Products of Defective Lysogeny in *Serratia marcescens* SMG 38 and Their Activity against *Escherichia coli* and Other Enterobacteria

By JEAN-PIERRE GRATIA

Microbial Genetics and Ecology Unit, Laboratory of Microbiology, Brussels University School of Medicine, CP 400/13, Boulevard de Waterloo 115, B-1000 Brussels, Belgium

(Received 25 July 1988; accepted 1 August 1988)

From a series of *Serratia marcescens* clinical isolates analysed with respect to bacteriocin production, one strain (SMG 38) was exceptional in that it produced two distinct phage-tail-like bacteriocins differing in morphology, sedimentation, heat sensitivity, and host range. The more active component (bc25) was effective against *Serratia*, while the other component (McG) inhibited growth of *Escherichia coli*, *Salmonella typhimurium* and *Shigella sonnei*, but not *Serratia*. Plaque formation on tested strains was negative except in the single case of the lysate of a subclone of SMG 38 which caused the production of a virulent phage, $\phi\epsilon$, in *E. coli* K12 RH 5108. This seems to be a rare event. Like the bacteriocin McG, phage $\phi\epsilon$ used the same receptor protein, coded at about 30 min (locus *fig*) on the *E. coli* chromosome, as does the temperate and serologically unrelated phage $\phi\gamma$. Both McG and UV-irradiated $\phi\epsilon$ killed sensitive bacteria. The survival rate depended on the input multiplicity and also on the indicator strain, and was increased by the presence of prophage $\phi 80$ in the cell. When survivors were allowed to resume their growth under normal conditions, they showed cell elongation whatever their RecA phenotype. No difference was observed between the two agents with respect to these observations, except that McG, unlike irradiated $\phi\epsilon$, was inactive against *Klebsiella pneumoniae* UNF 5023, which possessed the Fig receptor.

INTRODUCTION

As first observed by Hamon & Peron (1961), most *Serratia marcescens* strains produce colicin-like substances active against *Escherichia coli*; some strains also produce trypsin-resistant bacteriocins active against other strains of *Serratia marcescens*, but not against *E. coli*. Prinsloo (1966) distinguished subgroups of *Serratia marcescens* bacteriocins on the basis of host-range and biochemical properties. Bacteriocins are useful in typing of *Serratia marcescens*: the susceptibility of clinical isolates to bacteriocins produced by a set of ten strains of the same species allowed Traub (1980) to distinguish more than 70 types. These ten bacteriocins were all shown to be phage tails and, therefore, products of defective lysogeny. Based on differences in ultrastructure and other properties, these phage tail bacteriocins were divided into two subgroups; one of them used a receptor residing in the lipopolysaccharide (LPS) moiety of the outer membrane (Traub *et al.*, 1974). Lysates may be active against several species, but there is still no demonstration of a common receptor in *Serratia* and other enterobacteria.

In the course of an investigation into a series of clinical isolates of *Serratia marcescens* from Belgian hospitals, it was found that one particular strain, SMG 38, produces a phage tail bacteriocin with the unusual property of being active against several species including *Serratia marcescens* and *E. coli*. The nature of the agent and its affinity for receptors in different strains is described here. A virulent bacteriophage called $\phi\epsilon$ has been isolated: it is inactive against the *E. coli* mutants resistant to the bacteriocin but its host range is not identical to that of the

Abbreviations: bc25, bacteriocin from *Serratia marcescens* SMG 38 active against *S. marcescens* Sli; McG, bacteriocin from *S. marcescens* SMG 38 active against *E. coli*; MC, mitomycin C; TSB, tryptic soy broth (Difco).

bacteriocin. Killing activity and other cellular effects have been studied comparatively and the observations are discussed. A preliminary account of this work was given at the XIVth International Congress of Microbiology held in Manchester, UK, 7-13 September 1986.

METHODS

Media. Nutrient Agar (Difco; NA) was used for plating and standard techniques; Tryptic Soy Broth (Difco; TSB) was used for experimental cultures. Tests and dilutions were performed in T1 buffer (Matsushiro, 1963). The activity of bacteriocins and phages was assayed on TSB plus 1% (w/v) agar (Difco); agarose (Sigma) was also used in certain experiments. Soft agar comprised TSB plus 0.6% agar.

Bacterial strains. The *Serratia marcescens* strains used in this work included the ten phage tail bacteriocin producers used for bacteriocin typing according to Traub (1980), and the indicator strains S1i, S9i and S2li kindly provided by Dr W. H. Traub. Other strains of that species were clinical isolates collected by the Institut d'Hygiène et d'Epidémiologie (Belgian State) examined for both production of, and susceptibility to, the reference bacteriocins. A series of *E. coli* strains as well as other enterobacteria (*Shigella* spp., *Salmonella typhimurium*, *Klebsiella pneumoniae*) were used; they are described in Table 3. Therein are also presented mutants resistant to various bacteriophages, and heterospecific hybrids obtained in different ways endowed with various combinations of restrictions and sensitivity patterns.

Bacteriophages and phage techniques. Various temperate and virulent phages were used for comparisons, especially $\phi 80$ (Matsushiro, 1963), $\phi \gamma$ (Gratia, 1973) and hybrid derivatives. Phage $\phi \epsilon$ was isolated during the course of this work. High-titre stocks were prepared as usual by confluent lysis on cellophane (Gratia, 1984). Inactivation of phage development and lysis was performed using UV light from a germicidal lamp (Sylvania G8T5, USA).

Bacteriocinogeny, bacteriocin typing and characterization of the bacteriocins. The methods used for the production of bacteriocins by *Serratia* and for their assay were those described by Traub (1980). Induction was performed using mitomycin C (Sigma; MC) at a final concentration of $1 \mu\text{g ml}^{-1}$. Overnight induced cultures were centrifuged and the activity of the supernatants was assayed comparatively with or without prior treatment with 0.25% trypsin (BDH) at 37 °C for 30 min. Differentiation of the phage tails in a given lysate was achieved by modifying the conditions of incubation (temperature, pH) and by using centrifugation (Spinco centrifuge 20000 r.p.m. for 90 min with swinging rotor SW25.1). Fractions were serially diluted in T1 buffer and assayed by dropping the diluted samples on streaks of suspensions containing 10^7 bacteria ml^{-1} . Diffusibility was tested as follows. The producing bacteria were incubated on TSB plus 1% (w/v) agarose for 24 h at 37 °C. After removal of the bacteria and exposure to chloroform vapours, discs were cut from the gel containing the bacteriocin and laid on TSB agar (1%) at 4 °C for at least 72 h. Plates with discs and plates from which the discs had been removed were compared with respect to the inhibition of growth of sensitive bacteria overlaid in soft agar: D means that inhibition was observed over a large diameter where the bacteriocin had diffused from the disc to the bottom agar; ND means that inhibition was restricted to the disc containing the bacteriocin.

Killing activity of McG and inactivated $\phi \epsilon$. Diluted samples of McG preparations (fraction 11 of the induced lysate of SMG 38, Table 2) and of $\phi \epsilon$ inactivated by UV (less than 0.1% survival in terms of p.f.u.) were mixed with starved bacteria from exponentially growing cultures, and incubated for 1 h in T1 buffer at 37 °C. Counts of c.f.u. were made on NA plates before (N_0) and after treatment (N). The titre of McG units was defined on the basis of the growth inhibition range using parallel series of twofold dilutions of an McG preparation and of a sample with a known titre of UV-inactivated $\phi \epsilon$ particles. The input multiplicity m is the ratio between McG (or UV-inactivated $\phi \epsilon$) units and the initial number (N_0) of test bacteria (*E. coli* K12 1485).

Microscopic examination of the bacteria and cell-size measurements. Treated and untreated bacteria were pelleted and incubated in TSB for growth at 37 °C. At intervals, samples were fixed with alcohol on microscope slides and stained with toluidine blue for photography; images were projected onto a screen to measure the size of individual bacteria.

RESULTS

Screening of the *Serratia* strains

A series of 180 clinical isolates of *Serratia marcescens* from Belgium were compared with respect to either the sensitivity pattern towards the ten phage tail bacteriocins used as reference (Traub, 1980), or the production of colicin(s) and/or phage tails or trypsin-resistant bacteriocins upon treatment with MC. Among them, 125 (69.5%) produced a colicin of the same specificity

Table 1. Examples of *Serratia marcescens* bacteriocin sensitivity types differing in the production of bacteriocins active against other *S. marcescens* strains or against *E. coli*

Bacteriocin sensitivity type*	No. of strains	Detection of bacteriocins active against <i>S. marcescens</i> strains†:							Production of bacteriocins active against <i>E. coli</i> K12		
		S1i	S9i	S21i	SMG				Host range‡		Other characteristics§
					25	26	27	28	1485	1485R	
4 (O14:H1/12)	1	—	—	—	—	—	—	—	—	—	} T°S TpS D
	6	—	—	—	—	—	—	—	+	—	
	2	—	—	—	—	+	—	—	+	—	
	1	—	—	—	—	—	+	—	+	—	
	1	—	—	—	+	—	+	—	+	—	} T°R TpR ND
	1	+	—	—	—	—	—	—	+	+	
(SMG 38)											
33 (Co2.3:H1; --:H4)	2	—	+	+	—	—	+	+	+	—	} T°S TpS D
	1	—	+	+	+	+	+	+	+	—	
	1	+	+	+	+	+	+	+	+	—	
44 (O4/14:H4)	3	—	—	—	—	—	—	—	—	—	} T°S TpS D
	35	—	—	—	—	—	—	—	+	—	
	1	—	—	—	—	—	+	—	—	—	

* The types are those of Traub (1980) and correspond to the pattern of sensitivity to phage-tail-like bacteriocins produced by his ten bA + *S. marcescens* strains. Serotyping was done by S. Heinemann (H1/12 means H1 for some of the strains and H12 for the others; the same for O4/14).

† No difference was observed upon treatment of the tested lysates with 0.1% trypsin at 37 °C followed by heat up to 56 °C for 30 min. Indicator strains were from Dr W. H. Traub (S1i, S9i, S21i) and from our collection (SMG 25–28).

‡ Strain 1485R is a mutant selected by its resistance to one of the lysates active against *E. coli* 1485.

§ T°S, TpS, susceptible to, and inactivated by, heat (56 °C for 30 min) and trypsin (0.1%), respectively. D, diffusible in agar; ND, non-diffusible.

(inactive against a mutant of *E. coli* K12 1485 – 1485R – selected by its resistance to one of the lysates); 43 (23.9%) produced trypsin-resistant bacteriocins detected by their activity against one or several *Serratia* strains. There appeared to be some correlation between the bacteriocin sensitivity type and the pattern of production of both groups of bacteriocins (see examples in Table 1).

Among the type 4 *Serratia* strains, SMG 38 exceptionally produced poorly diffusible relatively heat-stable and trypsin-resistant bacteriocins active against *E. coli*, *Salmonella typhimurium* (LT2), *Shigella sonnei* (P9), and also against *Serratia marcescens* (S1i) (Table 1, line 6).

Lysates of MC-induced strain SMG 38 were analysed further. Samples were submitted to various conditions of pH and temperature (Table 2). The activity index after the various treatments differed for *E. coli* K12 1485 and *Serratia* S1i. For instance, after a short incubation at 60 °C, the activity against *Serratia* was much more affected than that against *E. coli*. Centrifugation at 45000 g clearly separated the two activities, and this was enhanced after passage of the pellet on *Serratia* S1i and of the supernatant on *E. coli* K12 1485. In the former case, the passage on S1i caused almost complete disappearance of the activity against that strain following specific adsorption to the corresponding receptor. The reverse was observed with the supernatant passaged on *E. coli*; it lost activity against that strain but retained full activity against *Serratia* S1i (lines 12 and 13 in Table 2).

Electron micrographs of the various preparations (Fig. 1) showed two types of phage tails distinguishable by thickness and shape. The heated and centrifuged preparations showed one type of phage tails, 16 nm thick and 140 nm long. This component, which was inactive against *Serratia* S1i but active against *E. coli*, *Salmonella typhimurium* and *Shigella sonnei*, was designated macrocin G (or McG).

Table 2. *Differentiation between the two bacteriocins produced by induced Serratia marcescens SM 38*

Preparation	Activity of the preparation* on:		Ratio (a)/(b)
	(a) <i>E. coli</i> K12 1485	(b) <i>S. marcescens</i> Sli	
1. Crude lysate† (pH 6.8)	64	256	0.25
2. +0.01 M-Tris/HCl (pH 8.1)	32	256	0.13
3. +HCl‡ (pH 5.5)	32	64	0.5
4. +HCl‡ (pH 4.2)	16	16	1
5. Prep. 1, 56 °C for 10 min	64	128	0.5
6. Prep. 1, 56 °C for 40 min	32	32	1
7. Prep. 1, 60 °C for 10 min	32	8	4
8. Prep. 1, 60 °C for 20 min	16	0	16
9. Supernatant of centrifuged prep. 1§	16	256	0.060
10. Pellet of centrifuged prep. 1§	256	8	32
11. Prep. 10, 60 °C for 10 min	128	2	32
12. Prep. 9/ <i>E. coli</i>	0	256 (bc2S)	≤0.004
13. Prep. 11/ <i>S. marcescens</i>	128 (McG)	0	≥128

* Highest active dilution.

† The lysate was treated with chloroform and centrifuged at low speed.

‡ Addition of drops of 0.5 M-HCl to the crude lysate.

§ Centrifugation at 45000 g for 90 min. The pellet, washed with saline, was resuspended in TSB at a 5 times concentration.

|| Passing by 30 min contact with 10⁹ bacteria ml⁻¹ of *E. coli* K12 1485 (sensitive to McG) or *Serratia* strain Sli (sensitive to bc25) followed by two runs of low-speed centrifugation.

Evidence for defective lysogeny

E. coli K12 strain RH 5108 was less sensitive to McG than was *E. coli* K12 1485. When these bacteria were pelleted after incubation with the *Serratia* SMG 38 lysate, both supernatant and sedimented bacteria (killed by chloroform) inhibited the growth of other strains which were fully sensitive to McG, either in liquid media or on the surface of agar plates. The same observation was made after serial contacts with sensitive bacteria. Plating sometimes resulted in the appearance of very tiny plaques. No plaque-forming particles could be obtained, except in one case, when a small plaque was formed upon treatment of *E. coli* K12 RH 5108 with the lysate of one *Serratia* SMG 38 subclone, and was found to contain a plaque-forming phage able to form larger plaques after dilution and plating with other bacterial strains. It was not possible to determine whether this phage (named $\phi\epsilon$) was formed in the SMG 38 subclone or in *E. coli* K12 RH 5108 upon infection with defective particles. The existence of defective elements was shown by electron microscopy of an McG preparation (see Fig. 1d). The tail structure was similar to defective virions of $\phi\epsilon$, except that the McG tails were shorter (141 ± 1 nm, $n = 56$) than those of $\phi\epsilon$ (179 ± 2 nm, $n = 39$). Adsorption specificity was the same for McG and $\phi\epsilon$.

Similarity of receptors for bacteriocin McG, virulent phage $\phi\epsilon$ and temperate phage $\phi\gamma$

The host range of McG and $\phi\epsilon$ resembled that of the temperate phage $\phi\gamma$ (Gratia, 1973) and included several enterobacterial species as mentioned above. $\phi\gamma$ was therefore used for comparisons with the *Serratia* agents, as well as other bacteriophages active against *E. coli* and, in some cases, also against *Salmonella typhimurium* (Table 3).

The main observation concerned the concordance in adsorption pattern between the temperate phage $\phi\gamma$ (or hybrid derivatives), bacteriocin McG, and the virulent phage $\phi\epsilon$. All bacterial mutants selected for their resistance against any of these agents revealed similar patterns of resistance. There was no exception in cross-resistance between Fig⁻ mutants (Fig⁺ and Fig⁻ denote the presence and absence, respectively, of the receptor for McG, $\phi\gamma$ or $\phi\epsilon$). The pattern of sensitivity to other bacteriophages was not modified by the *fig* mutation or vice-versa;

Fig. 1. Electron micrographs of negatively-stained preparations from lysates of *Serratia* SMG 38. (a) Preparation 1 (see Table 2) mainly contained thin tails corresponding to bc25. (b) Preparation 11 showed tails corresponding to McG. (c) The tail tip of $\phi\gamma$ (shown on the left for comparison) presents some likeness with, but is distinct from, the tip of McG (arrow on the right). (d) Some defective virions were observed among tails in McG preparations. Bars, 0.1 μm . Photographs were taken by G. Vanheule in the EM laboratory of Dr D. Dekegel (Institut Pasteur, Brabant), except for (c) (M. Wurtz, Microbiology Department of the Biozentrum, Basel).

the effect of the mutational phenotypes in polyresistant strains obtained in several steps was additive.

Susceptible bacteria (*E. coli*, *Shigella sonnei*, *Salmonella typhimurium*) were all rough in terms of outer membrane, suggesting that the receptor is a protein. This is in agreement with previous results (Gratia *et al.*, 1980) showing that all the neutralizing activity against $\phi\gamma$ was contained in the protein fraction of the extracts from sensitive *E. coli* or *Salmonella* strains, and that it was destroyed by trypsin and strongly inhibited by NaCl. No activity was found in LPS. Protein extracts of resistant mutants were completely inactive. The corresponding gene is located at about 30 min on the chromosome in both *E. coli* (*fig*) (Gratia *et al.*, 1980) and *Salmonella typhimurium* (*prk*) (Sanderson & Roth, 1983).

As found in various $\phi 80:\gamma$ hybrids (Gratia, 1982), adsorption was not related to seroneutralization. $\phi\epsilon$ and $\phi\gamma$ were serologically unrelated, in that the immune serum prepared against the latter did not affect the activity of the former. Absorption of this serum with McG did not alter its neutralizing activity against $\phi\gamma$ either. Serological relationships between McG and $\phi\epsilon$ have not yet been checked.

* Most of the other *E. coli* strains tested were sensitive to $\phi\gamma$, McG and $\phi\epsilon$ but exhibited differences in efficiency of plating (e.o.p.); *E. coli* C, B, K12 RH5108 (a gift of L. Desmet), CA7 ('coli V'), C6 ('coli ϕ '), Lisbonne (a gift of J. Beumer) and MH47 were among the most sensitive strains. *Shigella* spp. were provided by C. Godard. *Salmonella typhimurium* LT2 derivatives 4419 and 4526 (Colson & Van Pel, 1974) were given by C. Colson; LT2 derivatives SL1102 and SL/HK were given by H. Mäkelä. Hybrids CG10 and CG11 are conjugants having inherited from *Sh. flexneri* F6S a segment of variable length around *trp* (Godard, 1974), and GT41.1 is a $\phi\gamma$ -mediated transductant having received the *att*-80-*trp*-*cysB* segment from *Sh. sonnei* P9 lacking the *tonB*⁺ gene (unpublished). *Klebsiella pneumoniae* UNF 5023 (Dixon *et al.*, 1977) was a gift of C. Elmerich.

† Bacteriophages except ϕ 80 were kindly provided by C. Godard. +, Sensitive; —, resistant; $\pm$, partial sensitivity. A space indicates that the phage/strain combination was not tested.

‡ +, Normal or almost normal e.o.p.; —, no detectable activity; $\pm$, incomplete lysis even by clear mutants of $\phi\gamma$; $\sim$, very faint activity, so that e.o.p. was indeterminate; 50% (etc.), the e.o.p. was about half (etc.) the normal e.o.p. on restrictionless bacteria. *R* is the restriction index, defined as (e.o.p. on restrictionless bacteria)/(e.o.p. on the tested *r*⁺ strain). The phages were grown on *E. coli* K12 *r*⁺ m⁺ and therefore had K specificity. $\phi\gamma$:80 was chosen as the least comparable to $\phi\gamma$ among hybrids having inherited *hy*; it is morphologically and serologically related to ϕ 80.

§ $\phi\epsilon$ was inactivated with UV irradiation for these experiments.

|| Sensitivity to Mu1c (a gift of L. Desmet) was also observed.

¶ Isolated by P. Bordet; high frequency of smooth-rough variation.

** 2026 is a conjugant derived from *E. coli* B having received the SA system from *Salmonella* (Van Pel & Colson, 1974); 4419 and 4526 are restrictionless mutants of LT2 (Colson & Van Pel, 1974). *K. pneumoniae* UNF 5023 is restrictionless.

†† Isolate resistant to virulent phage HK068 (and temperate $\phi\gamma$) by mutation at about 30 min on the *Salmonella* chromosome (H. Mäkelä, personal communication).

‡‡ Normal confluent lysis, but no plaques upon dilution.

Fig. 2. Influence of the input multiplicity [m , the ratio (McG units)/(initial no. of bacteria, N_0)] on the survival rate (N/N_0 ; N corresponds to the number of c.f.u. after 1 h contact of bacteria with McG at 37 °C). Mixtures consisted of small samples of serially diluted suspensions of sensitive bacteria [●, *E. coli* K12 1485; ○, *E. coli* K12 1485(ϕ 80)] added to equal volumes of diluted or undiluted McG preparations. The bars represent the range of results; most of the points refer to experiments repeated three times.

Parameters of plating, i.e. lytic development and restriction-modification, as well as bacteriocin activity were easily distinguished by means of the strains used. For instance, plating efficiencies dependent or not dependent on restriction varied in $\phi\gamma$ and derivatives. Conversely, $\phi\epsilon$ was able to plate with almost the same efficiency on many Fig⁺ strains. This remarkable property is being exploited in further studies.

In *Klebsiella pneumoniae* UNF 5023, various efficiencies of plating were observed with hybrid derivatives of $\phi\gamma$ irrespective of restriction, while $\phi\gamma$ itself did not form plaques on that strain in spite of its ability to give rise to confluent lysis and to lysogenize this strain. Again, $\phi\epsilon$ was able to plate normally. But the most important observation concerned the discrepancy between bacteriocin activities: unlike $\phi\epsilon$, McG had no detectable effect on *K. pneumoniae* UNF 5023; conceivably, this strain was tolerant of McG but not of the killing activity of $\phi\epsilon$ even when the phage was arrested in its development.

Cellular effects of McG and UV-inactivated $\phi\epsilon$

Sensitive bacteria were brought into contact with McG at varying concentrations and examined with respect to irreversible killing and to possible lesions in the survivors. Comparisons were made with $\phi\epsilon$ and $\phi\gamma$ under conditions of no lytic development, i.e. after inactivation by UV light. Irradiated samples of these phages, which showed less than 10^{-3} p.f.u. survival, were mixed and incubated for 1 h at 37 °C with *E. coli* K12 1485 or derivatives, *K. pneumoniae* UNF 5023 and *Salmonella typhimurium* SL 1102 (Table 4, Fig. 3).

Killing activity. The number of survivors (N) was lower than the number of bacteria mixed with the preparation (N_0) by a factor depending on the respective concentration of the reagents. However, it also depended on the bacterial strains used. In *E. coli* K12 1485 the presence of a ϕ 80 prophage increased survival (Fig. 2, Table 4). This effect was not due to interactions between McG or $\phi\epsilon$ and the phage particles possibly produced by the ϕ 80-bearing strain and reabsorbed on the outer membrane, because we used washed preparations; moreover, the results were the same whether the bacteria were Rec⁺ or RecA⁻ in spite of the great difference in the amount of phage particles spontaneously produced. The same observation applies to lysogens carrying various ϕ 80 prophages whatever their host range [h from λ , ϕ 80 (h^+ or h), $\phi\gamma$]. As far as

Fig. 3. Frequency of elongated bacterial cells in cultures treated with McG or UV-inactivated $\phi\epsilon$. Samples of bacteria, either untreated (I, III or V) or treated with McG or inactivated $\phi\epsilon$ (II, IV, or VI), were examined under the microscope throughout a 4 h incubation at 37 °C in fresh TSB medium. After 2–3 h, several samples were stained for photography and measurements. In II and VI, no significant difference was observed whether McG or inactivated $\phi\epsilon$ was used; in IV, only $\phi\epsilon$ produced the effects shown, McG being inactive. The observations were highly reproducible in spite of the great variation in bacterial size. The number of experiments with McG and $\phi\epsilon$ used in parallel/average number of samples examined under the microscope for each experiment of treatment with McG (or inactivated $\phi\epsilon$)/total number of individual bacteria measured was as follows: I, 6/3/200; II, 6/4(4)/2570; III, 3/2/200; IV, 3/3(3)/310; V, 2/2/200; VI, 2/3(4)/385.

Table 4. Various forms of activity of McG and UV-inactivated $\phi\epsilon$

Agent (10 ⁹ units)*	Bacterial strain tested	Growth inhibition (dilution range)†	Survival (N/N ₀)‡	Cell elongation
$\phi\gamma$	<i>E. coli</i> K12 1485	—	1.1 ± 0.5 (3)	—
McG	<i>E. coli</i> K12 1485	++ (100–300)	5.1 ± 2.8 × 10 ⁻³ (9)	+
	<i>E. coli</i> K12 1485(ϕ 80)	+	1.54 ± 0.6 × 10 ⁻¹ (8)	+
	<i>Sal. typhimurium</i> SL 1102	+	1.02 ± 0.02 × 10 ⁻¹ (3)	+
	<i>K. pneumoniae</i> UNF 5023	—	0.91 ± 0.01 (3)	—
$\phi\epsilon$	<i>E. coli</i> K12 1485	++ (100–300)	1.5 ± 0.8 × 10 ⁻² (8)	+
	<i>E. coli</i> K12 1485(ϕ 80)	+	1.58 ± 0.9 × 10 ⁻¹ (9)	+
	<i>Sal. typhimurium</i> SL1102	+	1.29 ± 0.02 × 10 ⁻¹ (3)	+
	<i>K. pneumoniae</i> UNF 5023	+	6.5 ± 0.45 × 10 ⁻² (3)	+

* The number of units corresponds to the number of p.f.u. before UV inactivation of plaque-forming ability; the titre of McG is deduced from the equivalence to a known concentration of inactivated $\phi\epsilon$ with respect to bacteriocin activity.

† Dilution up to the lowest active concentration (drops of 10 μ l onto about 10⁴ bacteria; less than 20 surviving colonies). —, No detectable activity.

‡ The results are means ± SEM from determinations with several samples treated at multiplicities around 1000. The number of treated samples is indicated in parentheses.

$\phi\epsilon$ was concerned, the input multiplicity was higher than 1, ruling out the possibility that the survivors had never been in contact with either the phage or the bacteriocin particles. This was confirmed by the following observations.

Cell elongation. Treated bacteria were pelleted and incubated in TSB for several hours at 37 °C. At intervals, samples were taken for determination of c.f.u., and the bacterial cells were

examined microscopically. The numbers of c.f.u. did not change very much during the first 2–3 h of incubation, but the length of the cells increased progressively (Fig. 3). During further incubation, the number of long cells decreased whereas the numbers of c.f.u. increased. Thus bacterial cell division was impaired, albeit reversibly. These observations applied to both Rec⁺ and RecA⁻ bacteria.

In *K. pneumoniae* UNF 5023, the bacteriocin McG did not produce any of these effects. In contrast UV-inactivated $\phi\epsilon$ displayed lethal activity for this strain and also caused cell elongation of the survivors. Therefore, the two activities of $\phi\epsilon$ were correlated and tolerance of these bacteria to McG concerned a prior step.

DISCUSSION

At the time of the first observations on the lethal activity of bacteriocins, it was not anticipated that the same activity would be displayed by organized structures of viral origin massively produced by induced bacteria, especially in the case of colicin V, a low-molecular-mass proteinaceous substance (according to F. Baquero (personal communication), colicin V is more related to a microcin than are the other colicins). However, some parallelism with bacteriophage was considered at that time long before relationships between these agents were established. Now, a variety of bacteriocins produced by many species are known. There are two groups of bacteriocins (Bradley, 1967; Kageyama, 1975; Traub, 1980; Thiry, 1986), plus the microcins (Asensio *et al.*, 1976); and they are defined by their specific lethal activity, which implies a domain for recognition of a receptor and a nearby domain for binding with some target within the bacterial cell.

For unknown reasons, the bacteriocins active against *E. coli* are rather small proteinaceous molecules sensitive to trypsin (colicins or microcins), including those produced by many *Serratia* strains, some of which also produce phage tails active against *Serratia* (Hamon & Peron, 1961; Traub, 1980). On the other hand, the bacteriocins are, in general, active against strains of the same species as the producing strain. The present work concerns an exceptional case in two respects. Firstly, among the bacteriocins produced by *Serratia marcescens* SMG 38, one is not active against that species – unlike the other bacteriocin (bc25) – but is active against *E. coli*, *Salmonella typhimurium* and *Shigella sonnei*. Secondly, unlike the colicin-like bacteriocins produced by *Serratia*, this bacteriocin is a phage tail. It is named macrocin, more precisely macrocin G or McG on account of its relatedness with bacteriophage $\phi\gamma$ as to adsorption specificity.

The genesis of $\phi\epsilon$ is mysterious and requires further analysis of this phage. Its relationship with McG is suggested by a series of observations: (a) the adsorption specificity is identical; (b) the cellular effects of UV-inactivated particles of $\phi\epsilon$ are similar to those of McG; (c) electron micrographs do not show any qualitative difference between them.

The absence of any activity of McG towards *K. pneumoniae* UNF 5023, while UV-inactivated $\phi\epsilon$ was active, is intriguing. In all other sensitive strains tested, McG and UV-inactivated $\phi\epsilon$ had similar effects (with the same interference effect of prophage $\phi 80$). On the other hand, the efficiency of plating (e.o.p.) on UNF 5023 differed among the temperate $\phi\gamma$ -related phages, including $\phi 80 : \gamma$ hybrids, even in the absence of any restriction system (strain UNF 5023 is r⁻). The e.o.p. differences are unlikely to be due to differences in the adsorption organs, especially in hybrids serologically related to $\phi\gamma$. Therefore, strain UNF 5023 reveals a differential activity of some gene function(s) among $\phi\gamma$, hybrid derivatives and $\phi\epsilon$. As far as $\phi\epsilon$ is concerned, one may consider the possibility, among others, of interference between a phage gene product and a function of the membrane resulting in the killing activity of the $\phi\epsilon$ tail tips.

In connection with that problem, one is led to consider the phenomenon of interference exerted by prophage $\phi 80$ against bacteriocin activity. This is obviously an interference at the level of the adsorption site, the target of the bacteriocinic action, or at some intermediate step. Steric hindrance caused by adsorbed $\phi 80$ particles produced is considered unlikely, all the more so as the interference was also observed in the case of RecA⁻ lysogens which produced almost no

complete particles. Still more interference of prophage $\phi 80$ is observed by any worker using phage T1 whatever the lysogen, but differences appear when hybrid prophages are compared. Yet no satisfactory explanation has been proposed for any of these observations.

We are indebted to Dr W. Traub for interest, the gift of strains, and critical reading of the manuscript, and to Drs C. Colson, L. Desmet, C. Elmerich, H. Mäkelä, and especially C. Godard, who provided strains of bacteria and bacteriophages. We are obliged to Mrs S. Heinemann who provided the clinical isolates of *Serratia*, partly characterized for the serotype, and to Mrs G. Vanheule for having communicated micrographs from work in collaboration prior to publication.

This work was supported by a 'Crédit aux Chercheurs' from the FNRS (Belgium).

REFERENCES

- ASENSIO, C., PEREZ DIAZ, J. C., MARTINEZ, M. C. & BAQUERO, F. (1976). A new family of low molecular weight antibiotics from Enterobacteria. *Biochemical and Biophysical Research Communications* **69**, 7–14.
- BRADLEY, D. E. (1967). Ultrastructure of bacteriophages and bacteriocins. *Bacteriological Reviews* **31**, 230–314.
- COLSON, C. & VAN PEL, A. (1974). DNA restriction and modification systems in *Salmonella*. I. *Molecular and General Genetics* **129**, 325–337.
- DIXON, R. A., KENNEDY, C. & KONDOROSI, A. (1977). Complementation analysis in *Klebsiella pneumoniae* mutants defective in nitrogen fixation. *Molecular and General Genetics* **157**, 189–198.
- GODARD, C. (1974). Transfert simultané de caractères antigéniques et de résistance à des phages T de *Shigella flexneri* F6S Hfr à *E. Coli* K12. *Annales de Microbiologie* **125A**, 403–412.
- GRATIA, J. P. (1973). Etude génétique du bactériophage $\phi\gamma$ et d'autres coliphages apparentés à $\phi 80$. *Annales de Microbiologie* **124B**, 21–34.
- GRATIA, J. P. (1982). Differentiation in the specificities of sero-neutralization and adsorption to the host membrane in polyphenotypic hybrid viruses. *Archives internationales de physiologie et biochimie* **90**, 31–33.
- GRATIA, J. P. (1984). Prophage elements conferring high mutability of host neighbouring gene *tonB*, high spontaneous production of particles and cell elongation. *Annales de Virologie* **135E**, 369–388.
- GRATIA, J. P., HANNECART-POKORNI, E. & GODARD, C. (1980). Etude préliminaire du récepteur d'un bactériophage actif sur diverses Entérobactéries. *Archives internationales de physiologie et biochimie* **88**, 277–278.
- HAMON, Y. & PERON, Y. (1961). Etude de la propriété bactériocinogène dans le genre *Serratia*. *Annales de l'Institut Pasteur* **100**, 818–821.
- KAGEYAMA, M. (1975). Bacteriocins and bacteriophages in *Pseudomonas aeruginosa*. In: *Microbial Drug Resistance*, pp. 291–305. Edited by S. Mitsukashi & H. Hashimoto. Tokyo: University Press.
- MATSUSHIRO, A. (1963). Specialized transduction of tryptophan markers in *Escherichia coli* K12 by bacteriophage $\phi 80$. *Virology* **19**, 475–482.
- PRINSLOO, H. E. (1966). Bacteriocins and phages produced by *Serratia marcescens*. *Journal of General Microbiology* **45**, 205–212.
- SANDERSON, K. E. & ROTH, J. R. (1983). Linkage map of *Salmonella typhimurium*. *Microbiological Reviews* **47**, 410–553.
- THIRY, M. (1986). Etude de la détermination génétique de l'urédovoricine 20D3, pp. 1–27. PhD thesis, Liege University.
- TRAUB, W. (1980). Bacteriocin and phage typing of *Serratia*. In: *The Genus Serratia*, pp. 80–100. Edited by A. von Graevenitz & S. J. Rubin. Boca Raton, Florida: CRC Press.
- TRAUB, W., ACKER, G. & KLEBER, I. (1974). Studies on group A bacteriocins of *Serratia marcescens*. II. Further characterization of serogroup I and II bacteriocins. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abt. 1, Orig. Reihe A)* **229**, 482–489.
- VAN PEL, A. & COLSON, C. (1974). DNA restriction and modification systems in *Salmonella*. *Molecular and General Genetics* **135**, 51–60.
- WILKINSON, R. G., GEMSKI, P., JUN, P. & STOCKER, B. A. D. (1972). Non-smooth mutants in *Salmonella typhimurium*. *Journal of General Microbiology* **70**, 527–554.