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In Vitro Hemolytic Characteristic of *Vibrio parahaemolyticus*: Its Close Correlation with Human Pathogenicity

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An in vitro hemolytic characteristic of *Vibrio parahaemolyticus* is closely correlated with human pathogenicity.

A pathogenic halophile, *Vibrio parahaemolyticus*, has been well known as the causative agent of the most prevalent food poisoning in Japan. The organism has come to the attention of taxonomists outside Japan and was the subject of discussion at a recent international symposium (2, 4). In Japan, nationwide cooperative studies were initiated after the discovery of the causative agent during outbreaks of mackerel-borne food poisoning along the coast of the Pacific Ocean in 1959 (7). The 1959 outbreak was the second major occurrence since 1955 (5). Since then, extensive studies, including epidemiology and bacteriology, have been in progress, mainly by the Japanese workers. Evidence of the widespread distribution of *V. parahaemolyticus* in the sea of Southeast Asia and the Central Pacific has been reported by Yasunaga and Aoki et al. (*personal communication*), in the coastal areas of the United States by Baross and Liston (1), Colwell (*personal communication*), and Ward (9), and in Europe (i.e., in the Baltic Sea) by Nakanishi et al. (8).

TABLE 1. *Results of a monthly survey for primary and secondary contamination of mackerel with V. parahaemolyticus*

Month and temp	Primary contamination			Secondary contamination[a]					Totals
	Samples directly taken from a fishery (Koshigoe)			Samples collected from fish-selling shops					
	Directly after capture	Preserved in ice for 8 hr	Kept at room temp for 8 hr	"Futamatagawa"			"Koshigoe"		
				A	B	C	D	E	
May (19 C)	0[b]/20	0/20	0/20	0/10	5/10	0/10	0/10	0/10	5/110
June (26 C)	0 (1)/20	2 (1)/20	1/20	6/10	0/10	2/10	8/10	10/10	29 (2)/110
July (30 C)	3/20	0/20	5 (1)/20	7 (3)/10	7/10	7/10	10 (3)/10	9/10	48 (7)/110
August (32 C)	9 (2)/20								

[a] Higher incidence of isolates of this column denotes secondary contamination, i.e., partly due to the contamination by contact with utensils precontaminated with the organism or partly due to the longer periods of time needed for preservation and transportation after capture, thus permitting growth of the organism.

[b] Numerator expresses the number of positive fish detected by culture of skin, stomach, or enteric canal in enrichment media. The denominator is the number of fish examined. Figures in parentheses represent the number of positives detected by direct culture of the same organ on agar plates. The fish were captured by net. Each was put into a sterile polyethylene bag immediately after capture. The major criteria adopted for identifying *V. parahaemolyticus* were as follows: no growth in 0 and 10% NaCl peptone water, abundant growth at 3% NaCl peptone water, growth at 7% NaCl peptone water, fermentation of glucose with no gas production in Hugh-Leifson medium, indole (+), methyl red test (−), Voges-Proskauer (−), cytochrome oxidase (+), H_2S in triple sugar-iron agar (−), KNO_3 reduced, gelatin (+), D-tartarate (+), arabinose (+) or (−), sucrose (−), and motility (+).

Isolation of *V. parahaemolyticus* from diseased blue crabs in the Chesapeake Bay in the United States suggests these organisms to be pathogenic for invertebrate animals (6).

In the course of our studies on the direct isolation of this organism from sea fish, i.e., the mackerel, we faced the problem that the number of fish carrying *V. parahaemolyticus* during the summer season was too high, as shown in Table 1, to be the cause of the disease, which is actually encountered much less frequently. Table 1 shows the increase of the ratio of positive fish, e.g., summarized 5/110 to 48/110 from spring to summer. The occurrence of this food poisoning is actually restricted to the period from June to October.

Proceeding from the above described studies we observed that strains of *V. parahaemolyticus* isolated from human sources (including food poisoning cases and healthy carriers) and grown on a blood agar medium (*see* footnote, Table 2) exhibited hemolysis, whereas those from natural environments or commercial sources were nonhemolytic. Table 2 shows the highest percentage of the hemolytic strains were from human materials and the lowest percentage from natural and commercial environments.

Recently, Wagatsuma (*personal communication*) devised another medium for more clearly identifying hemolysis and making it amenable to observation without swabbing the colony. The constituents of Wagatsuma's medium are as follows: yeast extract, 0.3%; Bactopeptone (Difco), 1%; NaCl, 7%; K_2HPO_4, 0.5%; agar, 1.5%, distilled water added to a final volume of 1 liter. After dissolving by heating (heat sterilization should be avoided), mannitol is added to a concentration of 1%, 0.1% crystal violet alcohol solution to 0.1%, and human or rabbit defibrinated blood (or saline suspension of red blood cells) to 5%. Incubation period is (for an inoculum such as one drop of fresh broth culture) 20 to 24 hr at 37 C, but for streak plating (such as from clinical specimens) up to 48 hr. There are some peculiarities which are associated with hemolysis. First, horse blood cannot be hemolyzed. Second, hemolysis is affected by heat sterilization of the basal medium. Third, the NaCl concentration is involved in the development of hemolysis, and, if Wagatsuma's base medium with an NaCl concentration lower than 5% is used, nonhemolytic strains exhibit hemolysis and the distinction is lost.

At the 41st General Meeting of the Japan Bacteriological Society held in 1968, Fujino, the original discoverer of *V. parahaemolyticus* (3), announced that to distinguish the peculiar hemolysis of *V. parahaemolyticus* (specifically, the close correlation with human pathogenicity, strict dependence on human blood excluding other animal species when using Kato's medium, etc.), the hemolytic phenomenon detected by the study group of the Kanagawa Prefectural Public Health Laboratory should be called the "Kanagawa phenomenon." In addition, the use of Wagatsuma's medium is recommended for the identification of positive or negative "Kanagawa phenomenon." His proposals were approved unanimously by the investigators studying *V. parahaemolyticus*.

TABLE 2. *Correlation between hemolysis and pathogenesis of V. parahaemolyticus*

Source of strains	No. of strains examined	Hemolysis		Percentage of hemolytic strains
		Positive	Negative	
Suspected dysentery patients, hospitalized[a]	128	122	6	95.3
Food-poisoning patients with suspected symptoms	308	272	36	88.3
Healthy carriers	11	7	4	63.7
Sea- and river-water samples	187	1[b]	186	0.54
Fish, swabs from kitchen board and miscellaneous foods	209	1	208	0.48

[a] Because of the similarity of clinical symptoms, many of the food poisoning patients were diagnosed as dysentery.

[b] Tokyo Bay, station 17. The medium used (Kato, *unpublished observations*) was as follows: Brain Heart Infusion (Difco), 3.7%; NaCl, 2.5%; agar, 1.5%; *p*H adjusted to 7.4. After dissolving the above components by heating as quickly as possible, 0.1% crystal violet alcohol solution was added to a concentration of 0.1% and human red blood cell suspension (defibrinated blood or saline suspension of washed red blood cells) to 5%. Heat sterilization can not be done. Incubation was 15 to 18 hr at 37 C. On recording the results, weakly hemolyzing colonies were swabbed or rinsed away with saline containing 1% Formalin, thus allowing observation of the hemolytic zone beneath the colonies. Zones with either no or alpha hemolysis with slight greenish tone were taken as negative (nonhemolytic).

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