Effect of Carbohydrate Source on Kanagawa-Type Hemolysis of *Vibrio parahaemolyticus*

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Almost all strains of *Vibrio parahaemolyticus* produced Kanagawa-type hemolysis on media of high salt content in the presence of fermentable carbohydrates.

The Kanagawa-type hemolysis (KH), a well-defined clear hemolysis on specially prepared media containing mannitol, has been considered by Japanese workers as a special hemolysis of *Vibrio parahaemolyticus* that is closely related to the enteropathogenicity (3). However, several reports on food poisoning cases associated with KH-negative (K-) have appeared (4, 6). In a previous paper, we reported that KH is an expression of quantitatively different activity of a rather crude hemolysin production in the presence of fermentable carbohydrates on media of high salt content (1). Since this study was conducted with a small number of both KH-positive (K+) and K- strains, we investigated K+ and K- strains of *V. parahaemolyticus* to confirm our previous finding of variable KH activity in the presence of various carbohydrates.

The media and test methods used were the same as those described in our previous paper (1). Strains were isolated from human diarrheal stools, fish, seawater, and other marine materials and were confirmed to be strains of *V. parahaemolyticus* according to the criteria of Hugh and Sakazaki (2). Most strains were typed according to known K antigen types; the only exceptions were 3 K+ and 10 K- strains that were nontypable. Not more than one strain isolated from a single specimen was subjected to study, and strains that did not ferment all of the carbohydrates (except lactose and sucrose) that had been tested were excluded. KH was determined as described by Miyamoto et al. (3) after 20 h of incubation at 37°C on freshly prepared, modified Wagatsuma agar (Eiken, Japan) that contained mannitol, which had been incorporated with washed human erythrocytes (WBA). KH was recorded as positive (++, +), and the absence of hemolysis around bacterial growth was recorded as negative (-).

The KHs of 37 K+ and 57 K- strains were tested on freshly prepared WBA in which mannitol had been substituted by other carbohy-

drates, and the results were compared with those for the KH observed on WBA that contained mannitol (Table 1). Neither K+ nor K- strains produced a K+ reaction on WBA that contained nonfermentable lactose and sucrose or was without carbohydrates; the only exceptions were two K+ strains that were K+ in media that contained lactose. In the presence of media that contained glucose and other fermentable carbohydrates, 1 to 17 K+ strains were converted in varying degrees to K- by the added carbohydrates, whereas the others showed varying degrees of K+ reaction. Alternatively, quite large numbers of K- strains became K+ in media with fermentable carbohydrates. The incubation time evidently influenced the KH results, and most K- strains showed a tendency to react like K+, even in the presence of nonfermentable carbohydrates when incubation was prolonged up to 28 h. Table 2 shows the number of fermentable carbohydrates that produced a K+ reaction of *V. parahaemolyticus*. Thirty-four strains among 37 K+ strains and 52 among 57 K- strains exhibited a K+ reaction with three or more carbohydrates, and only two strains were constantly K- with any of the added carbohydrates.

These results clearly indicate that fermentable carbohydrates in media of high salt content play an important role in KH results (1). Almost all strains of *V. parahaemolyticus* were considered to have the ability to produce KH on media containing some fermentable carbohydrates, and the results on media that contained mannitol serve as only a model of the crude hemolysin test. It may be possible that the production of some hemolysins is responsible for the enteropathogenicity of this organism (5), but no confirmatory evidence on the relationship between hemolysins producing KH on media that contained mannitol and the enteropathogenicity of *V. parahaemolyticus* has been presented. Therefore, the determination of KH

TABLE 1. Effect of carbohydrates on KH of *V. parahaemolyticus*

Strain tested (no.)	KH ^a	No. (%) of strains showing KH on WBA ^b containing:									
		Mannitol	Glucose	Mannose	Maltose	Galactose	Trehalose	Arabinose	Lactose	Sucrose	None
K+ (37)	++	7 (18.9)	0	6 (16.2)	6 (16.2)	7 (18.9)	14 (37.8)	3 (8.1)	0	0	0
	+	30 (81.1)	36 (97.3)	17 (46.0)	28 (75.7)	15 (40.5)	15 (40.5)	17 (46.0)	2 (5.4)	0	0
	—	0	1 (2.7)	14 (37.8)	3 (8.1)	15 (40.5)	8 (21.6)	17 (46.0)	35 (94.6)	37 (100.0)	37 (100.0)
K— (57)	++	0	0	26 (45.6)	15 (26.3)	2 (3.5)	14 (24.6)	8 (14.0)	0	0	0
	+	0	49 (86.0)	23 (40.4)	29 (50.9)	15 (26.3)	27 (47.4)	17 (29.8)	0	0	0
	—	57 (100.0)	8 (14.0)	8 (14.0)	13 (22.8)	40 (70.2)	16 (28.1)	32 (56.2)	57 (100.0)	57 (100.0)	57 (100.0)

^a Symbols: ++, Zone wider than 2 mm around the bacterial growth; +, zone narrower than 2 mm; —, no hemolysis.

^b Mannitol in WBA was substituted by a 0.5% concentration of selected carbohydrates.

TABLE 2. Fermentable carbohydrates that produced KH on WBA

KH	No. of strains tested	No. of strains showing K+ reaction with carbohydrates ^a						
		0 ^b	1	2	3	4	5	6
K+	37	0	0	3	3	11	14	6
K—	57	2	1	2	11	22	15	4
Total	94	2	1	5	14	33	29	10

^a Glucose, mannose, maltose, galactose, trehalose, and arabinose were tested at a 0.5% concentration.

^b Number of carbohydrates producing a positive reaction.

itself at this time should not be considered as a method of proving enteropathogenicity.

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