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SHORT COMMUNICATION

CHARACTERIZATION OF *VIBRIO PARAHAEMOLYTICUS* ISOLATED IN THE USA[1]

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Recently *Vibrio parahaemolyticus* has been isolated in different areas of the world (Barros and Liston, 1968; Nakanishi et al., 1968; Ward, 1968; Krantz et al., 1969; Twedt et al., 1969; Barros and Liston, 1970; Fishbein et al., 1970; Kampelmacher et al., 1970; Bartley and Slanetz, 1971). We have obtained some strains of *V. parahaemolyticus* isolated in the USA by different workers. The present paper reports the biochemical and serological characterization of these strains and their comparison with strains isolated in this country.

The strains used in this study are listed in Table 1. When strains were received, they were inoculated and maintained on heart infusion agar containing 3% NaCl. Strains CDC 1334, CDC 3454, CDC 8198, A 5704, A 6202 and A 8633 were isolated from localized tissue infections of individuals living in coastal regions of the United States and were suspected to be *V. parahaemolyticus* (Twedt et al., 1969). Their serological characters were reported to be as follows: CDC 1334, O5 : K17; CDC 3454, untypable; CDC 8198, O3 : K33; A 5704, O1 : K25, A6202, O2,5 : K3,17; A 8633, O2, 3, 4, 6 :K2, 4, 8, 9, 11, 18, 33, 34, 37, 42, 43 (Twedt et al., 1969). Strains 4A-A, 5A and 5D-Bw were isolated by Dr. Colwell from the blue crab in Chesapeake bay (Krantz et al., 1969), and strain $OYG_1 3$ was isolated by Dr. Liston from an oyster in Puget Sound, northwest Pacific (Barros and Liston, 1968; Twedt et al., 1969).

TABLE 1. *Sources of strains of Vibrio parahaemolyticus*

Strain	Source	Reference
CDC 1334	Dr. R. M. Twedt	Twedt et al., 1969
CDC 3454	,,	,,
CDC 8198	,,	,,
A 5704	Dr. R. E. Weaver	,,
A 6202	,,	,,
A 8633	,,	,,
4A-A	Dr. R. R. Colwell	Krantz et al., 1969
5A	,,	,,
5D-Bw	,,	,,
$OYG_1 3$	Dr. R. M. Twedt	Barros and Liston, 1968

Some of the typical characteristics of the suspected strains of *V. parahaemolyticus* iso-

1 Part of this work was presented at 23rd Annual Meeting of the Kansai Branch of the Japan Bacteriological Society held at Osaka on Oct. 4, 1970.

TABLE 2. *Some characters of Vibrio parahaemolyticus isolated in the USA*

	Strain										
	CDC 1334	CDC 3454	CDC 8198	A 5704	A 6202	A 8633	4A-A	5A	5D-Bw	OYG_13	EB101
Gram stain	−	−	−	−	−	−	−	−	−	−	−
Polar flagellation	+	+	+	+	+	+	+	+	+	+	+
Motility (TSI[a])	+	+	+	+	+	+	+	+	+	+	+
Indole	+	−	+	+	+	+	+	+	+	+	+
Cytochrome oxidase	+	+	+	+	+	+	+	+	+	+	+
H_2S	−	−	−	−	−	−	−	−	−	−	−
Voges-Proskauer	−	+	−	−	−	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−	−	−	−	−	−
TSI agar[a]	−/A	A/A	−/A	−/A	−/A	−/A	−/A	−/A	−/A	−/A	−/A
SM agar[b]	−/A	−/A	−/A	−/A	−/A	−/A	−/A	−/A	−/A	−/A	−/A
Growth in peptone water with											
3% NaCl	+	+	+	+	+	+	+	+	+	+	+
7% NaCl	+	+	+	+	+	+	+	+	+	+	+
10% NaCl	−	+	−	−	−	−	−	−	−	−	−
Kanagawa phenomenon	−	−	−	−	−	−	−	−	−	+	−

[a] Triple sugar iron agar
[b] Saccharose mannite agar

TABLE 3. *Serological characters of Vibrio parahaemolyticus isolated in the USA*

Strain	O-antigen	K-antigen
CDC 1334	5	17
CDC 8198	1	33
A 5704	3	25
A 6202	—[a]	—
A 8633	4	42
4A-A	1	32
5A	5	—
5D-Bw	3	—
OYG_13	2	3

[a] Untypable

lated in USA are presented in Table 2. *V. parahaemolyticus* EB 101 (Type strain of *V. parahaemolyticus;* original strain of ATCC 17802, Fujino et al., 1953) was used as a control. Unlike EB 101 and other strains isolated in the USA, CDC 3454 had the following characteristics; Indole (−), VP (+), TSI agar A/A and positive growth in peptone water containing 10% NaCl. From these characteristics, we concluded that CDC 3454 was not *V. parahaemolyticus*, but *V. alginolyticus*.

The O- and K-antigenicities of the strains were studied using standard procedures developed in this laboratory and elsewhere (Miwatani et al., 1969; Sakazaki, 1965), and results are shown in Table 3. The O-antigenicity of A 6202 and the K-antigenicity of A 6202, 5A and 5D-Bw could not be typed. It is quite probable that these strains have different O- and K-antigens from those identified so far with *V. parahaemolyticus* strains isolated in this country.

It has been reported that there is a specific correlation between the O- and K-antigenicities of *V. parahaemolyticus* and that the O-antigen

TABLE 4. *Characterization of O-antigenicity of CDC 8198: Agglutination tests with antisera of CDC 8198 and O1*

Agglutinating antigen	Antiserum					
	CDC 8198				O1	
	Absorbed with					
	None	O3	O9	O1	O9	CDC 8198
O1	800[a]	800	800	—[b]	3200	800
O2	400	—	—	—	—	—
O3	400	—	—	—	—	—
O4	400	—	—	—	—	—
O5	200	—	—	—	—	—
O6	400	—	—	—	—	—
O7	200	—	—	—	—	—
O8	400	—	—	—	—	—
O9	200	—	—	—	—	—
O10	200	—	—	—	—	—
CDC 8198	1600	1600	1600	—	3200	—

[a] The agglutination test was carried out as described previously (Miwatani et al., 1969). The agglutination titer is expressed as the reciprocal of the highest dilution of antiserum showing visible agglutination.
[b] No detectable agglutination was observed at the lowest dilution of antiserum tested (1 : 200).

TABLE 5. *Characterization of K-antigenicity of CDC 8198: Slide agglutination tests with antisera of CDC 8198 and K33*

Agglutinating antigen	Antiserum				
	CDC 8198				K33
	Absorbed with				
	None	K26	K33	K33, O3	CDC 8198
K1	—	—	—	—	—
K25	—	—	—	—	—
K26	5[a]	—	5	—	—
K38	2.5	—	—	—	—
K41	—	—	—	—	—
K4	—	—	—	—	—
K5	—	—	—	—	—
K6	—	—	—	—	—
K7	5	—	—	—	—
K29	10	—	—	—	—
K30	—	—	—	—	—
K31	—	—	—	—	—
K33	40	20	—	—	—
K37	—	—	—	—	—
K43	—	—	—	—	—
K45	—	—	—	—	—
CDC 8198	200	100	100	100	—

[a] The agglutination test was carried out on a slide. The agglutination titer is expressed as the reciprocal of the highest dilution of antiserum showing visible agglutination.

can be specified, knowing the K-antigen of the strain (Sakazaki, 1965; Zen-Yoji et al., 1970). Following this specific correlation, K33 is demonstrated only in O3 and K25 is demonstrated only in O1. Thus, CDC 8198 (O1 :K33) and A 5704 (O3 :K25) do not fit this antigen schema. The antigenicities of CDC 8198 and A 5704 were studied further and results are presented in Tables 4–7. As shown in Table 4, when the antiserum of CDC 8198 was absorbed with either O3 and O9, agglutination was only observed with the homologous strain and O1. When it was absorbed with O1, no agglutination was observed with either O1 or CDC 8198. This suggests that the O-antigenicity of CDC 8198 is O1. However, when the antiserum of O1 was absorbed with CDC 8198, significant agglutination with O1 remained. This may be because the antigens of CDC 8198 lack some antigenic determinants, as has been reported for some strains of *V. parahaemolyticus* having O1 antigen (Torii et al., 1969). The results in Table 5 show that the K-antigenicity of CDC 8198 is K33. When the antiserum of CDC 8198 was absorbed with K26, agglutination was only observed with K33. When the antiserum of K33 was absorbed with CDC 8198, no agglutination was observed with either K33 or CDC 8198. The present data suggest that the main K-antigenicity of CDC 8198 is K33, but since agglutination occurred with CDC 8198 when the antiserum of CDC 8198 was absorbed with K33, it may also have another K-antigen(s). Similarly, the antigenicity of A 5704 was shown to be O3 :K25 (Tables 6 and 7). Recently, there have been other reports of exceptions to the specific correlation between the O- and K-antigenicities of *V. parahaemolyticus* (Terada, 1968; Kudo, 1969; Zen-Yoji et al., 1970; Zen-Yoji et al., 1971).

The Kanagawa phenomenon on Wagatsuma's medium is reported to be an indicator of human pathogenicity of *V. parahaemolyticus* (Miyamoto et al., 1969). We studied the Kanagawa phenomenon of the strains isolated in the USA. Only $OYG_1 3$ gave a positive reaction (Table 2). This strain was isolated from an oyster on the northwest Pacific coast,

TABLE 6. *Characterization of O-antigenicity of A 5704: Agglutination tests with antisera of A5704 and O3*[a]

Agglutinating antigen	Antiserum					
	A 5704				O3	
	Absorbed with					
	None	O1	O9	O3	O9	A 5704
O1	200	—	—	—	—	—
O2	400	—	—	—	—	—
O3	400	400	200	—	1600	400
O4	200	—	—	—	—	—
O5	400	—	—	—	—	—
O6	200	—	—	—	—	—
O7	200	—	—	—	—	—
O8	200	—	—	—	—	—
O9	200	—	—	—	—	—
O10	200	—	—	—	—	—
A 5704	>1600	3200	3200	—	3200	—

[a] Explanation as in Table 4.

TABLE 7. *Characterization of K-antigenicity of A 5704: Slide agglutination tests with antisera of A 5704 and K25*[a]

Agglutinating antigen	Antiserum				
	A 5704				K25
	Absorbed with				
	None	K29	K25	K25, O1	A 5704
K1	—	—	—	—	—
K25	>80	200	—	—	—
K26	—	—	—	—	—
K32	—	—	—	—	—
K38	—	—	—	—	—
K41	—	—	—	—	—
K4	—	—	—	—	—
K5	—	—	—	—	—
K6	20	—	—	—	—
K7	—	—	—	—	—
K29	>80	—	100	100	—
K30	—	—	—	—	—
K31	>80	—	—	—	—
K33	20	—	—	—	—
K37	>80	—	—	—	—
K43	—	—	—	—	—
K45	—	—	—	—	—
A 5704	800	400	400	200	—

[a] Explanation as in Table 5.

so it is possible that food poisoning due to *V. parahaemolyticus* may occur in this area of the USA. All the other strains tested showed a negative Kanagawa phenomenon. So although the latter strains have the characteristics of *V. parahaemolyticus*, they may not cause gastroenteritis.

The data presented in this paper show that with a very few exceptions strains of *V. parahaemolyticus* isolated in the USA have the same characteristics as those isolated in Japan. This, together with the recent outbreaks of food poisoning due to *V. parahaemolyticus* in the USA (Fishbein and Olson, 1971; Dadisman et al., 1972), suggests that there may be other cases of gastroenteritis due to *V. parahaemolyticus* in the USA besides those so far reported.

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