Colicinogeny in *Salmonella typhimurium*

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(Received 18 February 1980)

Colicin types Ia, Ib, E₁, E₂, B with M, K, S₄ and a new salmonellin-like colicin were found in 531 (11·8 %) of 4481 wild-type cultures of *Salmonella typhimurium*. Colicin typing added little useful information to phage typing and biotyping in strain differentiation, mainly because the most common types, Ia and Ib, are controlled by conjugative plasmids. Evidence from the mixed-Col⁻/Col⁺ pattern of colicinogeny in circumscribed outbreaks caused by strains of known phage type and biotype suggested that some Col factors are readily acquired by *S. typhimurium* from other enteric species. When a Col factor of the non-conjugative type, e.g. ColE₂, becomes established in strains of a successful phage type/biotype line, it may be a useful additional marker character.

INTRODUCTION

Colicinogeny in *Salmonella typhimurium* is dependent on specific Col plasmids (Ozeki *et al.*, 1962). Previous surveys have shown colicin I to be the predominant type in the 10 % of strains of this serotype that are colicinogenic (Fredericq, 1952; Papavassiliou, 1960; Hamon & Péron, 1966). These findings, however, referred to small collections of strains randomly collected from many outbreaks. This paper describes the colicin types of 4481 phage-typed and biotyped strains of *S. typhimurium*, the epidemiological relationship of which was already known (Anderson *et al.*, 1978; Barker *et al.*, 1980).

METHODS

Media. Nutrient Broth no. 2 (NB) and Nutrient Agar (NA) were from Oxoid. Cultures were usually incubated aerobically at 37 °C. Stock cultures were maintained at ambient temperature on Dorset's egg medium in screw-capped bottles, some for 3 years, others for up to 20 years.

Bacteria. The 4481 cultures of *S. typhimurium*, from man (2323), cattle (1336), other animals (520) and the environment or unspecified sources (302), were (i) the 2092 strains described by Anderson *et al.* (1978), (ii) the 2010 strains isolated in Scotland (Barker *et al.*, 1980) and (iii) additional cultures of phage types 141 (65), 160 (15), 49 (120), 204 (133) and 193 (46). This collection included four groups of cultures of major epidemic types isolated in Scotland: 507 of phage type 141 biotype 9f (PB 141/9f) from 1972–7 (Barker & Old, 1979), 244 cultures of PB 1/2a from 1974–6, 272 cultures of PB 56/17g from 1974–6 and 574 cultures of the related phage types 49, 204, 193 and biotype 26a from 1974–9 (Barker & Old, 1980). The definitive phage types of Anderson *et al.* (1977) and the biotype designations of Duguid *et al.* (1975) are used throughout.

Twelve indicator strains and nine standard producer strains used for colicin typing are described in Table 1.

Colicins. Cultures were examined for colicin production on NA by the overlay method with indicator strain *Escherichia coli* CL104, as described by Barker & Old (1979). Strains isolated between 1920 and 1973 were examined between 1966 and 1974; later cultures were examined within 4 months of isolation. When retested in 1979, cultures were still colicinogenic except five that failed to produce colicin I and one that did not produce colicin S₄.

The colicin type was obtained by comparison of the pattern of inhibition reactions of the test strain with those of standard producer strains (see Ozeki *et al.*, 1962). Colicin S₄ produced by *Shigella dysenteriae* P15 gave a

Table 1. *Strains used for colicin typing*

Strain	Description	Source/reference
Indicator strains		
CL104	<i>Escherichia coli</i> , streptomycin-resistant mutant of strain ϕ of Gratia (1925)	Ozeki <i>et al.</i> (1962)
CL135	<i>E. coli</i> K12-W1177/V, resistant to colicins B and M	Ozeki <i>et al.</i> (1962)
CL136	<i>E. coli</i> K12-30, producing colicin E ₁ -30	Ozeki <i>et al.</i> (1962)
CL137	<i>E. coli</i> K12-317, producing colicin E ₂ -317	Lewis & Stocker (1965)
CL146	<i>E. coli</i> K12-Row/K, resistant to colicin K	Ozeki <i>et al.</i> (1962)
CL148	Colicin E ₂ -resistant mutant of strain ϕ	Ozeki <i>et al.</i> (1962)
CL150	Colicin I-resistant mutant of CL104	Ozeki <i>et al.</i> (1962)
CL152	Colicins I- and E ₂ -resistant mutant of CL104	Ozeki <i>et al.</i> (1962)
CL184	Colicin I-resistant mutant of CL136	Ozeki <i>et al.</i> (1962)
CL234	<i>E. coli</i> K12-Row, carries ColIb-CT4 from <i>Sh. sonnei</i> Abbott and Shannon type 4	Stocker (1966)
CL235	<i>E. coli</i> K12-Row, carries ColIa-CT2 from <i>Sh. sonnei</i> Abbott and Shannon type 2	Stocker (1966)
S3259	<i>Salmonella typhimurium</i> , sensitive to colicin M	This collection
Producer strains		
CT1A	<i>Shigella sonnei</i> DRL10045, producing colicins K, X*	Abbott & Shannon (1958)
CT2	<i>Sh. sonnei</i> DRL10047, producing colicin Ia	Abbott & Shannon (1958)
CT4	<i>Sh. sonnei</i> DRL10049, producing colicin Ib	Abbott & Shannon (1958)
CT6	<i>Sh. sonnei</i> DRL10051, producing colicin E ₁	Abbott & Shannon (1958)
CT8	<i>Sh. sonnei</i> DRL10053, producing colicin E ₂	Abbott & Graham (1961)
CA18	<i>E. coli</i> , producing colicins B and M	Fredericq (1965)
P15	<i>Shigella dispar</i> P15, producing colicin S ₄	Fredericq (1965)
T20	<i>E. coli</i> , producing colicin B-K260	Pugsley & Reeves (1977)
32T19/VT5	<i>E. coli</i> , producing colicin M	Graham & Stocker (1977)

* Horák (1975) identified the colicin type of the *Sh. sonnei* strains of Abbott & Shannon and Abbott & Graham.

zone of inhibition with indicator strains CL148, CL150 and CL152; CL146 was always resistant to colicin S₄ and the variable reactions with CL135, CL136, CL137 and CL184 probably depended on the amount of colicin produced. With increase of temperature from 37 to 44 °C during the period of colicin production (24 h), a larger, more clearly defined zone was formed.

The strains producing the salmonellin-like colicin also produced colicin I. They were active on indicator strains derived from *E. coli* CL104 and *E. coli* K12, on 76 *E. coli* NCTC strains, on salmonellae of several serotypes – 245 cultures of *S. typhimurium* of 200 phage types, 5 cultures of *S. paratyphi* B and 35 cultures of 12 other serotypes – and on *Shigella sonnei* (3 cultures) and *Shigella flexneri* (3 cultures). Many resistant colonies appeared within the large, indistinct zone of inhibition. Some cultures of *S. typhimurium* of phage types 46 and 85 and one of *Salmonella brandenburg* were completely resistant.

RESULTS

Incidence of colicinogeny in S. typhimurium

Of the 4481 cultures examined, 531 (11·8 %) were colicinogenic; the colicin types were Ia (146 cultures), Ib (272), E₁ (11), E₂ (73), K (2), B with M (4), S₄ (15), Ia with B and M (1), E₁ with Ia (1), E₂ with Ib (2), a salmonellin-like colicin (3) and one unidentified that was not I, E₂ or M.

The 418 colicin Ia- or Ib-producing cultures were distributed among 109 of a total of 597 phage type/biotype (PT/BT) groups. Most of the PT/BT groups represented by more than a single colicinogenic strain also contained non-colicinogenic members. An exceptional group was PB 143/17a, all members of which produced colicin Ib; the 43 cultures came from cattle, man and other sources in 15 centres in Britain in 1967.

Cultures producing colicin E₁ were distributed in nine PT/BT groups and only three were thought likely to be epidemiologically related. The 73 ColE₂ cultures were distributed among

Table 2. Number of outbreaks and isolated cases of different colicin pattern that yielded 1597 cultures of *S. typhimurium* of epidemic types 141/9f, 1/2a, 56/17g and 49, 204, 193/26a

<i>S. typhimurium</i> epidemic type	No. of outbreaks (and no. of cultures of stated Col type) of colicin pattern:			No. of isolated cases infected with organisms of type:	
	all-Col ⁻	mixed-Col ⁻ /Col ⁺	all-Col ⁺	Col ⁻	Col ⁺
141/9f	30 (207)	6 (Col ⁻ 120, Ib 47) 3 (Col ⁻ 45, Ia 3) 1 (Col ⁻ 1, E ₂ 1) 1 (Col ⁻ 46, Ib 3, BM 1) 1 (Col ⁻ 20, Ib 1, ? 1)*	1 (Ib 10)	0	1 Ib
1/2a	ca 14 (167)	2 (Col ⁻ 31, Ia 4) 2 (Col ⁻ 19, Ib 2)	0	21	0
56/17g	ca 40 (180)	1 (Col ⁻ 10, Ia 2) 1 (Col ⁻ 3, Ib 1)	1 (Ib 13)	ca 40	7 Ia 16 Ib
49/26a	ca 30 (275)	1 (Col ⁻ 4, Ia 1, Ib 1) 2 (Col ⁻ 14, Ib 3) 1 (Col ⁻ 2, E ₂ 13)	1 (Ia 7)	ca 30	6 Ia 7 Ib
204/26a	ca 20 (104)	2 (Col ⁻ 6, Ib 5)	4 (E ₂ 41) 1 (E ₂ 13, E ₂ + Ib 1)	ca 10	1 E ₁ + Ia 2 Ia 2 E ₂ 3 Ib
193/26a	4 (18)	0	0	5	0

* ? indicates unidentified colicin type.

five PT/BT groups particularly PB 204/26a (57 cultures) and 49/26a (13 cultures). The two colicin K-producing cultures were probably also related to each other. The four cultures producing colicins B with M were of four PT/BT groups and not related by source or year of isolation.

Colicin S₄, first observed in *S. typhimurium* by Professor B. A. D. Stocker (personal communication), was identical to the colicin S₄ produced by *Sh. dispar* P15 (Dr P. Reeves, personal communication). The 15 strains producing S₄ were isolated between 1959 and 1964 in England (5 strains), Australia (4) and America (6). The Australian and English strains (excluding one of PB 12a/1a) belonged to PB 29/26i or closely related types, whereas all the American strains were of PB 13/29b. Differences in the specificity of colonies of CL148, picked as resistant to colicin S₄, indicated that the colicin produced by the American strains was similar to, but not identical with, that produced by the English and Australian strains of *S. typhimurium* and *Sh. dispar* P15.

The three cultures that produced the salmonellin-like colicin were isolated in 1964 in New Zealand (PB 43/3a) and in 1963 and 1965 in England (PB 99/26a and U/26a, respectively).

Colicinogeny in major epidemic types

The major epidemic strains in Scotland belonged to one of four PT/BT groups – 141/9f, 1/2a, 56/17g and 49, 204, 193/26a – within which epidemiological information on source and place of isolation allowed the recognition of separate family and herd outbreaks. The pattern of colicinogeny of cultures from such outbreaks was: (i) all non-colicinogenic (all-Col⁻), or (ii) all colicinogenic (all-Col⁺), or (iii) a mixture of colicinogenic and non-colicinogenic members (mixed-Col⁻/Col⁺).

Approximately 170 outbreaks were noted (Table 2); 138 yielded cultures (951) which were all-Col⁻, 8 yielded cultures (85) which were all-Col⁺ and, within each outbreak, produced colicin of the same type, and 24 yielded cultures (410) of mixed-Col⁻/Col⁺ pattern of which 321 were Col⁻ and 89 Col⁺. In three outbreaks of mixed pattern, cultures of two different Col⁺ types were isolated from different individuals (PB 141/9f Col⁻, ColIb and ColB with

M from cows in one herd; PB 141/9f Col⁻, ColIb and unidentified colicin from patients in Glasgow in 1975; PB 49/26a, Col⁻, ColIa and ColIb from cows in one herd). Usually, in any one outbreak of mixed pattern, the majority of individuals were infected with Col⁻ bacteria. One exceptional outbreak was caused by strains of PB 49/26a in which cultures from 13 calves produced colicin E₂ and 6 months later two different animals excreted non-colicinogenic bacteria of the same phage type and biotype.

In outbreaks of all-Col⁺ pattern, ColIb-carrying cultures (10) of PB 141/9f came from man (5), cattle (2) and milk (3) from one farm; ColIb-carrying cultures (13) of PB 56/17g were isolated over 15 months from cows in one herd and ColIa-carrying cultures (7) of PB 49/26a came from calves and environmental sources on one farm. Of the five outbreaks due to *S. typhimurium* PB 204/26a ColE₂, one occurred in a family in Edinburgh, another in a herd in Perthshire and three were herd outbreaks and associated milk-borne infections in man in Ayrshire. Non-colicinogenic *S. typhimurium* PB 204/26a caused outbreaks in Aberdeenshire, Perthshire and southern counties of Scotland including Ayrshire.

DISCUSSION

The percentage of colicinogenic strains (11.8 %) and most of the colicin types found (Ia, Ib, E₁, E₂, B and K) in the 4481 cultures examined were similar to those found in earlier surveys of *S. typhimurium* (Reeves, 1972). Strains producing colicin B were similar to the *E. coli* strains described by Fredericq & Smarda (1970) in producing colicin M with colicin B. Two new types were colicin S₄ and the salmonellin-like colicin. The properties of colicin S₄ closely resembled the description given by Atkinson (1973, 1976) of Salcol group 3b which she found in cultures of *Salmonella vejle* and *S. typhimurium* isolated in Australia in 1954 and 1957. The salmonellin-like colicin, active on salmonellae of many serotypes, was unlike the salmonellin described by Atkinson (1966) in being active on *E. coli*.

In this survey, epidemic types were recognized by phage type, biotype and epidemiological information and colicin typing contributed little additional differentiation except in recognition of a ColE₂ subline of *S. typhimurium* PB 204/26a. ColE₂ is a non-conjugative plasmid (Ozeki *et al.*, 1962) and its acquisition by *S. typhimurium* is probably rare; thus, colicin E₂-producing cultures were found in only five PT/BT groups, two of which (49/26a and 204/26a) are interconvertible (Threlfall *et al.*, 1978). Cultures of colicin types Col⁻, ColIb, ColE₂ and ColE₁ with ColIa were among the early isolates of *S. typhimurium* PB 204/26a, a type that first appeared in Scotland in 1974. Five outbreaks due to *S. typhimurium* PB 204/26a ColE₂ were of the all-Col⁺ pattern. The ColE₂-carrying cultures probably represent a subline that disseminated from a single E₂-recipient bacterium of phage type 49 or 204 and persisted in cattle until 1977. The only indication that a ColE₂ plasmid was lost during the course of an outbreak was in an epidemic among calves infected with *S. typhimurium* PB 49/26a in which the first cultures were ColE₂ and the later ones Col⁻. This may be an example of loss of a Col plasmid in progeny of ColE₂ bacteria, or the Col⁻ line may have been present but not isolated at the beginning of the outbreak, or there may have been reinfection from an outside source.

The carriage of Col plasmids was generally stable, both *in vivo* and *in vitro*. In outbreaks of the all-Col⁺ pattern, ColIa, ColIb and ColE₂ were stable during spread of the infecting strain to several host species and over a considerable period of time in a circumscribed community, a finding in agreement with Meynell *et al.* (1968) that these Col factors predominate in *Salmonella* due to their comparative stability. When cultures of the same phage type and biotype and mixed-Col⁻/Col⁺ pattern were isolated from the same host, or from different hosts in a single outbreak, the host might have been infected with bacteria of mixed colicin type or some of the bacteria might have gained or lost a colicinogenic factor during growth in the host. A study of *S. typhimurium* PB 141/9f over several years (Barker & Old, 1979) showed that, although the earliest cultures and the majority of later cultures were

Col⁻, colicinogenic cultures of types Ia, Ib, B with M, E₂ and an unidentified colicin were found within the PT/BT group. These probably arose by acquisition on at least one occasion of each different Col factor. Most mixed-Col⁻/Col⁺ outbreaks involved colicin I-producing strains and cultures from the majority of hosts were Col⁻. It would seem more likely, therefore, that many *S. typhimurium* strains acquired a Col factor from other enteric organisms in the host, giving rise to colicinogenic subtypes within a PT/BT clone, than that these outbreaks were initiated by Col⁺ bacteria and many *S. typhimurium* strains lost Col factors while resident in the host. The finding that colicinogenic *S. typhimurium* have remained at about 10 % over many years, despite the evidence that ColI is readily acquired, suggests uncertainty about the long-term stability of Col factors in competition with other plasmids carried by *S. typhimurium*.

Note added in proof. Since submission of this paper, a paper on the classification of E colicins (Males & Stocker, 1980) has been accepted for publication. Representative strains of *S. typhimurium* PB 49/26a and PB 204/26a were typed by Dr Males and the E₂ colicin they produced corresponded to colicin E₂-P9 in the new scheme.

I thank Professor J. P. Duguid, Dr J. S. Stevenson, Professor B. A. D. Stocker and Dr Janette Whyte for the gift of strains and information about their properties and Professor E. S. Anderson for information on phage types.

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