PHAGE β INTERACTION WITH *VIBRIO CHOLERA*E

K. A. I. SIDDIQUI AND F. KATHLEEN BHATTACHARYYA

Indian Institute of Chemical Biology, Jadavpur, Calcutta-700032, India

SUMMARY. The role of temperate phage β in determining the serology and eltor-lytic phage sensitivity in *Vibrio cholerae* was investigated. The only serological change found in six host strains was a change to roughness. This was accompanied by failure to adsorb several of the lytic phages. Various phage-sensitivity changes were induced by phage β in two hosts at the post-adsorption level. In strain HP47, three types of progeny were obtained of which one was universally resistant to lytic phages. These untypable lysogens were culturally stable but gave rise to segregants of the rare phage-type 6 on single colony selection.

INTRODUCTION

We have compared and characterised five temperate phages and investigated their role in determining the epidemiologically important host attributes of serotype and phage type in *Vibrio cholerae* (Siddiqui and Bhattacharyya, 1982). No serotype changes were detected after lysogenisation with these phages which were of two immunity classes, VcA-1 and the Kappa or K-class phages. Only K-class phages caused changes in phage type by inducing resistance to the eltor-group-III lytic phage. Thus the fluctuation seen in the prevailing phage types 2 and 4 in the present eltor cholera pandemic (Annual Report, Cholera Research Centre, 1975 and 1979; WHO, 1980), which differ only in their sensitivity to the group-III phage, may reflect shifts in the population of the K-class temperate phages.

In this paper we describe a third immunity class of temperate phage β which infects both classical and eltor biotypes of *V. cholerae*. This phage was found to be novel in that it induced a variety of changes in host lysogens.

MATERIALS AND METHODS

Cultures, phages and assay methods. Temperate phage β was isolated from 48-h culture supernates of Nicolle's strain of *V. cholerae* Vr 3 (Nicolle *et al.*, 1971). It was treated with pancreatic DNAase (type III), centrifuged at 5000 rpm for 10 min and filtered through a G5 sintered glass filter to remove bacterial cells. Details of other phages, strains and methods of culture and assay have been reported elsewhere (Siddiqui and Bhattacharyya, 1982). Lytic phage of the eltor typing series (Basu and Mukerjee, 1968) are prefixed with E to distinguish them from temperate phage; e.g., Group-III typing phage is designated E3.

Production of lysogens. Phage β was used to lysogenise six wild-type non-lysogenic strains of *V. cholerae* of both classical and eltor biotypes (table I) by infecting broth cultures. After 18 h, the cultures were plated out, and at least 48 colonies were examined by spot assay for the

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presence of phage and for sensitivity to all the lytic and temperate phages; their serotypes were also determined by slide agglutination with rough and smooth typing antisera. The methods used for these tests as well as for production of other lysogens have been described previously (Siddiqui and Bhattacharyya, 1982). Phage-negative progeny colonies were re-examined after UV induction for periods up to 20 s at a distance of 60 cm (Philip's 15 W TWV). Strains were examined for superinfection exclusion of lytic phage E3 as previously described (Siddiqui and Bhattacharyya, 1982).

Test for lysogeny. By the use of MAK757 as an indicator antiserum to K-class phage α was found to neutralise phage β . Its 100% inhibitory concentration for phage β (2×10^3 pfu/ml) at 37°C for 45 min was 1 in 500. Lysogens were tested for stability by serial growth in three broth cultures containing 20 times this concentration of K-antiserum. The rifampicin stability test described previously (Siddiqui and Bhattacharyya, 1982) was also performed.

Exclusion of lytic phage E3. Broth cultures of lysogens (*c.* 10^8 cfu/ml) were incubated with phage E3 (*c.* 10^7 pfu/ml) for 10 min and then centrifuged in the cold. The supernate was treated with chloroform and assayed for unabsorbed phage. The cells were resuspended in saline, treated with anti-E3 serum, then washed with normal saline, diluted and plated in indicator strain MAK757 to determine the number of productively infected cells.

Characterisation of lysogens and temperate phages by immunotyping. The induced lysogens and their phages were characterised by immunotyping with phage lysates from standard lysogens MAK757 (K), MAK757 (VcA-1) (Siddiqui and Bhattacharyya, 1982) and MAK757 (β) (Siddiqui and Ghosh, 1983). Phage lysates derived from these and from the test β -lysogens were spotted on soft agar lawns of all the cultures.

RESULTS

Phage sensitivity and serotype

In the cultures examined, phage β induced complete resistance to lytic phage E3 (table I). As many as 10% of colonies of some host strains also became untypable. The majority, but not all of the untypable progeny were autoagglutinating and serologically rough (table I). Rough progeny of some host strains retained sensitivity to some of the lytic phages but not to phages E3 or E5. Rough mutants were not detected (<0.37%) in untreated strain HP47 which was investigated in greater detail.

In recipient strain HP47 (eltor type 2), phage β generated three different classes of smooth progeny. The majority (HP47/2, table I) were converted to resistance to E3 (phage type 4). A minority (HP47/5) were phage-type 6, having become resistant in spot assay to phage E1 in addition to E3. This latter kind of lysogen could not be detected in a repeat experiment. Five per cent of progeny were untypable (HP47/6), having become resistant to all five lytic phages of the eltor typing series. This line was of particular interest because on plating, two kinds of segregants were observed, both of which retained the infecting phage even after growth in antiphage serum or rifampicin broth. Half of the segregants remained resistant to all typing phages and the rest reverted to sensitivity, but only to phages E2 and E5, thus becoming type 6. The segregation patterns of these lines were followed for seven single-colony platings. Whereas the type-6 segregant remained stable, the resistant colonies continued to segregate. Although the type-6 segregant was resistant to phage E1 by spot assay, it gave abnormally small plaques in soft agar at 1% of the efficiency of wild-type host HP47. Phage β was isolated from both this type-6 segregant and the resistant line; and when used to lysogenise wild-type strain HP47 a second time, all the lysogens tested became type 4, as in the original cross, which ruled out the likelihood of this finding being the result of a mutant or contaminating phage in the original lysates.

TABLE I
Changes induced in phage sensitivity and serology of six strains of *V. cholerae* by phage β

Parent (wild type) strains	Lysogens induced by phage β	Sensitivity to eltor phages*					Phage type	Sero type	Percentage of colonies in induced culture	Presence of infecting phage†
		E1	E2	E3	E4	E5				
HP47 (eltor)		+	+	+	-	+	2	IN	...	-
	HP47/1	+	+	-	-	-	UT	RO	5	-
	HP47/2	+	+	-	-	+	4	IN	75	+
	HP47/5	-	+	-	-	+	6	IN	3	+
	HP47/6	-	-	-	-	-	UT	IN	5	+
MAK757 (eltor)		+	+	+	+	+	1	OG	...	-
	MAK757/1	+	+	-	+	+	3	OG	20	+
	MAK757/2	-	-	-	+	-	UT	RO	5	-
VRC242 (eltor)		+	+	+	-	+	2	IN	...	-
	VRC242/1	+	+	-	-	+	4	IN	82	+
	VRC242/2	-	-	-	-	-	UT	RO	1	-
VRC276 (eltor)		+	+	+	+	+	1	OG	...	-
	VRC276/1	+	+	-	+	+	3	OG	50	+
T2 (classical)		-	-	+	-	-	2‡	OG	...	-
	T2/1	-	-	-	-	-	2‡	OG	50	+
	T2/2	-	-	-	-	-	UT‡	RO	1	-
H218 (classical)		-	-	+	-	-	1‡	IN	...	-
	H218/1	-	-	-	-	-	1‡	IN	50	+

UT=Untypable; OG=Ogawa; IN=Inaba; RO=rough.

* +=Sensitive; -=resistant.

† +=Phage detected; -=phage not detected in spot assay on lawns of universal indicator strain MAK757 before or after UV induction.

‡ =Classical strains were tested with the classical lytic phages (Mukerjee, Guha and Roy, 1957).

Phage exclusion by lysogens

Adsorption of lytic phage E3 to wild-type strains was efficient, being nearly 90% within 10 min. Rough progeny generated by phage β did not adsorb phage E3 (table II). All three true lysogens tested—type 4, untypable and type 6—adsorbed superinfecting phage E3 with an efficiency equal to that of the wild type but unproductively.

TABLE II
Superinfection exclusion of lytic phage E3 by β -induced progeny

Parent (wild type) strains	Lysogens induced by phage β	Phage E3 count (10^6 pfu/ml) in			Number of productively infected cells (10^6)
		Serotype	supernate		
HP47 (eltor)		IN	2.6		27.0
	HP47/1	RO	26.0		0
	HP47/2	IN	1.3		0
	HP47/5	IN	1.1		0
MAK757 (eltor)		OG	2.8		28.0
	MAK757/1	OG	2.2		0
	MAK757/2	RO	20.0		0

TABLE III
 Characterisation of *V. cholerae* temperate phage by immunotyping with K-class phages, phage VcA-1 and phage β

Parent (wild type) strains	Lysogens	Sensitivity of strains to		
		K-class phages	phage VcA-1	phage β
MAK757 (eltor)		+	+	+
	K-class*	—	+	+
	VcA-1	+	—	+
	β	—	+	—
VRC276 (eltor)		+	+	+
	K-class*	—	+	+
	VcA-1	+	—	+
	β	—	+	—
HP47 (eltor)		+	+	+
	K-class*	—	+	+
	VcA-1	+	—	+
	β	—	—	—
VRC242 (eltor)		+	+	+
	K-class*	—	+	+
	VcA-1	+	—	+
	β	—	—	—
T2 (classical)		+	—	+
	K-class*	—	—	+
	VcA-1†	...	...	...
	β	—	—	—
H218 (classical)		+	—	+
	K-class*	—	—	+
	VcA-1†	...	...	...
	β	—	—	—

* Lysogens produced by K-class phages α , 22, VcA-2 or 241 (Siddiqui and Bhattacharyya, 1982).

† VcA-1 lysogens of classical strains could not be produced because the parent strain was resistant to phage VcA-1.

Immunity patterns of lysogens

On the basis of phage immunotyping patterns, three distinct classes were recognised (table III). K-class lysogens remained sensitive to VcA-1 and phage β ; VcA-1 lysogens were resistant only to VcA-1 phage; β -lysogens were resistant to K-class phage but showed a host-dependent response to phage VcA-1. Thus, the β -lysogens of HP47 and VRC 242 were resistant to phage VcA-1 whereas those of MAK757 and VRC 276 were not.

DISCUSSION

In an earlier study we investigated the inter-relationship of five temperate phages of diverse origin and their role in determining host properties (Siddiqui and Bhattacharyya, 1982). They fell into two distinct, unrelated groups—the K-class phages and VcA-1. The phage β investigated here was found to form a third class. It showed non-reciprocal immunity with both K-class and VcA-1 phages, which, in the latter case, was host dependent (table III). A second phage- β effect was its tendency to select serologically rough variants in four of six strains at relatively high frequency.

Lysogeny could neither be confirmed nor ruled out in these rough progeny. Defects at the lytic phage E3 receptor level could be demonstrated in rough variants.

A third distinctive feature of β -lysogeny was the complexity it displayed in its interference with lytic phage infection. Three modes of interference were detected in strain HP47. The majority of β lysogens, like K-class lysogens, showed a single change to resistance to lytic phage E3. In addition, two novel phage types, both true lysogens, were recovered at low frequency (table II)—type 6 (HP47/5) and untypable or universally resistant (HP47/6). The type-6 progeny was stable and differed from the type-2 lysogen normal for this strain only in showing a highly restricted ability to support lytic phage E1 which was not detectable in routine typing. Wild type-6 strains were not available to us for comparison. The universally resistant lysogen HP47/6 shed type-6 segregants in about half the cell population at each transfer. The low frequency of these events may explain the rare occurrence of type-6 *V. cholerae* in clinical isolates.

Interference with lytic phage E3 replication in β lysogens, was by exclusion at the post-adsorptional level. The unusual interference seen in universally resistant lysogen (HP47/6) and its type-6 derivative was found to be due neither to a mutant phage nor to the presence of a second phage in the lysate. Because this lysogen never shed the normal type-2 segregant but only stable type-6 colonies, prophage integration must be presumed to be abnormal in this line.

It seems likely that the β -type of interaction occurs in nature. It is common to find untypable and type-unstable *V. cholerae* isolates that resolve into strains with broader phage sensitivity patterns (WHO Report, 1980). Furthermore, all β -lysogens were classified in exactly the same way as the β -resistant clinical isolates tabulated by Furniss, Lee and Donovan (1978).

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