Enrichment of Vibrio parahaemolyticus in a Simple Medium

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Received for publication 13 March 1974

A medium which contained 3% NaCl and 0.2% Teepol in \( \frac{1}{2} \) M phosphate buffer was prepared and was evaluated to be a useful enrichment medium for the isolation of Vibrio parahaemolyticus in marine specimens. Glucose salt Teepol broth produced a poorer result than direct culture.

Vibrio parahaemolyticus can be isolated without difficulty from marine specimens and human stools on thiosulfate citrate bile sucrose (TCBS) and bromothymol blue (BTB) Teepol agars, but enrichment is sometimes necessary for isolation from sparsely-infected materials. Reported enrichment media contain nutrients which favor the growth of not only V. parahaemolyticus but also marine and enteric bacteria (4, 5). We noted in a previous report that V. parahaemolyticus propagates in phosphate-buffered salt solution more readily than Escherichia coli and Salmonella (1). With this finding, we suspected that the enrichment of V. parahaemolyticus in marine specimens and human stools can be obtained in phosphate buffer containing NaCl, and prepared a medium composed of NaCl and phosphate buffer. We also added Teepol, a neutral detergent, which is more inhibitory to E. coli and V. alginolyticus than to V. parahaemolyticus. This report evaluates this medium, including preliminary experiments on the ingredients of the medium.

MATERIALS AND METHODS

**Strains.** V. parahaemolyticus 9337 is Kanagawa hemolysis positive and was supplied by Y. Miyamoto, Kanagawa Prefectural Public Health Laboratory, Japan. V. alginolyticus was isolated from a sea fish and E. coli 06 was isolated from a human stool in this laboratory, and both demonstrated typical characteristics.

**Enrichment media.** Salt peptone water (SP) and glucose salt Teepol broth (GSTB) were prepared as described previously (4, 5). SP (4) contained 3% NaCl in 1% peptone water (pH 7.2), and GSTB (5) contained 0.3% beef extract, 1% peptone, 3% NaCl, 0.5% glucose, 0.0002% methyl violet, and 0.4% Teepol in distilled water (pH 8.5). Based on preliminary experiments shown in Results, salt Teepol buffer (STB) was prepared to contain 3% NaCl and 0.2% Teepol (Shell Chemical Co.) in \( \frac{1}{2} \) M phosphate buffer (pH 8.5).

**Procedures.** To determine the optimum concentration of NaCl and Teepol in media, 0.1 ml of appropriate dilutions of a 24-h culture of test organisms in nutrient broth with 3% NaCl was inoculated into 5 ml of test media. Before and after incubation at 37 C for 24 h, 0.1 ml of decimal dilutions of test media was spread on solid media, and colonies were counted after 24 h of incubation. Counts of V. parahaemolyticus and V. alginolyticus were made on BTB Teepol agar, and E. coli counts were made on MacConkey agar. All counts were carried out in triplicate for each medium, and mean values were calculated.

For the test of field and clinical specimens, a small amount of marine and human fecal materials was suspended in a small amount of physiological saline. After shaking thoroughly, two loopsfuls were streaked on TCBS agar. For the enrichment, 0.2 ml of suspension was inoculated into 5 ml each of test media. After 24 h of incubation at 37 C, two loopsfuls were inoculated on TCBS agar plates, and suspected colonies were then transferred on nutrient agar slopes containing 3% NaCl, and characterized by the criteria of Hugh and Sakazaki (2).

RESULTS

**Effect of NaCl and Teepol on growth of test organisms.** The optimum concentration of NaCl on the growth of V. parahaemolyticus was studied, and 3% NaCl was found to produce the best result on growth in phosphate buffer and peptone water, with weaker growth in low and high concentrations of NaCl. A majority of the test strain was sterilized immediately after the inoculation in NaCl-free media (Table 1). The study on the effect of Teepol showed that Teepol is inhibitory on the growth of test organisms in both phosphate buffer and peptone water containing NaCl (Table 2). However, the inhibitory effect in phosphate-buffered NaCl solution was less marked with V. parahaemoly-
Table 1. Effect of NaCl on growth of Vibrio parahaemolyticus

| NaCl concn (°) | Buffer* | Peptone water* |
|---------------|---------|----------------|
|               | 0°      | 24°            |
| 0             | 9°      | 0              |
| 1             | 110     | 85.00          |
| 3             | 130     | 140.00         |
| 5             | 135     | 88.00          |
| 7             | 140     | 2.700          |

* Buffer = Phosphate buffer, 3/4 M, pH 8.5. Peptone water = 1%, pH 7.2.

Table 2. Effects of Teepol on growth of test organisms

| Organism             | Teepol concn (%) | Buffer | Peptone water |
|----------------------|------------------|--------|---------------|
| V. parahaemolyticus  | 0.4              | 64°    | 10,500        |
|                      | 0.2              | 56°    | 17,000        |
|                      | 0                | 75°    | 55,000        |
| V. alginolyticus     | 0.4              | 84°    | 1,700         |
|                      | 0.2              | 89°    | 7,600         |
|                      | 0                | 82°    | 24,000        |
| E. coli              | 0.4              | 90°    | 580           |
|                      | 0.2              | 83°    | 610           |
|                      | 0                | 98°    | 12,820        |

* Super, Shell Chemical Co.
* Each medium contains 3% NaCl.
* Hours of incubation at 37°C.
* Number of viable cells per 0.1 ml.

Table 3. Growth of test organisms in mixed culture in enrichment media

| Medium                  | Organisms* mixed with VP | Organisms counted |
|-------------------------|--------------------------|-------------------|
| Salt Teepol buffer      | VA                       | VP                |
|                         | VA                       | 100°              |
|                         | EC                       | 130°              |
| Salt peptone            | VA                       | VP                |
|                         | 250°                     |
| Glucose salt Teepol broth | VA                       | VP                |
|                         | 390°                     |
|                         | EC                       | 1,350°            |

* VP = V. parahaemolyticus, VA = V. alginolyticus, EC = E. coli.
* Hours of incubation at 37°C.
* Number of viable cells per 0.1 ml.

Table 4. Isolation of Vibrio parahaemolyticus from 110 marine specimens

| No. of colonies per plate | No. of colonies by direct culture* | No. of Colonies on enrichment media* |
|---------------------------|-----------------------------------|-------------------------------------|
|                           | STB                               | SP                                 | GSTB                               |
| 20                        | 0                                 | 5                                  | 4                                  | 0                                  |
| 11-20                     | 2                                 | 7                                  | 4                                  | 0                                  |
| 1-10                      | 41                                | 62                                 | 60                                 | 24                                 |
| 0                         | 67                                | 36                                 | 42                                 | 86                                 |
| No. positive              | 43                                | 74                                 | 68                                 | 24                                 |
| Isolation rate (%)        | 39.1                              | 67.3                               | 61.8                               | 21.2                               |

* On TCBS agar plate.
* STB = salt Teepol buffer, SP = salt peptone, GSTB = glucose salt Teepol broth.

and clinical specimens. Table 4 compares the results of enrichment on the isolation of V. parahaemolyticus in marine specimens collected at random. The enrichment in STB and SP significantly increased the rates of isolation more than direct culture, with similar results between STB and SP, but the enrichment in GSTB yielded a lower isolation rate than direct culture. In the study with clinical specimens obtained from hospitals and health centers, five cases among 878 fecal specimens were found positive after enrichment in STB, and SP, and two cases were positive by direct culture.

DISCUSSION

The results in this study clearly indicated that STB is a useful enrichment medium for the isolation of V. parahaemolyticus from marine
The enrichment in GSTB yielded a poorer result than direct culture, probably due to the overgrowth of marine bacteria in this nutrient-rich medium. GSTB originally described was pH 9.4 (5), but we adjusted the final pH to 8.5, since Ku and Chun (3) reported that the growth of *V. parahaemolyticus* was markedly suppressed and some strains failed to grow at pH 9.4. We found that the inhibitory effect of Teepol was almost identical with *V. parahaemolyticus* and *V. alginolyticus* in peptone water containing NaCl, but the inhibition was more marked with *V. alginolyticus* in phosphate buffer containing NaCl. Therefore, the addition of Teepol in GSTB was not thought to play a role in the inhibition of *V. alginolyticus*, a common marine bacterium.

We have noted that the propagation of *V. parahaemolyticus* in 3% NaCl solution (unpublished data), and in 3% NaCl added with 0.2% Teepol may be used for the same purpose with STB. Since STB does not contain ingredients which support the active growth of *V. alginolyticus*, a large amount of marine materials may be used for the enrichment of *V. parahaemolyticus*, with which much better results are obtained than SP. The evaluation on the enrichment of *V. parahaemolyticus* in fecal specimens needs more extended observation.

**ACKNOWLEDGMENT**

This research was supported by the U.S. Army Research and Development Group (Far East), Department of the Army, under grant DA-CRD-AFE-S92-544-71-G175. We thank Y. Miyamoto, Kanagawa Prefectural Public Health Laboratory, Japan, for the supply of *V. parahaemolyticus*.

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