Distribution of Vibrio parahaemolyticus and Related Organisms in the Atlantic Ocean off South Carolina and Georgia

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The distribution of Vibrio parahaemolyticus and related organisms in the Atlantic Ocean was determined during the summer of 1971 from samples collected at stations along four transects on the continental shelf off the South Carolina and Georgia coasts. No V. parahaemolyticus strains were isolated from any of the samples of seawater (surface and bottom), sediment, and plankton which were collected. A numerical taxonomy analysis of data on substrate utilization, including 154 organic compounds serving as single carbon sources, was carried out, and four groups of strains were observed. Each group showed well-separated distribution profiles from shore out to the continental shelf. That is, the groupings were observed to correspond to coastal, off-shore and intermediate distribution patterns for the strains. This study provides a useful example of the kind of ecological distributional analysis of bacteria which can be accomplished with numerical taxonomy.

The pathogenic halophile Vibrio parahaemolyticus has been shown to derive from the marine environment (17), and many workers, including food, sanitary (hygiene), and fisheries microbiologists, have investigated the distribution of this organism in the natural environment. The first full-scale survey of the distribution of V. parahaemolyticus in the marine environment was accomplished by Miyamoto et al. (12, 13), who studied the seasonal distribution of V. parahaemolyticus in seawater and plankton in Tokyo and Sagami Bay. In the study undertaken by these workers (12, 13), an unusual bloom of plankton, often occurring off the eastern coast of Japan, was implicated in the development of unusual blooms of V. parahaemolyticus, ultimately resulting in fish becoming contaminated with the pathogenic halophilic bacteria.

Aoso (1) isolated organisms related to V. parahaemolyticus and observed that there was an incidence of more than 20% in coastal seawater throughout the year. Zen-Yoji et al. (22), Sakai et al. (14), and Terayama (18) found V. parahaemolyticus in seawater and plankton collected in the summer off Oshima Island, which is located near Tokyo Bay. Horie et al. (7, 9) and Fukuda and Kitao (6) also isolated V. parahaemolyticus from sediment and from plankton collected in the coastal zone of Japan. In the United States, Baross and Liston (4) reported the isolation of V. parahaemolyticus from seawater, sediment, and shellfish in Puget Sound and off the Washington coast. Isolation of V. parahaemolyticus from moribund blue crabs was reported in Chesapeake Bay (11). Seasonal variation in the incidence of V. parahaemolyticus has also been studied (T. Kaneko, Ph.D. thesis, Georgetown University, Washington, D.C., 1973; 10), and an annual cycle of V. parahaemolyticus occurrence in the Rhode River area of Chesapeake Bay has been described (T. Kaneko and R. R. Colwell, manuscript in preparation). In the Gulf Coast area, V. parahaemolyticus has been isolated from shrimp (21). Bartley and Slanetz (5) have reported isolating the organism from seawater and oysters in Great Bay and Little Bay areas of New Hampshire during the fall months. Thus, the distribution of V. parahaemolyticus in coastal regions has been well described.

Knowledge of the distribution of V. parahaemolyticus in the open sea is limited, and the data are very sparse. Aoki (3) reported the isolation of V. parahaemolyticus from seawater, sediment, plankton, and fish collected in the middle Pacific Ocean, as well as in coastal regions off Honolulu, Hawaii. Aoki (2) also reported the occurrence of this organism from seawater, plankton, and fish samples collected.

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in the open sea in Southeast Asia. Yasunaga and Kuroda (20) isolated \textit{V. parahaemolyticus} from tuna caught in the open sea in the Indian Ocean. On the other hand, Horie et al. (8) were not able to isolate \textit{V. parahaemolyticus} from seawater and plankton samples collected in the pelagic zone of the Pacific Ocean. However, organisms belonging to biotypes 2 and 3 were found to be widely distributed in those samples.

Up to the present time, it has been commonly accepted that \textit{V. parahaemolyticus} can be readily isolated from seawater, sediment, and plankton samples collected in coastal regions during the warm months of the year, especially at the mouths of rivers (9). However, the evidence for the distribution of \textit{V. parahaemolyticus} in the open sea is contradictory. In this study, a systematic sampling was accomplished along the continental shelf off the South Carolina and Georgia coasts in the summer months to clarify the distribution of \textit{V. parahaemolyticus} from near-shore to off-shore areas. Numerical taxonomy was applied to elucidate the distribution of the major bacterial groups present in the samples and to demonstrate the value of this approach in microbial ecology.

**MATERIALS AND METHODS**

**Sampling.** Most of the sampling was done aboard the R/V EASTWARD, the Duke University Research vessel, from 21 to 27 August 1971. Four transects, Charleston, Wasson Sound, Sapelo Island, and Fernandina Beach, off the South Carolina and Georgia coasts, were included in this study (Fig. 1). The transects are located on the continental shelf. The length of the transects was ca. 60 to 70 miles (about 96.5 to 112.6 km), with each transect comprising five stations, station 5 on each transect being the station closest to shore, i.e., at a distance of 4 to 10 miles (about 6.4 to 16 km) from shore. Station 1 for each transect was located farthest from shore and at the continental shelf break. Additional sampling was accomplished during other cruises aboard the R/V EASTWARD, which included two stations off North Carolina on 8 April 1971. Details for locations, samples collected, etc., are given in Tables 1 and 2.

**Sampling procedures.** Water samples were collected with the Niskin sampler at 2 to 5 m below the surface and were transferred to presterilized glass bottles (250 to 500 ml).

Sediment samples were collected with a Shipkei grab and were transferred to presterilized wide-mouth glass bottles (300 ml).

Plankton samples were collected with a Clarke-Bumpus quantitative plankton sampler with a no. 20 net (77-μm mesh). The plankton net was towed ca. 5 m from the surface for 15 to 20 min. The plankton samples were each transferred to sterile, wide-mouth glass bottles. Fish and shrimp collected off North Carolina were caught by trawl, and the skins of these animals were swabbed for isolation of bacteria with presterilized cotton swabs.

Water temperatures were measured with a bathythermograph. Salinity was measured by the titration method (16). The surface water temperatures were between 27 and 31°C at all stations (Table 1). Salinities at station 5 and station 1 on each transect were 28 to 29% and 33 to 34% respectively.

**Bacteriological analyses.** All bacteriological analyses were carried out as soon as possible after collection of the samples. Procedures for determining bacterial counts and the isolation and identification of \textit{V. parahaemolyticus} have been described in a previous paper (10). However, in this study, the following criteria were employed in the identification of colonies on TCBS agar. Colonies that appeared on TCBS agar were regarded as presumptive vibrios (PV), “presumptive” being applied here as it is used in routine coliform determinations. PV in this study correspond to the vibrio-like organisms described by Kaneko and Colwell (10). “Typical” green colonies on TCBS were regarded as colonies of presumptive \textit{Vibrio parahaemolyticus} (PVP) or \textit{V. parahaemolyticus}-like organisms, as described by Kaneko and Colwell (10).

Since many marine bacteria do not grow at 37°C, two incubation temperatures, 25 and 37°C, were employed to determine counts of PV and PVP. Thus, the bacterial groups were described as PV (25°C), PV (37°C), PVP (25°C), and PVP (37°C). The PV (25°C) and PVP (25°C) counts were regarded as the total PV and PVP counts for a given sample.

Methods other than those described earlier (10) were employed for bacterial counts. The membrane filter method, with a 0.45-μm filter (Millipore Corp., Bedford, Mass.) on modified TCBS medium (total NaCl concentration of 7%) incubated at 37°C, was used for enumeration of PV and PVP.

**Numerical taxonomy.** Substrate utilization tests were employed basically as described by Stanier et al. (15). The basal medium employed for this study was composed of: NaCl, 2.4%; MgSO$_4$·7H$_2$O, 0.7%; MgCl$_2$, 0.53%; KCl, 0.07%; NH$_4$H$_2$PO$_4$, 0.05%; K$_2$HPO$_4$, 0.05%; and refined lustra (Difco), 1.5%. The pH was adjusted with tris(hydroxymethyl)aminomethane buffer to 7.2. A total of 154 organic compounds was tested as sole carbon source (Kaneko, Ph.D. thesis, 1973). The number and kinds of substrate compounds employed differed slightly from those used by Stanier et al. (15).

Seventy-four PVP strains isolated from Atlantic Ocean samples and 23 reference strains were employed in the numerical taxonomy. The reference strains were as follows: \textit{V. parahaemolyticus} strains SA-3 and SA-23, Bainbridge strains 4203 and 10734, FC 1011, K 4 (lida), and three strains isolated from Chesapeake Bay; \textit{V. alginolyticus} ATCC 17749; \textit{V. cholerae} ATCC 14035; \textit{V. anguillarum} ATCC 14181; \textit{V. marinofulvus} ATCC 14395; \textit{V. marinopraesens} ATCC 19648; \textit{V. ponticus} ATCC 14391; \textit{V. haloplanktis} ATCC 14393; \textit{V. ichthyodermis} NCMB 407; \textit{Photobacterium} pierantonii ATCC 14546; \textit{Lucibacterium harveyi} ATCC 14126; \textit{Beneckea pelagia} ATCC 25916; \textit{B. nereida} ATCC 25917; B.
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FIG. 1. Stations sampled during the R/V EASTWARD cruise (April to August, 1971). Stations C 1 to C 5 were located on the Charleston, South Carolina transect, W 1 to W 5 on the Wassow Sound transect, S 1 to S 5 on the Sapelo Island transect, and F 1 to F 5 on the Fernandina transect. NC 1 and NC 2 were stations off North Carolina.

neptuna ATCC 25919; and B. campbellii ATCC 25920.

Numerical analysis was done with the IBM 360/40 system, with disk and tape drives, located at the Georgetown University Computation Center. The programs used were GTP-1, -2, -3, -4 and -5 (George-town Taxonomy Programs 1-5) written by R. D’Amico and R. R. Colwell.

RESULTS

Fish skin samples collected off North Carolina in April 1971 yielded a total of 14 pure cultures which were classified as PVP. After extensive tests were done, none of these strains were identified as V. parahaemolyticus.

Results from samples collected off the South Carolina and Georgia coasts were comparable. Figures 2, 3, and 4 give the distribution patterns for the total viable, aerobic, heterotrophic bacterial counts (TVC), PV, and PVP of the water samples (cited as counts per 100 ml). In general, the bacterial counts along the transects showed a decrease with distance from shore. For example, a TVC of ca. 10^4 was observed at the station closest to shore, i.e., 4 to 10 miles from shore, whereas the counts were 10^4 at stations on the continental shelf break. Decrease in count with distance from shore was more clearly seen in the case of the PVP at both 25 and 37 C. That is, there was a marked decline in PVP counts, especially in the case of PVP (37 C) for each transect. In every case, a PVP (37 C) count of less than 10^2 was observed at station 1 for each transect. Bacterial counts obtained by membrane filter (Millipore Corp.) showed the same distribution pattern for PV and PVP (Fig. 5). At station 1, PV and PVP for the deep-water samples, which were collected at depths of 180 m, were less than 100 per 100 ml (Fig. 5). V. parahaemolyticus was not present in any of the water samples.

In the case of the sediment samples, results for two transects, Wassow Sound and Sapelo
TABLE 1. Location of stations on the R/V EASTWARD cruise in August 1971

| Station no. | Sampling date | Latitude  | Longitude  | Depth (m) | Water temp (°C) | Samples collected |
|-------------|---------------|-----------|------------|-----------|-----------------|------------------|
| C 5         | 8/21/71       | 32°39' N  | 79°40' W  | 10        | 28.7            | 27.5             |
| C 4         | 8/21/71       | 32°37' N  | 79°28' W  | 20        | 28.8            | 28.2             |
| C 3         | 8/21/71       | 32°36' N  | 79°16' W  | 25        | 28.7            | 24.5             |
| C 2         | 8/21/71       | 32°22' N  | 79°02' W  | 49        | 28.0            | 22.8             |
| C 1         | 8/21/71       | 32°18' N  | 78°50' W  | 180       | 27.6            | 11.2             |
| W 5         | 8/22/71       | 31°53' N  | 80°49' W  | 9         | 28.7            | 28.1             |
| W 4         | 8/22/71       | 31°41' N  | 80°34' W  | 17        | 29.0            | 27.0             |
| W 3         | 8/22/71       | 31°32' N  | 80°19' W  | 25        | 28.4            | 24.8             |
| W 2         | 8/22/71       | 31°23' N  | 80°02' W  | 40        | 28.4            | 22.8             |
| W 1         | 8/23/71       | 31°12' N  | 79°47' W  | 200       | 28.7            | 10.0             |
| W 0         | 8/23/71       | 31°04' N  | 79°21' W  | 700       | 28.5            | 10.0             |
| S 5         | 8/24/71       | 31°20' N  | 81°11' W  | 7         | 30.4            | 28.7             |
| S 4         | 8/24/71       | 31°13' N  | 80°53' W  | 17        | 27.5            | 26.4             |
| S 3         | 8/25/71       | 31°06' N  | 80°35' W  | 34        | 27.8            | 24.0             |
| S 2         | 8/25/71       | 30°58' N  | 80°12' W  | 30        | 27.5            | 23.4             |
| S 1         | 8/25/71       | 30°51' N  | 79°59' W  | 180       | 29.1            | 8.0              |
| F 5         | 8/27/71       | 30°43' N  | 81°10' W  | 15        | 27.2            | 25.5             |
| F 4         | 8/27/71       | 30°44' N  | 81°02' W  | 20        | 28.0            | 25.0             |
| F 3         | 8/27/71       | 30°43' N  | 80°40' W  | 27        | 27.7            | 22.5             |
| F 2         | 8/27/71       | 30°41' N  | 80°32' W  | 40        | 27.4            | 22.5             |
| F 1         | 8/27/71       | 30°41' N  | 80°03' W  | 190       | 29.3            | 9.0              |

* C 5 to C 1, on Charleston transect.
* W 5 to W 0, on Wasson Sound transect.
* S 5 to S 1, on Sapelo Island transect.
* F 5 to F 1, on Fernandina Beach transect.

TABLE 2. Location of stations on the R/V EASTWARD cruise off North Carolina in April 1971

| Station | Sampling date | Latitude  | Longitude | Depth (m) | Surface water temp (°C) | Sample collected |
|---------|---------------|-----------|-----------|-----------|-------------------------|-----------------|
| NC 1    | 4/8/71        | 34°29'00" N | 75°06'00" W | ca. 1000 | ca. 20                  | Shrimp          |
| NC 2    | 4/8/71        | 34°22'03" N | 76°05'03" W | ca. 100  | ca. 20                  | Plankton, fish, sargassum |

![Graph 1](http://aem.asm.org/)

**FIG. 2.** Total viable, aerobic, heterotrophic bacteria (TVC), presumptive vibrios (PV), and presumptive *Vibrio parahaemolyticus* (PVP) per 100 ml of surface water (2 to 5 m) of samples collected from off the Georgia coast. Wasson Sound transect.

![Graph 2](http://aem.asm.org/)

**FIG. 3.** Total viable, aerobic, heterotrophic bacteria (TVC), presumptive vibrios (PV), and presumptive *Vibrio parahaemolyticus* (PVP) per 100 ml of surface water (2 to 5 m) in samples collected from off the Georgia coast. Sapelo Island transect.
Island, are given in Fig. 6 and 7. The TVC for station 5 on both transects were \(10^4\) to \(10^6\) per g (wet weight) of sediment. The distribution patterns for TVC were found to be related to the transect, whereas PVP (37°C) showed no change in counts at those stations between 5 and 3. At the latter stations, the bottom temperature was >24°C. However, PVP counts were too low to be enumerated by the methods used in the case of station 1 where the bottom temperature was \(\leq 10\)° C. *V. parahaemolyticus* was not present in any of the sediment samples.

Two transects were followed in the plankton sampling. Plankton sample data appeared to vary with the stations. In general, the plankton communities were more heterogenous in their distribution compared with results of similar plankton studies carried out in Chesapeake Bay (10). The off-shore plankton samples indicated occasional phytoplankton blooms, with crustaceans not always predominant. The distribution patterns for the plankton TVC, as well as PV and PVP, are given in Fig. 8 and 9. The TVC of the plankton samples were approximately \(10^7\) to \(10^9\) gm at the stations sampled, without a clear pattern of decrease in count with distance from shore as was observed for the water samples. This was also the case for the plankton PV and PVP counts. The PVP (37°C) also did not give the expected pattern of distribution, i.e., decrease with distance from shore. *V. parahaemolyticus* was not present in any of the plankton samples.

A total of 74 PVP strains was selected from the Atlantic Ocean isolates and were included in the numerical taxonomy analysis along with the 23 reference strains, including nine reference strains of *V. parahaemolyticus* (Fig. 10). Four groups, or clusters, were obtained, namely, groups I, II-B, III, and IV, respectively. Group I appeared to comprise a single group, with group I-A (*V. parahaemolyticus*) distinguishable as a subgroup, with a difference in inability to grow at 43°C noted for I-A and I-B. Other characteristics, however, were very nearly identical with respect to substrates utilized by strains of each group, as well as in results of
The intergroup $S$ value of group III with group I-A was only 53.4%. All strains of group III did not grow at 37°C on initial isolation. Group IV was comprised of 16 PVP strains that did not grow at 37°C but grew well at 25°C. Most of the isolates from off the North Carolina coast were included in this group. The intergroup $S$ value was 80.5%, and the intergroup $S$ value with group I-A was 54.8%.

Thus, from the numerical analysis, with isolates from the Atlantic Ocean, none of the strains were found to be included in group I-A (the *V. parahaemolyticus* cluster of strains), and 25 of the 74 strains examined did not cluster with any of the four groups obtained in the analysis. None of the strains of the groups observed in this analysis showed high similarity values with the *Beneckea* species.

**DISCUSSION**

The distribution of *V. parahaemolyticus* in various geographical areas has been studied by a number of investigators, with most of the work being done in coastal regions. It is generally accepted that *V. parahaemolyticus* inhabits coastal waters, especially at the mouth of rivers. Studies of the distribution of *V. parahaemolyticus* in the open sea are few and contradictory. One of the difficulties is that isolates identified as *V. parahaemolyticus* from open ocean samples were poorly described. Hence, any conclusions as to the presence of *V. parahaemolyticus* in the ocean are not yet definitive. Nevertheless, there have been several reports on the isolation of *V.
**parahaemolyticus** from samples collected in the open sea, i.e., in the Pacific and Indian Oceans (2, 19, 20). According to Horie et al. (8), *V. parahaemolyticus* is not found in samples of seawater and plankton collected from the pelagic zone of the Pacific Ocean, although organisms belonging to other *Vibrio* biotypes are found. Observations made in this study carried out off the South Carolina and Georgia coasts support the observation of Horie and co-workers. The interesting fact is that *V. parahaemolyticus* was not detected in seawater, sediment, or plankton samples collected at station 5 of each of the transects which were close to shore, i.e., at distances of only 4 to 10 miles from shore. However, organisms belonging to the PVP group were isolated from several samples, collected at several of the stations. It is
our conclusion that the strains identified as PVP may in fact often be misidentified as *V. parahaemolyticus*, if only a few standard bacteriological tests for identification are done. Based on the numerical taxonomy analysis of the extensive substrate utilization data of this study, there are four major groups present in samples collected in coastal and open ocean waters, i.e., up to 70 miles from shore. These groups revealed clear-cut distributional patterns with respect to location and distance from shore (Fig. 11). That is, organisms of group I-A, the *V. parahaemolyticus* strains, and group II-A, the Chesapeake Bay luminescent strains, which were isolated during a study of the distribution of *V. parahaemolyticus* in Chesapeake Bay (Kaneko and Colwell, manuscript in preparation), were restricted to the estuary and were easily isolated from Chesapeake Bay samples collected in the warm months of the year. Groups I-A and II-A strains were not found in samples collected at any of the stations in the Atlantic Ocean. Organisms of group I-B, very closely related to *V. parahaemolyticus*, were isolated only at station 5. The distribution of this group appears to be restricted to near-shore areas of the coastal zone. These organisms could be easily misidentified as *V. parahaemolyticus* unless growth at 43°C was tested. Organisms belonging to group II-B were more widely distributed, being found in samples of stations 5 to 2, compared with the distribution of group I-A organisms, and being more typical of the coastal type than strains of groups III and IV. Group III strains were found only at two stations, 3 and 2. Distribution of this group was found to be very limited, and the distribution pattern seems to be between those of groups II-B and IV. Group IV was found at those stations between 4 and 1. It appears to be an open-sea type of microorganism. These strains did not grow at 37°C, which is typical of many marine bacteria.

As seen in the distribution patterns for PV and PVP in the water and sediment samples (Fig. 2-7), organisms belonging to groups I-B and II-B comprised the PVP (37°C) count, the population of which was observed to decrease with distance from shore. Furthermore, these organisms were not detected in sediment samples collected at station 1 where the temperatures were ≤10°C and where PVP (25°C) strains could be isolated. Thus, with respect to the distribution of PVP (37°C) strains in sediment, the temperature of the bottom appears to be a determining factor when the temperature is ≤10°C. On the continental shelf, where the depth is ca. 180 to 200 m, the temperature throughout the year is ≤10°C.

![Figure 11](http://aem.asm.org/) Incidence of selected bacterial groups in the Atlantic Ocean and Chesapeake Bay samples. The abscissa indicates relative distance from shore to the continental shelf break off the Georgia Coast, that is, the number is the station number of the transect (see Fig. 2). The distances involved are 4 to 10 miles from the shore at Station 5 and 60 to 70 miles at station 1, i.e., at the continental break. C, Chesapeake Bay, in this case, typically estuarine.

The distribution pattern of PVP (37°C) strains in surface water was found to be influenced by factors limiting the distribution, as for group I-B, but may not be the water temperature in the summer months, since the water temperature of surface water at all the stations sampled was 27 to 31°C. Salinity is not likely to be a factor either, since the salinity at station 5 was 28 to 29‰ and at station 1 was 33 to 34‰.
with no major difference noted. What factors restrict the distribution of these organisms? \(V.\) \(parahaemolyticus\) is affected by its association with zooplankton, as has been found to be important in the continuation of the annual cycle of this organism in the estuary (10). Adsorption of \(V.\) \(parahaemolyticus\) onto zooplankton is perhaps the most important factor in determining its distribution in nature (Kaneko, Ph.D. thesis, 1973; 10; Kaneko and Colwell, manuscript in preparation). Limiting factors for organisms isolated from the Atlantic Ocean are not known. This is an area of research which requires further study.

The application of numerical taxonomy to microbial ecology has proven to be both practical and useful. It is especially valuable when the organisms under study are frequently misidentified by applying only a few tests for identification and classification. The type of analysis carried out in this study was found to permit a more careful characterization of the distribution pattern of several bacterial species than could have been obtained with conventional classification methods.

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