of colicin distribution is part of a larger effort to determine the ecological significance and examine the molecular evolution of Col plasmids in E. coli populations. Of the colicins identified in the present survey, plasmids carrying colicins E1 and Ia were chosen to serve as representatives of low- and high-molecular-mass Col plasmids, respectively. These plasmids have been characterized with a combination of restriction fragment and Southern analysis.

Methods

Bacterial strains, plasmids and growth media. The ECOR collection (Ochman & Selander, 1984) is available from the *E. coli* Reference Center (Pennsylvania State University, USA). The Pugsley colicin collection, kindly provided by A. Pugsley, contains over 20 Col

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Table 1.	Colicin-bearing and	colicin-insensitive	strains	used
	in thi	s study		

E. coli K12 W3110 was the host for the colicin plasmids. The colicin-insensitive strains are mutants of W3110 (Pugsley, 1985).

Colicin-bearing strains				
Strain	Colicin type	Plasmid type*		
BZB2101	Α	I	_	
BZB2102	В	II		
BZB2103	D	I		
BZB2104	E 1	Ι		
BZB2125	E2	I		
BZB2106	E3	Ι		
BZB2107	E4	1		
BZB2108	E5	I		
BZB2109	E6	I		
BZB2110	E7	Ι		
PAP247	E8	I		
PAP1407	E9	I		
BZB2114	Ia	II		
BZB2115	Ib	11		
BZB2116	K	I		
PAPI	M	II		
BZB2123	N	I		
PAP2	S4	II(?)		
PAP222	v	I		

Colicin-insensitive strains

Strain	Colicin tolerance or resistance				
BZB1013	B, D				
BZB 1030	A, E1, E2-E9				
BZB1190	К				
BZB1191	М				
BZB1192	B, D, Ia, Ib, M, V				
PAP308	A, N				

* I, Plasmid not self-transmissible; II, plasmid self-transmissible.

plasmids obtained from natural isolates of bacteria and transferred to E. coli K12 W3110, as well as a number of colicin-insensitive mutants (Pugsley, 1985). The Pugsley strains used in this study are listed in Table 1. A derivative of W3110, BZB1011 (gyrA), and strain K12 1228 (gyrA r^{-m}) were used as bacteriophage and colicin-sensitive indicator strains. LB was used as the broth and plate medium. Mitomycin C at a concentration of $0.1-0.2 \,\mu g \, m l^{-1}$ was used to induce colicin production.

Colicin phenotypes. Three replicates each of a patch test and an overlay test (modified from Pugsley & Oudega, 1987) were used in colicin phenotype determinations of the ECOR collection. Since the mitomycin C treatment employed to induce colicin production will induce bacteriophage replication as well, a positive result from either of these tests indicates that the ECOR strain is producing a colicin, serving as a host to a temperate bacteriophage, or both. Two tests were required to distinguish between colicins and bacteriophage. (1) A supernatant fraction was prepared for each colicin-positive strain. The supernatant was serially diluted and dilutions spotted onto a lawn of strain 1228. If the halos became increasingly opaque as the degree of dilution increased, without the appearance of individual phage-like plaques, the strain was scored as positive for colicin production. If individual plaques were produced the strain was scored as positive for phage. (2) Colicin-positive strains were further tested for phage presence by adding 100 μ l of the spot test extract to a flask containing 10 ml of LB and 10⁸ cells of strain 1228. The culture was incubated overnight and a supernatant fraction was prepared. The supernatant was then spotted onto a sensitive lawn. Clearing of the lawn would occur if a bacteriophage was present in the ECOR strain.

To determine the identity of the colicins detected, each colicinpositive strain was tested with a patch test (with three replications) against lawns of the Pugsley colicin collection (Pugsley, 1984). This test takes advantage of the specific immunity provided to a colicin-bearing strain against the colicin it produces. If the unknown colicin is the same as one of the Pugsley collection, then that Pugsley strain should be immune and the patch would not be clear. If none of the Pugsley collection were immune, then the colicin is unique or produces more than one colicin.

To examine the sensitivity of the ECOR strains to colicins, for three replicate tests, crude colicin extracts of the Pugsley strains were prepared and spotted on to lawns of each of the ECOR strains.

Col plasmid transfer. Plasmids ColE1 and ColIa from the ECOR collection were transferred to a common host, strain CSH50 [Δ (*lac-pro*) ara rpsL gryA]. Matings (for conjugative ColIa plasmids) and transformations (for non-conjugative ColE1 plasmids) were carried out using standard techniques (Pugsley & Oudega, 1987). ColIa plasmids that transferred at low frequencies were allowed to incubate, without shaking, for 48 h. Selection of transconjugants and transformants was accomplished on tetrazolium lactose plates with 100 µg ml⁻¹ nalidixic acid (Berquist, 1987). After 10 h of growth, mitomycin C was spread on the plate. After an additional 5 h of growth, a colicin-sensitive lawn was overlaid. Transconjugants and transformants were revealed by zones of growth inhibition seen in the overlaid sensitive lawn.

DNA manipulations. Col plasmid transfer was confirmed by phenotype (see above) and by analysis of plasmid DNA using agarose gel electrophoresis. Plasmid DNA was prepared by the alkaline lysis method described in Sambrook *et al.* (1989). Restriction enzymes were obtained from Bethesda Research Laboratories and were used according to manufacturer's instructions. Standard electrophoresis and hybridization techniques were used (Sambrook *et al.*, 1989).

Probes. (1) A colicin-E1-specific probe consisted of a SstII/EcoRI fragment internal to the cea gene, located on the ColE1 plasmid sequenced by Chan et al. (1985) (see Fig. 1 a and b). This fragment was isolated following separation on a 0.7% low-melting-temperature agarose gel and cleaned with glass milk according to the manufacturer's protocols (GeneClean, Bio 101). (2) A colicin E2-E9-specific probe consisted of a 1.1 kb AvaII/BstY fragment internal to the ceaB gene. located in the colicin E2 operon sequenced by Cole et al. (1985) (Fig. 1a and c). The 1.1 kb AvaII/BstY fragment was chosen because it was restricted in homology to only colicins E2-E9 (see Results) and because the AvaII/BstY digestion produced a fragment that was easily separated from the remainder of the plasmid DNA on a 0.7% agarose gel. (3) A ColE1 plasmid probe consisted of undigested pColE1-K53 plasmid DNA (Pugsley, 1984). (4) A ColIa plasmid probe consisted of EcoRI-digested pAPBZ106 plasmid DNA (Pugsley, 1984). Probe DNA was radioactively labelled with [32P]dATP according to the Prime Time procedure (International Biotechnologies).

Results

Phenotypic analysis of colicins

Twenty-five of the 72 ECOR strains (35%) exhibited a phenotype consistent with colicin production. The colicin-positive strains and their relevant characteristics

B D F H J L N P R T V (b) A C E G I K M O Q S U

(a) A B C D E F G H I J K L M N O P Q R S T U V

Fig. 1. (a) Plasmid DNA preparations of the Pugsley collection of colicin strains separated by electrophoresis on a 0.7% agarose gel. Lanes: A, λ HindIII; B, ColA; C, ColB; D, ColD; E, ColE1; F, ColE2; G, ColE3; H, ColE4; I, ColE5; J, ColE6; K, ColE8; L, ColE9; M, Colla; N, Collb; O, ColK; P, ColM; Q, ColN; R, ColV; S, ColC7; T, ColB17; U, ColE1; V, & HindIII. (b) High stringency hybridization of the ColE1-specific DNA probe to the plasmid DNA from the Pugsley collection of colicin strains. Lanes as in (a). (c) High stringency hybridization of the ColE2-E9-specific DNA probe to the plasmid DNA from the Pugsley collection. Lanes as in (a).

are presented in Table 2. Colicin production was significantly more prevalent among human isolates than among animal isolates. Human isolates accounted for 55% of the ECOR strains, while they accounted for 80%of the colicin-positive strains ($\chi^2 = 6.05$; degrees of freedom, 1; P < 0.05).

Table 2. Colicin-positive strains of the ECOR collection

ECOP			Caliain		
no.	Source	Phage	>25 kb	<25 kb	phenotype*
3	Dog	+	+	+	Ia
11	Human	+	+	+	E(E2)
12	Human	+		+	E(E1)
14	Human	+	+	+	Ia
15	Human	+	+	+	Ia
24	Human	+	+	+	E(E1)
25	Dog	+	+	+	В
28	Human	+	+	+	Ia
31	Leopard	+		+	E(E1)
34	Dog	+	+	+	Ia
36	Human	+	+	+	E(E1)
38	Human	-	+	+	Ia
39	Human	+	+	+	E(E1)
40	Human	+	+	+	E(E1)
41	Human	+	+	+	K and/or N
42	Human	+	+	+	В
48	Human	+	+		Ib
50	Human	+	+	+	E(E1)
55	Human	+	+	+	?``
60	Human	+	+	+	E(E2)
61	Human	+		+	Ib
62	Human	+	+		В
63	Human	+		+	E(E1)
66	Ape	+		+	?
71	Human	+	+	+	E(E1)

* Designations in parentheses refer to whether the strain contains a plasmid hybridizing to the colicin-E1-specific or E2-E9-specific probes.

Each colicin-bearing strain carries one or more plasmid (Table 2). Indeed, plasmids ranging in size from roughly 4 to 200 kb were found in all but 10 of the ECOR strains (86%). Also common are temperate bacteriophage. Most of the ECOR strains are hosts to temperate bacteriophage (71%), while all but one colicinpositive strains carry one or more inducible phage (96%) (Table 2).

To identify the colicins further, a modification of the colicin typing scheme described by Pugsley & Oudega (1987) was employed. The outcome of these tests allowed the assignment of 22 of the 25 colicin-bearing strains to one or more colicin groups (Table 2). The two exceptions were ECOR 55 and 60. These strains were clearly producing bacteriocins, however, they did not produce a recognizable pattern of immunity/susceptiblity when tested against the Pugsley colicin collection.

Several strains could not be assigned unambiguously to a single colicin group. ECOR 41 produces a colicin which is neutralized by both K and N immunity proteins. In addition, strains ECOR 11, 12, 24, 31, 36, 39, 40, 50, 60, 63 and 71 have been assigned as colicin E. Due to the presence of bacteriophage and the occasional occurrence of immunity to both colicins E1 and E2 it was not possible to distinguish between these two E colicin types reliably by phenotype.



Colicin	ECOR strains susceptible			
	· · ·			
Α	1.4			
D	2.8			
El	1.4			
E2-E9	75.0			
Ia	0			
Ib	0			
К	1-4			
М	0			
Ν	0			
S4	0			
v	0			

 Table 3. Susceptibility of the ECOR strains to different colicins

Genotypic analysis of colicins E1 and E2

In an effort to characterize the E group colicins further, plasmid DNA preparations of each of the ECOR strains were probed with E1- or E2–E9-specific probes. To verify probe specificity each probe was hybridized to plasmid DNA prepared from the entire Pugsley collection of strains (Fig. 1). The results of the ECOR probings are given in Table 2 (parentheses in column 6).

Colicin susceptibility among the ECOR strains

The ECOR collection showed a broad range of colicin sensitivity (Table 3). The majority of the ECOR strains were susceptible to at least one of the colicins in the E2–E9 group and were resistant to all other colicins tested. ECOR strains 13 and 57 were the only strains susceptible to colicins outside of the E2–E9 group. Sixteen of the 72 ECOR strains (22%) were resistant to all of the colicins tested. There were significant levels of variation among the replicate tests in the degree of sensitivity shown by the ECOR strains to colicins from the E2–E9 group. However, within this group, sensitivity to colicin E4 was consistently the most common.

Col plasmid characterization

Two plasmid types, ColE1 and ColIa, were chosen to serve in efforts aimed at exploring the population dynamics and molecular evolution of Col plasmids in E. *coli*. To isolate the Col plasmids from the additional plasmids carried by the ECOR strains and to simplify subsequent molecular manipulations of the plasmids, ColE1 and ColIa plasmids were transferred to a common host.



Fig. 2. (a) HaeIII digestion of ColE1 plasmid isolates separated by electrophoresis on a 6% acrylamide gel. Lanes: A, ϕX HaeIII DNA size marker; B, ECOR 71; C, ECOR 63; D, ECOR 50; E, ECOR 40; F, ECOR 39; G, ECOR 31; H, ECOR 24; I, ECOR 12; J, ColE1. (b) High stringency hybridization of the ColE1 plasmid DNA probe to the HaeIII-digested ColE1 plasmid isolates. Lanes as in (a).

ColE1 isolates

Eight of the nine ColE1 plasmids were transferred into strain CSH50 by transformation. Strain ECOR 36, phenotypically a colicin E1 producer, had a plasmid of approximately 10 kb in size that hybridizes with colicin-E1-specific probe DNA (data not shown). However, this plasmid failed to transfer either as isolated plasmid or with the additional small plasmids this strain carries.

The eight remaining ColE1 plasmid isolates were digested with four restriction endonucleases (*HaeIII*, *TaqI*, *MspI*, *HinfI*) and their restriction fragment patterns were examined. A representative *HaeIII* digest is given in Fig. 2(*a*). The laboratory standard ColE1 (Pugsley, 1985) and strains ECOR 12, 24 and 50 comprised one plasmid group, sharing 36 of 37 fragments examined. Strains ECOR 39, 40 and 63 comprised a second group, sharing 48 of 48 fragments examined. Strains ECOR 31 and 71 each have their own unique patterns.

All of the ColE1 plasmids share a common set of restriction fragments, over the four restriction endonucleases examined; with differences between plasmid groups due to the presence or absence of one or more fragments. The shared fragments consist of twice the length required to encode the colicin E1 operon, thus presumably including not only the colicin operon, but additional plasmid sequences as well. The level of polymorphism revealed for the ColE1 plasmids is quite high. Plasmid nucleotide diversity (Nei, 1987), or basepair heterozygosity, is estimated to be 0.204 ± 0.059 when calculated from the number of restriction fragment differences pooled over the four restriction endonucleases examined.

High stringency hybridization of the laboratory standard ColE1 plasmid probe to the transferred restriction fragments of the eight ColE1 plasmid isolates revealed that the majority of all fragments have extensive sequence homology to the probe. Fig. 2(b) provides a representative hybridization pattern for the ColE1 plasmids examined. The homology extended beyond those fragments shared between the four classes of ColE1 plasmids to include many of the unique fragments as well. This result argues that ColE1 plasmids share a common plasmid background and that small insertion/ deletion events (in the order of 1-2 kb in length) and accumulated restriction site polymorphisms on to this common plasmid background can account for the restriction fragment differences observed among the ECOR ColE1 plasmid isolates.

ColIa isolates

Five of the six ColIa plasmids were transferred by conjugation into strain CSH50. Strain ECOR 38, phenotypically a colicin Ia producer, contained a highmolecular-mass and several low-molecular-mass plasmids. Repeated attempts to transfer the plasmid responsible for the colicin Ia phenotype failed. The six



Fig. 3. (a) EcoRI digestion of ColIa plasmid isolates separated by electrophoresis on a 0.7% agarose gel. Lanes: A, λ HindIII; B, ColIa; C, ECOR 3; D, ECOR 14; E, ECOR 15; F, ECOR 28; G, ECOR 34; H, λ HindIII. (b) High stringency hybridization of the ColIa plasmid DNA probe to the *Eco*RI-digested ColIa plasmid isolates. Lanes as in (a).

transferred ColIa plasmids were digested with four restriction endonucleases (*Eco*RI, *Hin*dIII, *Bam*HI, *Sal*I) and the restriction fragments were examined. A representative digest is given in Fig. 3(*a*). Strains ECOR 14 and 15 showed identical fragment patterns for each of the four digests. The remaining plasmids each had unique fragment patterns for each of the four digests. Unlike the case with ColEl plasmids, there is no common set of fragments shared by all ColIa plasmids.

High stringency hybridization of the ColIa plasmid probe to the transferred ColIa plasmid restriction fragments reveals that little of the plasmid DNA is homologous between these natural isolates. Fig. 3(b)provides a representative hybridization pattern for the ColIa plasmids examined. The observed differences in restriction patterns cannot be due simply to the accumulation of small insertion/deletions or polymorphic restriction sites, but rather must be due to the transfer of the colicin Ia operon between plasmids with very different evolutionary ancestry. This result is, again, quite different from that observed for ColE1 plasmids.

Discussion

The most obvious result from this study is that colicins were found at high frequencies in isolates of *E. coli* from

Table 4. Colicins in the ECOR and Achtman E. colicollections

		Colicin*								
	В	E1	E2	la	Ib	v	K/N	D	U	Total†
ECOR	3	9	2	6	2	0	1	0	2	25/72
Achtman	15	42	3	13	2	16	15	6	34	146/234

* Nineteen colicins were screened, only those detected are reported (E2 includes E2–E9, K/N indicates K and/or N, U refers to colicins with unknown identities).

† Total number of colicins detected/total number of strains surveyed.

natural sources. Thirty-five percent of the ECOR strains possess Col plasmids. In contrast, less than 2% of the ECOR strains possess characterized antibiotic resistance plasmids (Miller & Hartl, 1986; Lin Chao, personal communication). Two previous studies have also indicated that colicins are a common factor in bacterial populations. Achtman *et al.* (1983) examined 234 *E. coli* K1 strains isolated from diseased humans. They reported that 51% of the strains are colicinogenic. Table 4 provides a comparison of the number and kinds of colicins observed in the Achtman and ECOR strains.

The Achtman collection contains a higher frequency of colicins than the ECOR strains (51% versus 35%) and a different distribution of colicin types (G = 26.24; degrees of freedom, 9; $P \ll 0.01$). If the frequency of colicin-positive strains for only the human isolates of the ECOR collection are compared to the Achtman collection, then the frequency of colicinogeny in the two collections is identical (50%). In addition, E. coli isolated from animals with clinical conditions (Singh et al. 1989), had a frequency of colicinogenicity (22%) similar to that found in the animal isolates of the ECOR collection (16%). Thus these data argue that E. coli isolated from human hosts are more likely to be colicinogenic than those from animal hosts. The different distributions of colicin types in the Achtman and ECOR collections may be due, in part, to the bias towards disease isolates in the Achtman collection. For example, colicin V is found in high frequency in the Achtman collection and is absent in the ECOR collection. The ColV plasmid is found primarily among virulent enteric bacteria (Waters & Crosa, 1991), thus it is not surprising that a collection biased towards isolates from diseased hosts should have a higher frequency of a colicin implicated in pathogenicity.

Little is known about the ecological function of colicins in bacteria, although it has been suggested that they play a role in competition, the establishment of new strains in bacterial communities and pathogenicity (Smith, 1974; Chao & Levin, 1981; Pugsley, 1984;

Waters & Crosa, 1991). In this light it is intriguing to note that the majority of ECOR strains were resistant to most of the colicins detected in this study. ColE1 was the most common colicin in the ECOR collection, yet only one of the ECOR strains was susceptible to this colicin. Whether the widespread occurrence of colicin resistance among E. coli is the direct result of selection acting against the colicins is unknown. It is worth noting that bacteriophage and colicins often share comparable paths of entry into the cell (Konisky, 1982). Thus, selection for resistance to bacteriophage could have the indirect result of producing a strain resistant to a number of colicins as well. Bacteriophage are certainly found in high frequency in E. coli. Seventy-one percent of the ECOR strains possess one or more temperate bacteriophage. Further, it appears that wild-type E. coli are resistant to most temperate bacteriophage (Rhyzard Korona, personal communication).

ColE1 plasmids are roughly 6 kb in length. The results from the restriction fragment and Southern analyses suggested that ColEl plasmids isolated from multiple bacterial strains share the majority of this 6 kb of DNA, with short insertions and deletions creating 1–2 kb size differences between the plasmids. Because the majority of the 6 kb is shared among ColE1 plasmid isolates, these plasmids may represent a stable, or cohesive, plasmid lineage. The association of the term 'plasmid stability' with plasmid segregation requires the introduction of a new term, 'cohesive lineage', to describe the evolutionary stability of plasmid lineages described here.

Colla plasmids are roughly 150 kb. With the exception of the ColIa plasmids isolated fom strains ECOR 14 and 15, most of the 150 kb is not shared between the Colla plasmid isolates. These plasmids presumably share colicin Ia-homologous sequences and the region shared may be as long as that observed among the ColE1 isolates. However, the shared DNA constitutes only a small fraction of the entire plasmid. The hybridization results strongly indicated that the colicin operon region, or some larger fragment encompassing the operon region, has been transferred between distinct plasmid lineages. The ColIa plasmids do not constitute a cohesive plasmid lineage. It has been reported that ColV plasmids constitute a family of related plasmids that share the IncFI-correlated replication region, the four colicin Vrelated genes and an aerobactin ion uptake system (Waters & Crosa, 1991). Although the mechanism of transfer of these elements between distinct plasmid backgrounds has not been determined, it is likely to involve recombination of large blocks of DNA sequence, rather than transposon-mediated exchange (Waters & Crosa, 1991). Colla plasmids appear to represent a second family of Col plasmids, related by sharing at least the colicin Ia operon. Fine-scale restriction mapping is

currently underway to determine the extent of homology between members of this plasmid family.

Selander et al. (1987) provide a phylogeny for the ECOR collection based upon MLEE. As colicins are usually plasmid-borne, it is not surprising that colicin distribution among the ECOR strains does not correspond to chromosomal identity. For example, strains ECOR 61 and 62 are closely related in terms of their chromosomal loci and yet have different Col plasmids, while strains ECOR 12 and 50 have identical ColE1 plasmids and yet their chromosomes are highly divergent, as assayed by MLEE. Colicinogenicity is detected in the four major divisions of the ECOR lineages. This wide phylogenetic distribution implies either (1) an ancient origin of Col plasmids and subsequent loss of plasmids in many lineages or (2) frequent transfer of Col plasmids, or colicin operons, between unrelated host lineages. Although none would argue that plasmid carriage of colicins provides the opportunity for exchange of colicins between chromosomal lineages, it is unclear how common plasmid transfer is in vivo.

It is possible to address this question with the results of the restriction fragment analysis of ColE1 plasmids. Given that a cohesive ColE1 plasmid lineage was observed, the relationship among the ColE1 plasmids (i.e. their phylogeny) can be compared to the relationship of their hosts. If the plasmids and hosts have evolved together for some period of time, then the two phylogenies should overlap. If plasmid transfer is frequent and the evolutionary history of the plasmids is different from that of their hosts, then the phylogenies will not match.

Fig. 4(a) provides a phylogeny of the colicin-E1positive ECOR strains based upon MLEE (taken from Selander *et al.*, 1987). Fig. 4(b) provides a phylogeny for the corresponding ColE1 plasmids based upon restriction fragment differences. Both trees were constructed using the neighbour-joining method of Saitou & Nei (1987). A comparison of the branching patterns of the two trees revealed that one clear transfer event can be inferred. Deep branches in the MLEE tree separate strain ECOR 50 from strains ECOR 12 and 24. Their ColE1 plasmids have identical restriction fragments and thus cluster tightly on the plasmid tree. In this analysis it is assumed that Col plasmids do not experience variable rates of evolution and, therefore, the different branching patterns reflect at least one plasmid transfer event.

The remaining branching patterns of the two trees are remarkably similar, implying that the majority of the ColE1 plasmids have evolved in the same host for a long period of time. It should be noted that strain ECOR 63, which clusters with strain ECOR 71 in the MLEE tree and with strains ECOR 39 and 40 in the plasmid tree, has not been cited as an example of a second plasmid transfer



Fig. 4. (a) Phylogenetic tree for the ColE1-positive ECOR strains, based upon MLEE (taken from Selander *et al.* 1987). (b) Phylogenetic tree for the ColE1 plasmids isolated from the ECOR strains, based upon restriction fragment differences.

event. This is because the branch responsible for the cluster of strains ECOR 71 and 63 in the MLEE tree is quite short relative to the branches that distinguish between them.

Given the limited sequence homology between the ColIa plasmids, it is not possible to construct a ColIa plasmid phylogeny from the restriction fragment data. However, it should be noted that the two ColIa plasmids with identical restriction fragments (isolated from strains ECOR 14 and 15) occur in strains that are highly differentiated with respect to chromosomal genotype (Selander *et al.*, 1987). Thus, at least one transfer event is implied from the limited ColIa plasmid data.

It has been proposed that plasmids are unstable, mobile components of the bacterial gene pool (reviewed by Davey & Reanney, 1980). This view is based principally upon results from investigation of antibioticresistance-encoding (R) plasmids. However, little is known about the population dynamics and molecular evolution of most naturally occurring plasmids. The results of this study suggest that there may be quite different evolutionary forces experienced by different plasmids. The group of ColIa plasmids examined exhibit patterns of evolution that may be similar, in several respects, to the rapidly evolving R plasmids, with the colicin operon carried by multiple, unrelated plasmid lineages and between plasmid transfer of the colicin operon, a dominant theme in the evolution of this plasmid family.

The ColE1 plasmid exhibits quite a different evolutionary strategy. The colicin operon is maintained in a small, cohesive plasmid lineage. There is no evidence for transfer of the colicin operon between plasmid lineages and, in particular, no transfer from the small, nonconjugative background into large conjugative plasmid backgrounds. However, the ColE1 plasmids are stable only in the sense that multiple isolates share a common plasmid background. The plasmid DNA itself is rapidly evolving through a combination of small insertion/deletion events and accumulated base pair polymorphisms, at a level roughly tenfold higher than that observed for genes located on the *E. coli* chromosome (Dykhuizen & Green, 1986; Milkman & Crawford, 1983; Stoltzfus, *et al.*, 1989).

We thank Anthony Pugsley for kindly providing the colicin plasmid bank and Judith Mongold for critically reading earlier versions of this ms. We thank two anonymous reviewers and Bauke Oudega for their many helpful comments. This rearch was supported by the Sloan Foundation for the Study of Molecular Evolution (M.A.R.), the Canadian Medical Research Council (D. M.G.), a National Institutes of Health Grant (GM33782) to B. R. Levin and by the Department of Biology at Yale University, USA (M.A.R.)

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