Isolation and use of eight phages for typing *Yersinia enterocolitica* O3

Y. KAWAOKA*, T. MITANI, K. OTSUKI and M. TSUBOKURA

Department of Veterinary Microbiology, Faculty of Agriculture, Tottori University, Tottori-shi, Tottori 680, Japan

Summary. Subdivision of 137 isolates of *Yersinia enterocolitica* O3 into eight phagovars has been achieved. Some geographical differences were found in the sources of these phagovars and also of two biovars.

Introduction

The species *Yersinia enterocolitica* was subdivided by Nicolle *et al.* (1973) into 10 phagovars (I to X) with 12 phages (I to XII). Amongst 55 O-serovars of *Y. enterocolitica*, O3 was the most frequent isolate from patients in many countries except North America (Aldová and Láznicková, 1979; Alonso *et al.*, 1979; Vandepitte and Wauters, 1979; Shayegani *et al.*, 1981; Zen-yoji, 1981; Bottone, 1983). There are also four biovars of *Y. enterocolitica*, distinguishable by their action on D-xylose and lecithin, and their production of indole (Wauters, 1970; Brenner, 1981). Most O3 strains are biovar 4 and belong to phagovar VIII or IX of Nicolle *et al.* (1973). The O3 strains of phagovar VIII have been isolated in Europe (Aldová and Láznicková, 1979) and Japan (Tsubokura *et al.*, 1982; Fukushima *et al.*, 1984), and those of phagovar IX in South Africa (Nicolle *et al.*, 1973) and Canada (Toma and Lafleur, 1981). Moreover, O3 strains have been isolated from various animals: man, pigs, dogs, cats, monkeys and rats (Hurvell, 1981). Except for North America, the strains isolated in any one country have been thought to belong to only one biovar and phagovar (Shayegani *et al.*, 1981; Toma and Lafleur, 1981; Bottone, 1983). Further subdivision seemed desirable epidemiologically.

Materials and methods

Bacteria

We received 137 isolates of *Y. enterocolitica* O3, of various origins (table IV), from Drs D. P. Falcao, H.

Fukushima, W. Knapp, H. J. Koomhof, T. Maruyama, H. H. Mollaret, G. Wauters and S. Winblad. They were identified as *Y. enterocolitica* by their positive reactions with urea, ornithine, sucrose, maltose and cellobiose, and negative reactions for L-rhamnose, melibiose, raffinose, KCN and Voges-Proskauer (Brenner, 1981).

Medium

In all tests, bacteria were cultivated on nutrient-agar plates (Kawaoka *et al.*, 1983).

Isolation of phages

The samples from which phages were isolated are shown in table I. Faeces (1 g) was suspended in 10 ml of nutrient broth. The suspended faeces and other samples were centrifuged at 10 000 rpm for 10 min, and the supernates were filtered (0.45 µm Millipore). Phage isolation was attempted by direct plating of the filtrate (0.1 ml) and a suspension of the propagating bacterium (10⁸ cells, 0.1 ml) in 0.5% soft agar. When phage was not isolated by direct plating, the same specimen (1 ml) was mixed with an overnight culture (5 ml) of a propagating strain in nutrient broth. After incubation for 24 h at 25°C, a few drops of chloroform were added, and the culture was shaken vigorously and then centrifuged. A 0.1-ml portion of the aqueous phase was mixed with bacterial suspension (10⁸ cells, 0.1 ml) and 0.5% soft agar (3 ml) and poured on to a nutrient agar plate. Unless quite different plaques were detected on this plate, only one plaque was picked. The phages were cloned more than three times by plaque isolation.

Phages I to XII of Nicolle *et al.* (1973) were obtained from Dr H. H. Mollaret. They were propagated as described previously (Kawaoka *et al.*, 1982).

Phage typing

Of 28 phages that were isolated, eight were selected because of their stability and propagating ability. Their propagating strains and origins are shown in table I.

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* Correspondence should be sent to Dr Y. Kawaoka, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, 332 N. Lauderdale, P.O. Box 318, Memphis, TN 38101, USA.

Table I. Origins and propagating strains of phages

Phage*	Origin	Propagating strain† of <i>Y. enterocolitica</i> (serovar)
HM-1	raw sewage	Ye 3827 (O3)
HM-3	raw sewage	MY 79 (O9)
HR-1	river water	Ye 3827 (O3)
HM-2	raw sewage	Ye 3827 (O3)
HU-1	raw sewage	Ye 3827 (O3)
HC-2	chicken faeces	Ye 135 (O1)
HS-6	raw sewage	Ye 135 (O1)
HT-3	raw sewage	Ye 135 (O1)

* The phages were isolated by direct plating, or after preliminary propagation, at 25°C (see text).

† The three propagating strains were received from Dr H. H. Mollaret.

Phage-typing was performed with the routine test dilution of the phage (highest dilution that gives confluent lysis of its propagating strain), as described previously (Tsubokura *et al.*, 1982). The phages were spotted on a lawn of the test culture and the plates were incubated for 24 h at 25°C.

Serotyping

Strains were serotyped by slide-agglutination with cross-adsorbed O-antisera.

Biochemical tests

Each strain was tested at 25°C for biochemical characteristics sufficient to identify the species and to determine its biovar. Carbohydrates were examined up to 7 days; action on amino acids and citrate, and the Voges-Proskauer reaction, were recorded at 4 days.

Results

The phage susceptibility of 137 isolates of *Y. enterocolitica* O3 revealed eight different patterns (table II) which enabled us to designate eight new phagovars (1 to 8) of serovar O3. This permitted further subdivision of each of four phagovars previously described by Nicolle *et al.* (table III). In conjunction with their phagovars, we have defined nine groups (A to I) within serovar O3.

Table IV shows that although 90 (66%) of our 137 isolates belonged to group A, the others could be differentiated into eight groups. The 16 strains of group B were isolated mainly in Japan, and constituted 25% of the 61 Japanese isolates. Similarly, all 15 strains of groups F, G, H and I were from Japan. In contrast, the 13 strains of groups D and E came from Brazil, France, South Africa and Sweden, but not from Japan.

Thus, there was a difference in geographical distribution of the phagovars of *Y. enterocolitica* O3. Moreover, this difference in distribution was apparent even when the isolates were divided only into their two biovars: the 122 isolates of biovar 4 came from various countries, only 46 of them from Japan, whereas all 15 of the biovar 3 isolates were from Japan (tables III and IV).

Discussion

Baker and Farmer (1982) developed a phage-typing system for inter-species differentiation of *Y. enterocolitica*, *Y. kristenseni*, *Y. frederikseni* and *Y. intermedia*; but this was not suitable for subdivision of *Y. enterocolitica* O3.

The phage set established in the present investigation subdivided strains of *Y. enterocolitica* O3

Table II. Designation of eight phagovars of *Y. enterocolitica* O3 by the range of phages that lysed them

Phagovar of <i>Y. enterocolitica</i> O3	Lysis with phage							
	HM-1	HM-3	HR-1	HM-2	HU-1	HC-2	HS-6	HT-3
1	+	+	+	+	+	—	—	—
2	+	+	+	+	+	—	+	—
3	+	+	—	+	+	—	—	—
4	+	+	+	—	+	—	—	—
5	+	+	+	+	—	+	—	—
6	+	+	+	+	+	+	—	—
7	+	—	—	—	—	—	—	—
8	—	—	—	—	—	+	+	+

+ = Confluent lysis; — = no lysis or incomplete lysis.

Table III. Grouping of *Y. enterocolitica* O3

Biovar* of <i>Y. enterocolitica</i>	Phagovar† of <i>Y. enterocolitica</i>	O3 phagovar‡	O3 group§
4	VIII	1	A
4	VIII	2	B
4	VIII	3	C
4	IX	1	D
4	IX	4	E
3	II	5	F
3	II	6	G
3	X	7	H
3	X	8	I

* Wauters, 1970.

† Nicolle *et al.*, 1973.

‡ Our designation (table II).

§ Our designation, by derivation from the other columns.

into eight phagovars. In conjunction with the previous phagovars of Nicolle *et al.* (1973), this has enabled us to designate nine groups, A to I (table III). Recently we have shown the biochemical heterogeneity of O3 strains isolated in Japan (Fukushima *et al.*, 1984). The present study showed that this serovar is even more heterogeneous in phage susceptibility; and our nine new groups may be useful epidemiologically.

In spite of extensive studies, the mode of

transmission and other epidemiological features of *Y. enterocolitica* infection have not been fully elucidated. The number of O3 strains tested in this study is not sufficient to establish a firm correlation between origin and phage type. However, some geographical differences were found (table IV). Japan was the country of origin of all our isolates of groups F, G, H and I, and of most of our group B isolates, whereas our South African isolates were of groups D and E which correspond to phagovar IX of Nicolle *et al.* (1973). This finding is consistent with those of previous studies (Nicolle *et al.*, 1973; Toma and Lafleur, 1981) in which phagovar IX was found mainly in Canada and South Africa. Unfortunately, we could not examine the Canadian strains; it would be interesting to know which of our nine groups occur in that country.

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Table IV. Relationship between origin and O3 group in 137 strains of *Y. enterocolitica* O3

Origin		Number of strains of <i>Y. enterocolitica</i> O3 in each of our newly designated O3 groups*								
Isolate from	Country	A	B	C	D	E	F	G	H	I
Man	Japan	21	4					5		
	France	10		1	1					
	Sweden	10			1					
	South Africa				5	5				
	Hungary	10								
	West Germany	7		2						
	Belgium	7	1							
	Brazil	5								
Pig	Japan	6	4				4	3		1
	Belgium	6								
	Brazil	1								
Dog	Japan	3	5					1		
Cat	Japan	1	2							
Monkey	Brazil	3								
Rat	Brazil					1				
River water	Japan								1	
Total		90	16	3	7	6	4	9	1	1

* see table III.

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