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Isolation of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* from Estuarine Areas of Southeastern Alaska

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The first reported isolations of halophilic vibrios, including *Vibrio parahaemolyticus*, from three seafood processing areas in Southeastern Alaska are described.

The occurrence of *Vibrio parahaemolyticus* in North America was first reported in 1968 by Baross and Liston (3) in oyster and sediment samples collected from Puget Sound, Washington. Since that time, numerous isolations of this organism in North America have been reported (4, 5, 8-12). To date, no isolations have been reported outside the temperate zone or north of the 60th parallel. The distribution of *V. parahaemolyticus* seems to be temperature dependent; few have been found in waters with temperatures below 8 to 10 C (4, 7).

As part of a comprehensive investigation of seafood processing in Alaska, the presence of various microorganisms of public health significance was determined. This included the occurrence of *V. parahaemolyticus* and the related biotype *V. alginolyticus* from samples of seawater, sediments, and waste discharges from shrimp, crab, and salmon processing facilities.

As a control measure to ascertain the natural background of halophilic vibrios, additional seawater and sediment samples were collected from remote waste-free areas not influenced by seafood processing operations. The three study areas selected for *Vibrio* enumeration were Petersburg, Hoonah, and Chatham, all of which lie along the southern coastal region of Alaska, north of the 55th parallel, and approximately 500 miles south of the Arctic circle. Surface water temperatures in this region normally range from 7 to 11 C during the summer months but are greatly influenced by runoff. At Petersburg, the average water temperatures from all stations sampled ranged between 9.0 to 9.5 C during both high and low tides. At Hoonah and Chatham, the corresponding values were 9.5 to 11.1 C and 7.7 to 11.2 C, respectively.

Samples of seawater and waste water were collected according to accepted procedures (1). Solid seafood waste was collected in sterile 170-g (6 oz.) plastic bags. Bottom sediments, consisting of the top 1 to 2 cm of mud, were obtained using a Peterson grab, which was thoroughly rinsed and air-dried prior to use. All vibrio samples were placed in styrofoam containers and transported in ice chests containing slush ice. The maximum allowable time between sample collection and examination was 8 h.

Isolation and identification of vibrios followed established quantitative and semi-quantitative procedures (2, 4, 13). The majority of confirmed isolates were obtained using the three-tube, most-probable-number technique outlined in the *Bacteriological Analytical Manual* (2). All suspected isolates were screened using triple sugar iron agar, motility media, cytochrome oxidase production, and sensitivity to vibriostatic agent 0/129 (2,4-diamino-6-7-diisopropyl pterdine). If suspected isolates were cytochrome oxidase positive, motile, and showed an acid-butt/alkaline-slant (*V. parahaemolyticus*) or an acid-butt/acid-slant (*V. alginolyticus*), negative gas, and H_2S on triple sugar agar, biochemical testing was continued.

Two of 10 samples collected from shrimp and crab processing facilities at Petersburg yielded *V. parahaemolyticus* (Table 1). The concentrations of *V. parahaemolyticus* and *V. alginolyticus* from these samples were 36/100 g in shrimp waste and 4/100 ml in the waste water discharge. Although no *V. parahaemolyticus* was encountered at Hoonah and Chatham, *V. alginolyticus* was found in 9 of 20 samples (45%) examined at these two locations. The greatest number of *V. alginolyticus* was obtained from waste sediments collected inside the main cannery dock at Chatham. Considering the close association and phenotypic similarity of *V. parahaemolyticus* to *V. alginolyticus* (6, 7), it is surprising none of the former were found at this location. The fact that processing of salmon had ceased 1 to 2 days prior to sampling at the loca-

TABLE 1. *Location and number of samples positive for V. parahaemolyticus and V. alginolyticus*

Location	Sample[a]	No. of samples examined	No. of samples positive for:		Organisms total count/100 ml or 100 g	
			V. parahaemolyticus	*V. alginolyticus*	*V. parahaemolyticus*	*V. alginolyticus*
Petersburg (shrimp and crab)	Sediment (control)[b]	1	0	0	NF[c]	NF
	Seawater (control)	1	0	0	NF	NF
	Sediment near discharge	3	0	0	NF	NF
	Shrimp waste	3	1	1	36	36
	Waste water	2	1	1	4	4
Hoonah (crab)	Sediment (control)	1	0	0	NF	NF
	Seawater (control)	1	0	0	NF	NF
	Sediment near discharge	3	0	2	NF	36, 36
	Crab waste	3	0	2	NF	36, 20
	Waste water	2	0	1	NF	15
Chatham (salmon)	Sediment (control)	1	0	0	NF	NF
	Seawater (control)	1	0	0	NF	NF
	Sediment near cannery	4	0	3	NF	430, 91, 91
	Seawater near cannery	4	0	1	NF	2

[a] Samples collected during August 1973.
[b] Control samples collected remote from the plant(s).
[c] NF, Halophilic vibrios not found.

tion may have had some residual effect on recovery of *V. parahaemolyticus*.

Background stations (controls) remote from any canning or processing operation at Petersburg, Hoonah, and Chatham all failed to yield vibrios by the three methods employed. Since vibrios were only found in the waste discharges, seawater, and sediments near the plants, it is suggested that they originated from the freshly caught fish and shellfish entering the plant. After processing the catch, there was ample opportunity for introduction of vibrios into sediments near the plants via waste discharges, which consisted mostly of waste portions of fish and shellfish. In Alaska, seafood wastes are disposed of by grinding and dumping off the plant dock or through outfalls located a few meters from shore. Limited data indicate that marine environments contaminated with animal wastes and high in chitinous materials contain higher densities of *V. parahaemolyticus* and related species than waters of low organic content (3, 4, 7).

Isolates of *V. parahaemolyticus* from Petersburg were serologically typed and subjected to the Kanagawa hemolysin test on Wagatsuma agar (2). All isolates submitted were confirmed as serotype 08:K39 and negative for the Kanagawa phenomenon.

Although the number of halophilic vibrios isolated during this survey was small, their occurrence in Alaska further demonstrates the ubiquitous nature of these microorganisms in coastal areas of the Pacific Ocean. Although *V. parahaemolyticus* seems to be present in Southeastern Alaska, their numbers are small in relationship to other related biotypes, possibly a result of lower seasonal water temperatures.

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