Abortive phage-infection and UV-protection markers on Col I plasmids from epidemic strains of *Salmonella*

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Summary. Cultures of *Escherichia coli* carrying ColI plasmids received in conjugation from strains of *Salmonella typhimurium* and *S. agona* were examined for abortive infection (Abi) of phage BF23 and for enhanced resistance to the lethal action of UV-irradiation (Uvr). The Abi character of stored cultures of *E. coli* was also compared with the reaction of the same stock culture tested 5 years before. Seven of the eight potential types differentiated by three characters were represented among 160 ColI plasmids: ColIa Abi⁺ Uvr⁺ (3 plasmids), ColIa Abi⁻ Uvr⁺ (1), ColIa Abi⁻ Uvr⁻ (2), ColIb Abi⁺ Uvr⁺ (85), ColIb Abi⁺ Uvr⁻ (5), ColIb Abi⁻ Uvr⁺ (4), ColIb Abi⁻ Uvr⁻ (60). Recognition that different plasmid types could be carried by strains of a clone proved useful in the interpretation of the epidemic spread of strains of *S. typhimurium* of phage type/biotype 141/9f in Scotland and in tracing the ancestry of a recently emerged rhamnose non-fermenting mutant strain of *S. agona*.

Introduction

Epidemiological investigations of salmonella infections require accurate strain identification, and phage typing is the method of first choice for differentiating epidemic strains. The discriminating power of phage typing, however, may be greatly enhanced when it is used in conjunction with a second method such as biotyping (Anderson *et al.*, 1978). In many cases epidemic strains of the same phage type and biotype are probably clonal in origin (Barker, 1986a). During epidemic spread clonal strains sometimes acquire additional plasmids that may serve to differentiate subtypes within the clone.

Although colicinogeny is one such acquired character that can be determined easily, colicin production has been considered of little value as a primary typing method because most serotypes of *Salmonella* produce a limited range of colicins (Barker, 1980). ColI is the most common colicin plasmid found in *Salmonella* and consideration of other characters sometimes associated with ColI has extended the number of types recognised. The plasmids may be identified by immunity pattern as subtype ColIa or ColIb (Stocker, 1966) and both subtypes may be further differentiated by the character of abortive phage infection (Strobel and Nomura, 1966; Yousuf *et al.*, 1987). In addition, some ColI plasmids carry genes conferring on the

host bacterium resistance to UV irradiation (Howarth, 1965). These additional marker characters of abortive infection (Abi) and UV-protection (Uvr) differentiated epidemic ColIb⁺ strains of *S. agona* isolated in Dundee in 1979 and 1980 (Barker, 1986b).

The experiments reported in this paper describe the stability of the Abi character and the UV-protection character of 160 ColI plasmids of known colicin type. The Col plasmids from strains of *S. typhimurium* and *S. agona* have been assigned colicin, Abi and Uvr types. The value of this greater degree of differentiation of plasmid types is demonstrated with reference to epidemic strains of *S. typhimurium* and *S. agona* of known phage type (PT) and biotype (BT).

Materials and methods

Bacterial strains

The phage type and biotype of the 132 colicin I-producing strains of *S. typhimurium* and of 28 strains of *S. agona* are given in table I.

Shigella sonnei strain CT4 of Abbott and Shannon (1958) carried ColIb-P9. *E. coli* strain K12 14R519, F⁻ and NaI^R (Anderson, 1975) was used as the recipient of ColI plasmids in conjugation experiments. Stock cultures were maintained at ambient temperature on Dorset's egg medium in screw-capped bottles, some cultures of *Salmonella* for as long as 25 years and some of the recipient cultures of *E. coli* for as long as 5 years.

ColI was transferred from the producing culture of *Salmonella* to *E. coli* strain 14R519 during overnight mixed culture in static conditions in Nutrient Broth (Oxoid) at 37°C. Colonies of *E. coli* were selected on Nutrient Agar (Oxoid) containing nalidixic acid (40 µg/ml); individual colonies were tested for colicin production by the overlay method of Ozeki *et al.* (1962).

Bacteriophage and phage restriction

Bacteriophages BF23 and T5 were supplied, respectively, by Professor B. A. D. Stocker and Professor N. Symonds. They were propagated on *E. coli* strain K12 14R519.

Phage lysate (10^{10} plaque-forming units/ml) was diluted in nutrient broth and titrated on lawns of bacteria on plates of nutrient agar by the method of Males and Stocker (1980).

Source of UV irradiation and test for sensitivity

The UVC source and details of the method of estimating the exposure time (log. s) giving 50% killing of the irradiated culture have been described when used in the determination of the Uvr character of ColIb plasmids carried by strains of *S. agona* isolated in Dundee (Barker, 1986b).

Results

In 160 conjugation experiments the ColI plasmid was transferred from 132 strains of *S. typhimurium* and 28 strains of *S. agona* to cultures of *E. coli* strain 14R519. Colicin production was taken as evidence of carriage of the plasmid. Col⁺ cultures of the

recipient lines were examined for abortive infection of phage BF23 within 6 months of transfer of the plasmid (Yousuf *et al.*, 1987). Of the 160 ColI plasmids, 93 expressed the Abi⁺ phenotype (table I).

After an interval of 1–5 years after receipt of the plasmid, Col⁺ cultures of *E. coli* were examined for sensitivity to UV-irradiation. At each dose level of irradiation, i.e., exposure for 0.60, 0.95 and 1.15 log. s, the survival of *E. coli* strain 14R519 without a Col plasmid was considerably less than that of the same line of *E. coli* carrying ColIb-P9. The time required for 50% killing of the Col⁻ line of *E. coli* in 32 replicate tests was 0.69 log. s (range 0.55–0.80) and of *E. coli* carrying ColIb-P9 in 10 replicate tests was 1.07 log. s (range 1.00–1.11). Recipient strains of *E. coli* carrying ColI plasmids derived from *S. typhimurium* and *S. agona* segregated into 67 that survived exposure to UV to give a time for 50% killing of ≤ 0.8 log. s (and similar, therefore, to *E. coli* strain 14R519 without a Col plasmid, i.e., were Uvr⁻) and 93 that survived for ≥ 0.85 log. s (i.e., were Uvr⁺). Of the recipient strains carrying Col plasmids expressing the Uvr⁺ phenotype, 43 showed a survival pattern similar to that of *E. coli* strain 14R519 ColIb-P9, for which the time for 50% killing was ≥ 1.0 log. s and 50 survived to give a count resulting in the 50% killing time falling within the range 0.85–0.99 log. s indicating an intermediate level of protection to UV.

When cultures that had been stored for up to 5 years were re-examined for colicin production and abortive infection, 159 produced colicin and 90

Table I. Abortive infection (Abi) and sensitivity to ultraviolet irradiation (Uvr) of *E. coli* strain 14R519 with ColI plasmids from strains of *S. typhimurium* and *S. agona*

Strain*	Phage type	Biotype	Colicin type	Number tested	Number with reaction pattern			
					Abi ⁺ Uvr ⁺	Abi ⁺ Uvr ⁻	Abi ⁻ Uvr ⁺	Abi ⁻ Uvr ⁻
<i>S. typhimurium</i>	135	25h	Ib	6	0	0	0	6
<i>S. typhimurium</i>	141	1f	Ib	5	5	0	0	0
<i>S. typhimurium</i>	143	17a	Ib	41	40	1	0	0
<i>S. typhimurium</i>	56	17g	Ia	3	2	0	0	1
<i>S. typhimurium</i>	56	17g	Ib	13	5	0	0	8
<i>S. typhimurium</i>	141	9f	Ia	3	1	0	1	1
<i>S. typhimurium</i>	141	9f	Ib	61	11	4	0	46
<i>S. agona</i>	I or V	1a	Ib	8	4	0	4	0
<i>S. agona</i>	XVI	5a	Ib	20	20	0	0	0

* Strains of *S. typhimurium* of known PT/BT were: from localised outbreaks 135/25h (Barker *et al.*, 1980), 141/1f (Barker and Old, 1979) and 143/17a (Anderson *et al.*, 1978); or from extended outbreaks 56/17g (Barker *et al.*, 1980) and 141/9f (Barker and Old, 1979). Strains of *S. agona* were from Zaire (Barker *et al.*, 1982).

were Abi⁺. One culture of *S. typhimurium* of phenotype Abi⁺ Uvr⁺ when first tested, was Abi⁻ on retesting, but was still protected against UV (time for 50% killing of 1.03 log. s).

Abi and Uvr character of ColI plasmids from phage-typed and biotyped strains of S. typhimurium and S. agona

The ColI plasmids from 132 strains of *S. typhimurium* and 28 strains of *S. agona* were assigned an Abi type (by the reaction in the test made on the *E. coli* recipient stock culture when first tested) and a Uvr type. The Abi and Uvr types have been matched with the colicin type, phage type and biotype of each strain of *Salmonella* (table I).

Among the 41 cultures of *E. coli* carrying ColIb plasmids that originated in *S. typhimurium* strains of PT/BT 143/17a, 40 were Abi⁺ Uvr⁺ and one was Abi⁺ Uvr⁻. Other lines of *E. coli* that received this Col plasmid from the same strain of *S. typhimurium* during independent conjugation experiments were also Uvr⁻. Independent conjugation experiments with another strain of *S. typhimurium* of PT/BT 143/17a gave rise to cultures of *E. coli* that were Abi⁺ Uvr⁺, Abi⁺ Uvr⁻ or Abi⁻ Uvr⁺. Tests for abortive infection on several colonies of the ColIb-recipient line of *E. coli* indicated that the stock culture of *S. typhimurium* harboured plasmids of both Abi⁺ and Abi⁻ phenotypes.

Details of the colicin type of the ColI plasmids carried by strains of *S. typhimurium* of PT/BT 141/9f isolated in Scotland between 1972 and 1977 have been reported (Barker and Old, 1979). The Abi and Uvr characters of these plasmids are given in table I and, with details of each outbreak, in table II. Colicinogeny among strains of *S. agona* was rare except among cultures received from Zaire where patients were infected with ColIb⁺ strains of biotypes 1a and 5a (Barker *et al.*, 1982). The Abi and Uvr characters of the ColIb plasmids carried by these strains of *S. agona* are given in tables I and III. The additional information gained by determining the Abi and Uvr types of these ColI plasmids and its contribution to the epidemiological analysis of the strains are discussed below.

Discussion

The ColI plasmid was known to be generally stable in *S. typhimurium* (Barker, 1980) and was found to be rarely lost from cultures of *E. coli* to which it had been transferred. The *abi* gene, determining the character of abortive infection, is

probably located on the ColI plasmid carried by strains of *S. typhimurium* (Yousuf *et al.*, 1987), as it is on ColIb-P9 also (Boulnois, 1981; Pinkerton *et al.*, 1981). In this study, the characters Uvr, conferring enhanced resistance to UV-irradiation, and Abi were found in association with both ColIa and ColIb plasmids. They were frequently present together in that 88 of them were Abi⁺ Uvr⁺ and 62 Abi⁻ Uvr⁻, whereas five were Abi⁺ Uvr⁻ and five Abi⁻ Uvr⁺ (table I). The Abi character or the Uvr character was occasionally not expressed by a plasmid previously found to be Abi⁺ or that was thought to be Uvr⁺ from the evidence of similar plasmids found in other strains of the same PT/BT group, e.g., strains of *S. typhimurium* of PT/BT 143/17a. Thus, each character may be lost independently. Plasmids were not analysed to determine whether phenotypic loss resulted from excision of the gene or from a mutation leading to the presence of a non-functional gene. Again, cultures that did not produce colicin were not examined for the Abi⁺ and Uvr⁺ phenotypes although one conjugative plasmid (pRES-K317 found in *E. coli* strain K317) has been described (Males and Stocker, 1980) that was phage-restricting and conferred protection to UV-irradiation, but did not code for colicin production.

ColI plasmids derived from cultures of the same phage type and biotype isolated from sources that appeared related were usually of the same Abi/Uvr pattern; e.g., six ColIb⁺ isolates of *S. typhimurium* PT/BT 135/25h from a localised outbreak in Edinburgh were Abi⁻ Uvr⁻ and five ColIb⁺ isolates of *S. typhimurium* PT/BT 141/1f were Abi⁺ Uvr⁺ (table I).

The colicin type, Abi and Uvr characters of ColI plasmids carried by cultures of the same phage type and biotype from apparently unrelated sources were, in some instances, the same and suggested a possible epidemiological connection between the outbreaks, whereas among other groups of cultures the plasmids varied in one or more characters indicating that separate outbreaks were probably caused by different lines of infecting bacteria. Thus, 16 cultures of *S. typhimurium* of PT/BT 56/17g carried ColI plasmids with the following combinations of characters: ColIa Abi⁺ Uvr⁺, ColIa Abi⁻ Uvr⁻, ColIb Abi⁺ Uvr⁺, ColIb Abi⁻ Uvr⁻ (table I). The number of different plasmid types carried by the infecting strains suggested that the several outbreaks had originated from diverse sources.

The cultures of *S. typhimurium* of PT 141 (551 strains) isolated in Scotland from 1972 to 1977 were of one of three distinct clones of BT 1f, 9f and 31bd (Barker and Old, 1979). Colicinogenic strains of

types 1f ColIa (1 strain), 1f ColIb (5), 9f ColIa (3) and 9f ColIb (62) were isolated and 69 cultures were available for further examination. Abortive infection and sensitivity to UV differentiated the ColIa and ColIb plasmids carried by strains of biotype 9f into six subtypes (table I). Correlation of subtype character with epidemiological information considerably extended the degree of precision in tracing the likely route of spread of epidemic strains of *S. typhimurium* of PT/BT 141/9f (table II).

Early colicinogenic strains among the 110 isolates from human and other sources in Edinburgh in Aug. 1972 included 37 strains that were ColIb Abi⁻ Uvr⁻ and nine strains isolated from human, bovine and milk sources associated with an outbreak in 1973 at Farm 7, Midlothian that were also ColIb Abi⁻ Uvr⁻. Later isolates did not carry ColIb plasmids with this Abi/Uvr pattern. From Jan. 1973 to Dec. 1977 strains of type 141/9f caused many infections in cattle and cases of human infection. The ColI types found were: ColIa Abi⁺ Uvr⁺ (Penicuik Farm 4); ColIa Abi⁻ Uvr⁺ (Lanarkshire Farm 21); ColIa Abi⁻ Uvr⁻ (Glasgow); ColIb Abi⁺ Uvr⁺ (Edinburgh, Midlothian Farms 10 and 16, Wigtownshire Farm 12, and Glasgow); and ColIb Abi⁺ Uvr⁻ (Wishaw Farm 6, Midlothian Farm 10, Wigtownshire Farm 14) (table II). Thus, a source connected with the Edinburgh outbreak probably carried infection to Farm 7 where all victims were infected with *S.*

typhimurium carrying an Abi⁻ Uvr⁻ ColIb plasmid, and no person or animal from Farm 7 was the source of a subsequent outbreak. Related sources in these subsequent outbreaks yielded isolates of mixed colicin types, i.e., Col⁻ and Col⁺ and infection probably originated with a non-colicinogenic line of PT/BT 141/9f that acquired a Col plasmid from other enteric organisms resident in the host (Barker 1980).

Most of the 419 cultures of *S. agona* examined by Barker *et al.* (1982) were of biotype 1a and colicinogeny was generally rare in that serotype. A rhamnose non-fermenting mutant line of biotype 5a and colicin type Ib, however, became established in Zaire in 1979 at a time when other patients in that area were excreting ColIb⁺ strains of biotype 1a. The Rha⁻ strains also differed from the Rha⁺ strains in phage type (table III). The ColIb plasmids carried by isolates of biotype 1a were of the types Abi⁺ Uvr⁺ and Abi⁻ Uvr⁺, whilst those carried by the 20 isolates of biotype 5a were Abi⁺ Uvr⁺. Thus, the presence of a plasmid of similar colicin type and Abi/Uvr pattern in isolates of both biotypes provided further indication that, although of different phage types, the rhamnose non-fermenting line probably arose from an ancestral bacterium of biotype 1a, as suggested before (Barker *et al.*, 1982).

In conclusion, the presence of the marker characters Abi and Uvr carried by some ColIa and

Table II. Abortive infection (Abi) and sensitivity to ultraviolet irradiation (Uvr) associated with ColI plasmids in *S. typhimurium* strains of PT/BT 141/9f from outbreaks in Scotland 1972-1977

Year	Place of outbreak*	Number of isolates			Number with reaction pattern			
		All	Col ⁺	Col type	Abi ⁺ Uvr ⁺	Abi ⁺ Uvr ⁻	Abi ⁻ Uvr ⁺	Abi ⁻ Uvr ⁻
1972	Edinburgh restaurant	53	5	Ib	0	0	0	5
	Edinburgh (man)	57	32	Ib	0	0	0	32
1972-73	Penicuik Farms 4, 5	16	1	Ia	1	0	0	0
1973	Wishaw Farm 6	3	1	Ib	0	1	0	0
	Midlothian Farm 7	9	9	Ib	0	0	0	9
	Edinburgh (man)	20	4	Ib	4	0	0	0
1973-75	Midlothian Farm 10	31	4	Ib	2	2	0	0
1973-76	Wigtownshire Farm 12	50	3	Ib	3	0	0	0
1974	Wigtownshire Farm 14	3	1	Ib	0	1	0	0
	Midlothian Farm 16	1	1	Ib	1	0	0	0
	Lanarkshire Farm 21	2	1	Ia	0	0	1	0
1975	Glasgow (man)	22	1	Ib	1	0	0	0
1976	Glasgow (man)	30	1	Ia	0	0	0	1

* For further details see Barker and Old (1979).

Table III. Characters of *S. agona* strains isolated in Zaire in 1979 and 1980

Year of isolation	Number of isolates	Phage type	Biotype	Plasmid type
1979	4	V	1a	ColIb Abi ⁺ Uvr ⁺
1979	2	V	1a	ColIb Abi ⁻ Uvr ⁺
1979	2	I	1a	ColIb Abi ⁻ Uvr ⁺
1980	7	XV	1a	Col ⁻
1979	6	XVI	5a	ColIb Abi ⁺ Uvr ⁺
1979	1	NT	5a	ColIb Abi ⁺ Uvr ⁺
1980	13	XVI	5a	ColIb Abi ⁺ Uvr ⁺
1980	1	XVI	5a	Col ⁻

NT = non-typable.

ColIb plasmids greatly extended the value of plasmid typing of otherwise identical strains of *Salmonella*. ColI plasmids of mol. wt $c. 61.5 \times 10^6$ are relatively large and may well carry genes for other characters that may be used to differentiate

the plasmids and monitor their spread in host bacteria.

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