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Isolations of Organisms Related to *Vibrio parahemolyticus* from American Estuarine Sediments[1]

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In 1966, Dack (Food Technol. **20**:39) suggested that organisms of significance in other parts of the world should be considered in American outbreaks of food-borne disease of unrecognized causes. Ready acceptance of Dack's thesis was to some extent predicated upon my observation that *Clostridium botulinum* had remained long undetected in many areas only for lack of any organized search (Appl. Microbiol. **13**:502, 1965; **14**:837, 1966; **15**:629, 1967; **15**:964, 1967).

Frozen sediment samples from two coasts of the U.S. (retained from earlier surveys of U.S. waters for the presence of *C. botulinum*) were thawed. Material from any given sample was transferred into a sterile 125-ml beaker to a depth of 0.25 inch (0.63 cm), covered with sterile physiological saline, and swirled. The contents from each such beaker were used to inoculate the AE medium of Horie et al. (Bull. Japan. Soc. Sci. Fisheries **29**:785, 1963) and plates of the AAC medium of Horie, Saheki, and Okuzumi (Bull. Japan. Soc. Sci. Fisheries **33**:126, 1967) were inoculated. After 24 hr of incubation at 37 C, AE tubes were streaked on AAC plates. All suspect colonies that developed were transferred to slants of AAC, and the resultant cultures were subjected to a number of the differential tests outlined by Horie et al. (Bull. Japan. Soc. Sci. Fisheries **30**:786, 1964). The results are presented in Table 1. Cultures were also tested serologically by use of the only commercially available diagnostic antisera for *V. parahemolyticus* (Nichimen Co., Ltd., Chemicals Department, Takaro-Cho, Chuo-Ku, Tokyo, Japan). Initially, slide reactions were used, but positive tests were verified in capillary tubes. The results are presented in Table 2. The sources of positive samples are given in Table 3.

The characteristics and reactions given in Table 1 are in general agreement with those listed as typical by Horie et al. (1964), but there are differences in detail. An examination of Table 2 shows that many of the cultures most nearly satisfying the Japanese outline of cultural characteristics failed to react serologically. Conversely, a number of serologically reactive isolates (including four isolates giving clear patterns of reactivity and several others showing defective reactivity according to the Japanese scheme) do not appear in Table 1 because their cultural characteristics conformed to Horie's scheme in only two or three of the items listed.

Examination of Table 2 indicates still other disparities. Multiple group reactions (in the case of isolate 34I, four groups) are common, but they are usually weak and often are associated with blurred K-antigen patterns (e.g., 385I and 385II, with all K-antigens of Group II). Other oddities are also evident, such as a strong K2 (Group II) and an equally strong K10 (Group IV) in culture 174 and the absence of all associated K-activity for the strong Group II reaction of culture 34I.

On the basis of such results, one is led to the conclusion that American isolates are undoubtedly related to the Japanese organisms, but problems of antigenic intermediacy and new serotypes, of physiological and cultural differences, and of degrees of pathogenicity remain to be examined. The possibility of a complex of relationships cannot be dismissed. Actually, complete identity of these isolates with Japanese organisms would have been more surprising than were results given here. This view is supported by the work of researchers at the Torry Research Station, Aberdeen, Scotland (G. Hobbs, *personal communication*); these researchers have observed mouse deaths with typical *V. parahemolyticus* symptoms (green peritoneum, etc.) upon injection with the known organism and with large numbers of cells of either of two marine

[1] Contribution from the Institute of Marine Sciences, University of Miami.

TABLE 1. *Cultural and reaction characteristics of isolates*

Characteristics	Isolate no.										
	32A	32B	34I	44	85	90	99	174	369	385	647
Gram's stain	—	—	—	—	—	—	—	—	—	—	—
Cell morphology	Small rod	Small rod	Small rod	Rod	Small rod	Small rod	Small rod	Small rod	Small rod	Small rod	Small rod
Colony appearance (gross)	Dark center, green	Dark center, rough, green	Green, 1 mm	Green, concentric brown	Dark center, green	Green, 1 mm	Dark center, green	Dark center, green	Dark center, green	Green, 1 mm	Dark center, green
Litmus milk reaction	Proteolytic	Proteolytic	Proteolytic	—	Proteolytic	Proteolytic	Proteolytic	Proteolytic	Proteolytic	Proteolytic	Proteolytic
Mannitol (acid)	±	±	±	−	+	±	+	±	±	±	±
Arabinose (acid)	+	±	+	—	+	+	+	+	+	±	+
Galactose (acid)	—	±	±	—	±	+	±	±	±	±	±
Maltose (acid)	—	±	±	+	±	+	±	±	±	±	—
Lactose (acid)	±	±	±	±	±	±	±	±	±	±	—
Sucrose (acid)	±	±	±	+++	±	±	±	—	±	±	—
Glucose (acid)	+	+	+	+++	+	+	+	+	+	+	+
Indole production	—	—	—	—	—	—	—	—	—	—	—
V-P reaction	—	—	—	+	—	—	—	—	—	—	—
NaCl tolerance (10%)	—	—	—	—	—	—	—	—	—	—	—
NaCl tolerance (0.5%)	±	±	±	+++	+	±	±	+	±	±	±
Citrate utilization	+	+	+	—	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+
Mouse deaths with green peritoneum (4 mice), hr	8, 8, 4, 4	7, 7, 8, 8	17, 17, 8, 20	—, —, —, —	7, 5, 17, 16	16, 16, 17, 17	17, 5, 5, —	17, —, —, —	7, 7, 5, 5	5, 16, 7, 7	17, 7, 16, 5

vibrios (untested serologically for relationships with *V. parahemolyticus*) isolated from British waters. Only one other vague reference to *V. parahemolyticus* from European waters has reached the author, and this has yet to be verified by a reply to direct enquiry sent to the Pasteur Institute, Lille, France. It is possible, therefore, that this report represents the first Western isolation of a *V. parahemolyticus*-like organism.

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TABLE 2. *Serological reactions of isolates*

Culture no.	Group reactions	K reactions
29 B	V ++	K15−, K17±
81	II +	K2+, K3−, K28−
PC81 B	II ++	K2−, K3−, K28+
94	VI +	K18−, K19−, K20−, K21−, K22−, K23±, K24−
385 I	II +	K2+, K3+, K28+
	IV +	K8−, K9−, K10−, K11+, K12−, K13−
385 II	II +	K2+, K3+, K28+
	IV +	K8−, K9−, K10−, K11+, K12−, K13−
174	II +	K2++, K3−, K28−
	IV +	K8−, K9−, K10++, K11−, K12−, K13−
	VI +	K18+, K19−, K20−, K21−, K22−, K23−, K24−
341	I +	K1±, K25±, K26±, K32−
	II ++	K2−, K3−, K28−
	IV +	K8−, K9−, K10+, K11−, K12−, K13+
	VI +	K18+, K19−, K20+, K21−, K22+, K23±, K24±
32A	VI ±	K18−, K19−, K20++, K21±, K22+, K23−, K24−
2	IV ±	
20	V ±	
27	II ±	
31	IV ±	
PC81	II ±	Not tested
83	II ±	
100	II ±	
101	VI ±	
195	II ±	
200	II ±	
270	II ±	
29A	I ±	
	II ±	Not tested
99	III ±	K4−, K5−, K6−, K7−, K29−, K30+, K31−
	V ±	K15±, K17±
647	I ±	K1−, K25−, K26−, K32±
	II ±	K2±, K3−, K28−
23, 24, 29, 30C, 31A, 32B, 34II, 44, 77, 80A, 81A, 85, 88, 90, 98, 244, 286, 369, 385	Negative	

TABLE 3. *Origins of positive sediment samples*

Sample no.	Geographic source	Sediment type
2	Hog Key, Everglades, Fla.	Mud and sand
20	Biloxi Bay, Miss.	Mud
27	Belhaven, N.C.	Mud
29A	Bath River, Bath, N.C.	Sand
29B	Bath River, Bath, N.C.	Sand
31	Albemarle Sound (south), N.C.	Sand
32A	Joe Kemp Key, Fla.	Mud
32B	Joe Kemp Key, Fla.	Mud
34I	Bradley Key, Fla.	Mud
44	St. James City, Fla.	Mud and sand
81	Steinahatchee R. (mouth), Fla.	Shell and mud
$PC81_1B$	Steinahatchee R. (mouth), Fla.	Shell and mud
$PC81_2$	Steinahatchee R. (mouth), Fla.	Shell and mud
83	Aucilla R. (tidal), Fla.	Mud and clay
85	Bald Point, Fla.	Sand and shell
90	Panama City, Fla.	Mud and sand
94	Matawan R., Keyport, N.J.	Sand
99	St. Petersburg Beach, Fla.	Sand
100	Clearwater Beach, Fla.	Sand and shell
101	Crystal Beach, Fla.	Sand and shell
174	Hunting Island, S.C.	Sand
195	Fanstinas Beach, Ala.	Mud
200	Fort Morgan, Ala.	Sand
270	Padre Island, Tex.	Sand
369	Sullivan's Hollow, S.C.	Sand
385	Fernandino Beach, Fla.	Sand
385I	Brazos River (tidal), Tex.	Mud
385II	Brazos River (tidal),	Mud
647	Gloucester Point, Va.	Sand