Modified Selective and Differential Isolation Medium for \textit{Vibrio parahaemolyticus}

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The semiselective salt-starch-agar formulation of Baross and Liston was modified as the result of a systematic study of the effect of each constituent on the growth of \textit{Vibrio parahaemolyticus} and competitive species characteristic of the marine environment. The selection of nutrient constituents depended on an analysis of their effect on generation time. The addition of inhibitors depended on an analysis of minimal inhibitory concentrations. The modified formulation included: peptone, 2.0%; yeast extract, 0.2%; corn starch, 0.5%; NaCl, 3.0%; agar, 1.5% (pH 8.0). Penicillin at 2 to 5 units/ml increased selectivity without significantly inhibiting \textit{Vibrio} in pure suspensions. Over 62% of the most sensitive strain (YM-K33) was recovered at a concentration of 5 units of penicillin per ml. The per cent recovery of \textit{V. parahaemolyticus} from fish homogenate compared favorably with other selective formulations. At an initial concentration of $10^4$ cells/ml, recovery varied with the strain used from 60 to 119%, whereas at $10^6$ cells/ml the range was 36 to 94%. Applications of the medium for \textit{Vibrio} quantification are discussed.

The first description of \textit{Vibrio parahaemolyticus} by Fujino et al. (5) in 1953 followed the isolation of the organism on conventional media. After its identification as a halophile by Takikawa (13) in 1958, many early investigators continued to isolate \textit{V. parahaemolyticus} on conventional media supplemented with 3% salt (1, 11). The growth of competitive organisms, however, made isolation and identification of \textit{V. parahaemolyticus} from sea fish and sea fish products difficult. Consequently, new selective media were proposed and used by Japanese investigators (2, 6–8, 10). It is not apparent that these new media resulted from systematic studies of the effect of each of their constituents on the growth of \textit{V. parahaemolyticus} or competitive species typical of the marine environment. In addition, in our hands many of these media exhibited inadequate recovery of strains of \textit{V. parahaemolyticus}. Recently, Baross and Liston (3, 4) formulated a semiselective salt-starch-agar (0.5% soluble starch, 0.3% peptone, 0.1% yeast extract, 0.5% NaCl, 1.5% agar; pH 7.5) upon which they recovered \textit{V. parahaemolyticus} from sea fish and seawater. The present study was initiated to make an appraisal of these constituents to formulate an improved \textit{V. parahaemolyticus} isolation medium by modification.

**MATERIALS AND METHODS**

\textbf{Cultures}. Seven representative strains of \textit{V. parahaemolyticus} (SAK-K3, KA-K4, SJ-K7, KA-K18, SJ-K18, SJ-K28, and YM-K33) used in this study as well as five strains of suspected \textit{V. parahaemolyticus} isolated from tissue infections (CDC A6614, CDC A8633, CDC A5704, CDC A5002, and CDC A4871) have been described and their maintenance conditions have been outlined (14). (A typographical error appears in Table 1, p. 512 of reference 14. Under "Strain designation" for SJ, "K1 K18" should be "K1 K18" and "K3 K32" should be "K3 K32").

Five additional strains of \textit{V. parahaemolyticus} were obtained from Y. Miyamoto, Kanagawa Prefectural Public Health Laboratory, Yokohama, Japan. Three of these (4750, 9121, and 9187) were isolated from patients' stools, were Kanagawa-positive, and were considered virulent by Miyamoto. The fourth (M229) was isolated from the stools of a healthy carrier. The fifth (S-42-87) was recovered from seawater, was Kanagawa-negative, and was considered avirulent.

Ten species typical of the competitive marine microflora (\textit{Staphylococcus aureus} C31; \textit{Escherichia coli} GF; \textit{Alcaligenes faecalis} SEC 55; \textit{Pseudomonas aeruginosa} KR1; \textit{Streptococcus liquefaciens} Y5; \textit{Bacillus cereus} 27A; \textit{Aerobacter aerogenes} ATCC 211; \textit{Salmonella senftenberg} 775-W; \textit{Proteus vulgaris} ATCC 4669; and \textit{Shigella flexneri} 4AD) were chosen from our stock collection and were maintained on Trypticase Soy Agar (BBL).
Growth rate studies. The selection of nutrient constituents depended on their effect on the growth rate of *V. parahaemolyticus* expressed as generation time. Strains of *V. parahaemolyticus* were grown on Trypticase Soy Agar plus 2.5% NaCl for 18 hr at 35°C. Growth was suspended, and cultures were washed three times by centrifugation at 2000 x g and suspended in buffered saline (1.165% NaCl, 5.36% NaH2PO4, 7.2% KH2PO4, pH 7.2). The optical density was adjusted to 0.3 at 620 nm in a Coleman Junior spectrophotometer. The suspension was diluted 1:10 in buffered saline, and 0.5 ml was inoculated into 49.5 ml of experimental broth, placed in duplicate 300-ml nephelo-culture flasks (no. 520, Bellco Glass, Inc., Vineland, N.J.) with a sidearm (19 by 130 mm) for measuring turbidity. Culture flasks were incubated at 35°C and 160 rev/min in a gyratory shaker (New Brunswick Scientific Co., New Brunswick, N.J.). Viable cell counts were prepared from serial dilutions, and turbidities of flasks were determined at appropriate intervals from 0 through 24 hr. The generation time for a given medium, determined from the maximum tangent of a semi-log plot of turbidity versus time, equaled the shortest time for doubling optical density. Duplicate determinations were averaged.

Nutrient media and constituents examined included Trypticase Soy Broth, Trypticase, Thiotone, Phytone, and gelatin (BBL); Tryptic Soy Broth, Brain Heart Infusion, yeast extract, peptone, Casamino Acids, proteose peptone, beef extract, dextrose, and soluble starch (Difco); potato starch and 1-cysteine hydrochloride (Fisher Scientific Co., Pittsburgh, Pa.); and cornstarch (Corn Products Co., Englewood Cliffs, N.J.).

Inhibition studies. Preliminary selection of inhibitory agents was based upon the quantity of growth appearing in experimental broth containing inhibitors after 18 hr at 35°C. Test media were inoculated from 18-hr Brain Heart Infusion or Brain Heart Infusion plus 2.5% NaCl broth.

The final choice of inhibitors depended upon an analysis of the minimal inhibitory concentrations (MIC). The MIC's of inhibitors were determined by the gradient agar plate technique (12). Strains of *V. parahaemolyticus* and competitors were grown, harvested, and suspended to known optical density. Suspending diluent for competitors was either buffered distilled water (1.25 ml of a 3.4% solution of KH2PO4 adjusted to pH 7.2 and added to 1 liter of distilled water) or 0.1% peptone. The adjusted suspension of each was diluted so that the inoculum spread over the surface of duplicate test plates would contain 10⁶ viable cells. Plates were air dried and incubated for 18 hr at 35°C. The MIC for each agent was determined by measuring the position of the line of growth inhibition.

Inhibitory substances tested included potassium tellurite, sodium deoxycholate (Difco); potassium penicillin G, erythromycin (Eli Lilly & Co., Indianapolis, Ind.); Teepol 610 (Shell Chemical Co., New York); gentamicin sulfate (Schering Corp., Bloomfield, N.J.); sodium colistimethate (Warner-Lambert Research Institute, Morris Plains, N.J.); hydroxyurea, neomycin sulfate (Nutritional Biochemicals Corp., Cleveland, Ohio); thallous acetate (Matheson Coleman & Bell, Cincinnati, Ohio); and sodium azide (Eastman Organic Chemicals, Rochester, N.Y.).

Recovery studies. The efficiency and sensitivity of the modified isolation medium were determined by a comparative examination of the recovery of strains of *V. parahaemolyticus* from pure culture suspensions and mixed natural flora. The latter condition provided the competitive microflora of the marine environment. Strains were grown, harvested, and suspended to known optical density in buffered saline. They were then diluted to an initial concentration of 10⁶ to 10⁹ cells/ml in buffered physiological saline or fish homogenate (frozen haddock fillets, thawed, suspended in four volumes of buffered physiological saline, and blended at high speed in a Waring Blender for 2 min). Viable cell counts were prepared from serial dilutions of the initial suspensions. Plate counts were compared after 18 hr of incubation at 35°C. Counts were verified by subculturing 10 representative colonies into media designed to test sucrose fermentation, salt tolerance, and indole and acetoin production (11, 14). Selective media formulations compared with the modified isolation medium included BTB-Teepol agar and TCBS agar [described by Sakazaki (10)] and salt-starchagar [described by Baross and Liston (3, 4)].

### RESULTS

A preliminary comparison of growth rates, expressed as generation times, of *V. parahaemolyticus* SJ-K18 grown on various conventional media suggested that a simple peptone-salt medium supported the most rapid growth (Table 1).

In subsequent investigation, the comparative abilities of several different nitrogen sources to stimulate the growth rates of strains of *V. parahaemolyticus* were tested (Table 2). The concentration of peptone that supported the most rapid growth of strains in the simple buffered salt medium was 2.0%. Proteose peptone and Thio-

| Table 1. Growth rates of *Vibrio parahaemolyticus* SJ-K18 in several conventional media |
|---|---|---|
| Medium | Constituents | Generation time (min) |
| 1 | Trypticase Soy Broth + 2.5% NaCl | 21 |
| 2 | Tryptic Soy Broth + 2.5% NaCl | 29 |
| 3 | Brain Heart Infusion + 2.5% NaCl | 30 |
| 4 | 2.0% Trypticase + 3.0% NaCl | 33 |
| 5 | 2.0% Trypticase + 0.5% yeast extract + 3.0% NaCl | 25 |
| 6 | 2.0% peptone + 3.0% NaCl | 19 |
| 7 | 2.5% Casamino Acids + 0.5% yeast extract + 3.0% NaCl | 24 |

hydroxyurea, neomycin sulfate (Nutritional Biochemicals Corp., Cleveland, Ohio); thallous acetate (Matheson Coleman & Bell, Cincinnati, Ohio); and sodium azide (Eastman Organic Chemicals, Rochester, N.Y.).
growth, the buffered peptone-salt medium was supplemented with additives providing a carbohydrate source or modifying carbohydrate metabolism (Table 3). Maximum growth rates were achieved with the addition of 0.2% yeast extract and 0.5% glucose or soluble starch. Both an anaerobic atmosphere and a reducing environment (provided by addition of 0.1% cysteine hydrochloride to the medium) decreased the growth rate of \textit{V. parahaemolyticus} strains.

The buffered peptone-salt medium supplemented with yeast extract supported growth of species other than \textit{V. parahaemolyticus}. Consequently, in preliminary experiments we compared the effectiveness of a series of inhibitory agents upon the 18-h growth of strains of \textit{V. parahaemolyticus} and some competitors. The addition of 0.005% potassium tellurite, 0.25% sodium desoxycholate, 0.2% Teepol 610, 6% NaCl, or 6% K$_2$HPO$_4$ to the base formula was less useful than the presence of penicillin in an alkaline pH. This was also true of the application of an anaerobic atmosphere to the base formula. As a result of pH titration experiments, pH 8.0 was selected as optimal.

The MIC's of five antibiotics and four inhibitory chemicals were determined from single- and double-gradient plates (Table 4). In preparation of agar plates, phosphate was deleted from the medium. Except for penicillin, MIC's of the inhibitors tested were not significantly greater for the \textit{V. parahaemolyticus} strains than for the competitors. Except for an occasional species, no advantage was attached to their use.

### Table 2. Effect of different nitrogen sources on the growth rate of \textit{Vibrio parahaemolyticus} strains

| Experiment | Constituents (%)$^a$ | Generation time (min) |
|------------|----------------------|----------------------|
|            | Strain | Peptone | Protose | Thiolute |          |
| 1          | YM-K33 | 1.0     | 0.2     | 1.0      | 17       |
|            |        | 1.5     | 0.5     | 1.5      | 16       |
|            |        | 2.0     | 1.0     | 2.0      | 15       |
|            |        | 2.5     | 0.3     | 2.0      | 14       |
|            |        | 3.0     | 0.3     | 2.5      | 15       |
|            |        | 3.5     | 1.0     | 2.0      | 15       |
| 2          | YM-K33 | 1.0     | 0.2     | 1.0      | 20       |
|            |        | 2.0     | 0.2     | 2.0      | 20       |
|            |        | 0.2     | 0.3     | 0.3      | 20       |
|            |        | 0.3     | 0.3     | 20       | 17       |

* Dissolved in 3% NaCl, 0.25% K$_2$HPO$_4$ (pH 7.2).

### Table 3. Effect of additional energy sources on the mean generation time of \textit{Vibrio parahaemolyticus} strains

| Experiment | Constituents (%)$^a$ | Generation time (min) |
|------------|----------------------|----------------------|
|            | Strain | Yeast extract | Glucose | Soluble starch | 1-Cysteine |
| 1          | SJ-K28 | 0.2 | 0.2 | 0.5 | 17 |
|            |        | 0.5 | 0.2 | 0.5 | 15 |
|            |        | 1.0 | 0.2 | 0.5 | 17 |
| 2          | YM-K33 | 0.2 | 0.2 | 0.2 | 17.25 |
|            |        | 0.2 | 0.2 | 0.5 | 17.25 |
|            |        | 0.2 | 0.2 | 0.5 | 19.25 |
| 3          | YM-K33 | 0.2 | 0.2 | 0.5 | 17.75 |
|            |        | 0.2 | 0.2 | 0.5 | 17.75 |
|            |        | 0.2 | 0.2 | 0.5 | 19.75 |
| 4          | YM-K33 | 0.2 | 0.2 | 0.5 | 16.75 |
|            |        | 0.2 | 0.2 | 0.5 | 15.75 |
|            |        | 0.2 | 0.2 | 0.5 | 15.75 |
| 5          | SJ-K28 | 0.2 | 0.2 | 0.5 | 14.75 |
|            |        | 0.2 | 0.2 | 0.5 | 19.5 |
|            |        | 0.2 | 0.2 | 0.5 | 16.5 |
| 6          | YM-K33 | 0.2 | 0.2 | 0.5 | 18.5 |

* Dissolved in 2.0% peptone, 3% NaCl, 0.25% K$_2$HPO$_4$ (pH 7.2).

$^a$ In an atmosphere of 95% N$_2$ - 5% CO$_2$. 

### Note
- Peptone were equally useful, but not better, sources of nitrogen compared with peptone. The addition of beef extract, Phytone, Trypticase, gelatin, or Casamino Acids to the basic formula or the substitution of each for all or a portion of the peptone neither shortened nor lengthened \textit{V. parahaemolyticus} generation time. None bettered the maximum cell crop.

In an effort to increase growth rate or total
TABLE 4. Minimal inhibitory concentrations of inhibitors against strains of Vibrio parahaemolyticus and some competitive species

| Strain       | Genti-micin sulfate (µg/ml) | Sodium colistimethate (µg/ml) | Sodium sulfathiazole (mg/ml) | Hydroxyurea (mg/ml) | Neomycin (mg/ml) | Potassium tellurite (mg/ml) | Thallous acetate (mg/ml) | Sodium azide (mg/ml) | Penicillin (units/ml) |
|--------------|-----------------------------|------------------------------|-----------------------------|---------------------|-----------------|------------------------|------------------------|---------------------|---------------------|
| KA-K4        | >1.0                         | >10.0                        | <0.1                        | 0.2                 | 3.4             | >10                    | >10                    | >100                | 457                 | 12.8                |
| SJ-K7        | >1.0                         | >10.0                        | <0.1                        | 0.2                 | 3.3             | >10                    | >10                    | <100                | 407                 | 20                  |
| SJ-K18       | >1.0                         | >10.0                        | <0.1                        | 0.5                 | 3.5             | >10                    | >10                    | <100                | 509                 | 6.9                 |
| SJ-K28       | >1.0                         | >10.0                        | <0.1                        | 0.2                 | 4.0             | 74.3                   | >10                    | <100                | 611                 | 8.4                 |
| YM-K33       | >1.0                         | >10.0                        | 7.4                         | <0.1                | 5.6             | >10                    | >10                    | <100                | 611                 | 20                  |
| Staphylococcus aureus | 0.8 | >10.0                        | <0.1                        | >0.8                | 2.0             | >10                    | >10                    | <100                | 83                  | >1,000              | <0.5                |
| Escherichia coli | 0.8 | <1.0                         | <0.1                        | <0.1                | 1.7             | <1.0                   | <1.0                   | <10                 | 46                  | >120                | 2.25                |
| Alcaligenes faecalis | 1.0 | <1.0                         | <0.1                        | <0.1                | 0.4             | <1.0                   | <1.0                   | <10                 | 36                  | >10                 | >20                 |
| Pseudomonas aeruginosa | >1.0 | >10.0                        | >1.0                        | >0.8                | >10             | 59.0                   | 438                    | >100                | >100                | >100                | >20                 |
| Streptococcus liquefaciens | >1.0 | >10.0                        | >1.0                        | >0.8                | >100            | >1,000                 | >1,000                 | >1,000              | >1,000              | >1,000              | >5                  |
| Bacillus cereus | ND* | >10.0                        | 0.7                         | 0.6                 | 1.1             | 7.9                    | 54                     | >120                | >120                | <1,000              | <0.5                |
| Aerobacter aerogenes | 0.4 | <1.0                         | 0.8                         | 0.2                 | <1.0            | <1.0                   | <1.0                   | <10                 | >120                | <1,000              | >5--<10             |
| Salmonella senftenberg | >1.0 | <1.0                         | >1.0                        | 0.4                 | 3.5             | <1.0                   | <1.0                   | <100                | >1,000              | <1,000              | 2.8                 |
| Proteus vulgaris | >1.0 | >10.0                        | >1.0                        | >0.8                | 2.6             | <10                    | 20                     | 540                 | >100                | <1,000              | >20                 |
| Shigella flexneri | 1.0 | <1.0                         | >1.0                        | <0.1                | 3.3             | <1.0                   | 32                     | >120                | <1,000              | 12.8                |

* Base medium was 2.0% peptone, 0.2% yeast extract, 0.5% soluble starch, 3.0% NaCl, 1.5% agar (pH 8.0). Inhibitors were added aseptically from a previously sterile or filter-sterilized stock solution.

* Not done.

When penicillin was tested, however, the MIC's exhibited against four species (S. aureus, E. coli, B. cereus, and S. senftenberg) were significantly less than the mean MIC for the V. parahaemolyticus strains. For another three species (S. liquefaciens, A. aerogenes, and S. flexneri), the MIC's were of the same order of magnitude as for the Vibrio strains. Penicillin appeared to be a useful inhibitory additive for the basic isolation medium. Nevertheless, A. faecalis, P. aeruginosa, and P. vulgaris still failed to be repressed by levels of penicillin that were inhibitory to the majority of V. parahaemolyticus strains.

The recoveries of strains of V. parahaemolyticus and some competitive species from pure buffered suspensions by the basic isolation agar containing different concentrations of penicillin were compared (Table 5). Over 62% of the most sensitive Vibrio strain (YM-K33) were recovered at a concentration of 5 units of penicillin per ml.

Four competitive species yielded good recoveries at that inhibitor concentration (A. faecalis, P. aeruginosa, P. vulgaris, and S. flexneri).

The inability of the selective agents tested completely to inhibit unwanted competitors necessitated the addition of a differential indicator system. All strains of V. parahaemolyticus (14) but only an occasional strain of the four tested interfering competitor species hydrolyze starch. Therefore, partially soluble cornstarch was substituted for the soluble starch in the basic isolation medium. The rapid growth of V. parahaemolyticus on this medium permitted viable plate counting of colonies with large hydrolytic zones at 18 hr. The occasional competitive strain that hydrolyzed starch required 48 hr of incubation on the modified Vibrio isolation medium and at 18 hr did not exhibit hydrolytic zones.

The final formulation of the modified isolation medium included: peptone, 2.0%; yeast extract, 0.2%; cornstarch, 0.5%; NaCl, 3.0%; agar,
Table 5. Mean per cent recoveries of strains of Vibrio parahaemolyticus and some competitive species on medium supplemented with penicillin

| Strain          | Penicillin concentration |
|-----------------|-------------------------|
|                 | 3 units/ml (%) | 4 units/ml (%) | 5 units/ml (%) | 6 units/ml (%) |
| KA-K4           | 91             | 80             | 90             | 79             |
| SJ-K7           | 107            | 93             | 112            | 76             |
| SJ-K18          | 78             | 70             | 84             | 49             |
| SJ-K28          | 85             | 82             | 85             | 68             |
| YM-K33          | 93             | 82             | 62             | 54             |
| Staphylococcus aureus | <1         | <1             | <1             | <1             |
| Escherichia coli | <1            | <1             | <1             | <1             |
| Alcaligenes faecalis | 91       | 68             | 51             | 16             |
| Pseudomonas aeruginosa | 107     | 71             | 81             | 66             |
| Streptococcus liquefaciens | <1     | <1             | <1             | <1             |
| Bacillus cereus  | <1            | <1             | <1             | <1             |
| Aerobacter aerogenes | 14        | <1             | <1             | <1             |
| Salmonella senftenberg | 6        | 3              | 1              | <1             |
| Proteus vulgaris | 112           | 102            | 95             | 94             |
| Shigella flexneri | 94            | 89             | 94             | 67             |

* Base medium was 2.0% peptone, 0.2% yeast extract, 0.5% soluble starch, 3.0% NaCl, 1.5% agar (pH 8.0). Inhibitors were added aseptically from a previously sterile or filter-sterilized stock solution. Medium without penicillin was used as control.

Table 6. Mean per cent recoveries of strains of Vibrio parahaemolyticus on several isolation media

| Strain | Media                  | Modified isolation medium | Modified isolation + penicillin (2 units/ml) | Modified isolation + penicillin (5 units/ml) | Salt-starch-agar | Salt-starch-agar + penicillin (2 units/ml) |
|--------|------------------------|----------------------------|---------------------------------------------|---------------------------------------------|------------------|--------------------------------------------|
|        | BTB-Teepol             | Saline Fish                | Saline Fish                                 | Saline Fish                                 | Saline Fish      | Saline Fish                                |
| KA-K4  | 36a                    | 21                         | 42 46                                       | 118 77                                      | 68 34           | 28 55                                      |
| SJ-K7  | 12                     | 2                          | 1 2                                        | 52 28                                       | 48 29           | 12 61                                      |
| RS-K18 | <1                     | >1                         | <1 1                                        | 1 4                                         | <1 <1           | <1 <1                                      |
| SJ-K28 | 90                     | 74                         | 48 18                                       | 88 82                                       | 86 89           | 20 60                                      |
| M-229  | 80                     | 76                         | 65 56                                       | 89 54                                       | 99 89           | 81 76                                      |
| 9121   | 98                     | 67                         | 59 67                                       | 123 78                                      | 106 119         | 104 124                                    |
| 9187   | 117                    | 91                         | 108                                         | 107 99                                      | 103 108         | 115 109                                    |

* Values expressed as mean per cent recovery.

1.5% (pH 8.0). Penicillin was at levels of 0, 2, and 5 units/ml.

The per cent recoveries of *V. parahaemolyticus* from initial suspensions of 10^6 cells/ml in physiological saline or fish homogenate upon BTB-Teepol, TCBS, salt-starch-agar, and the modified isolation medium are compared in Table 6. In all cases, the last recovered *Vibrio* strains more efficiently. Although paired plates of salt-starch-agar were always incubated both anaerobically [as described by Baross and Liston (3, 4)] and aerobically, the latter condition invariably gave better recovery. These aerobic results are reported in Table 6. The presence of fish homog-enate adversely affected recoverability of certain strains (notably KA-K4 and SJ-K7), but this effect appeared most often with the more efficient BTB-Teepol and modified isolation agars. The addition of penicillin either to modified isolation medium or to the original salt-starch-agar formulation decreased recoverability, but the effect was more noticeable with the latter.

The sensitivity of both BTB-Teepol and modified isolation agar varied with the strain (Table 7). At an initial concentration of 10^6 cells/ml, recovery from fish homogenate varied with the strain used from 69 to 96% for BTB-Teepol and from 60 to 119% for the modified isolation.
percent recoveries of strains of *Vibrio parahaemolyticus* on BTB-Teepol and modified isolation medium

| Strain | Conc of strain (organisms/ml) | BTB-Teepol | Modified isolation medium | Modified isolation medium + 2 units of penicillin/ml |
|--------|-------------------------------|------------|---------------------------|---------------------------------------------------|
|        |                               | Saline     | Fish                      | Saline | Fish | Saline | Fish | Saline | Fish |
| 9121   | 10⁴                            | 98         | 88                        | 112    | 119  | 115    | 103  | 109    | 99   |
|        | 10³                            | 78         | 85                        | 99     | 92   | 108    | 89   | 108    | 89   |
|        | 10²                            | 90         | 82                        | 112    | 111  | 108    | 89   | 102    | 88   |
|        | 10¹                            | 94         | 109                       | 91     | 94   | 120    | 88   |         |      |
| SAK-K3 | 10⁴                            | 72         | 73                        | 90     | 75   | 84     | 70   | 101    | 77   |
|        | 10³                            | 62         | 62                        | 99     | 65   | 101    | 77   | 103    | 77   |
|        | 10²                            | 43         | 72                        | 68     | 79   | 80     | 61   | 89     | 61   |
|        | 10¹                            | 40         | ND¹                       | 86     | 36   | 89     | 23   |         |      |
| S-42-87| 10⁴                            | 79         | 96                        | 92     | 109  | 102    | 61   |         |      |
|        | 10³                            | 61         | 58                        | 65     | 53   | 53     | 28   | 101    | 32   |
|        | 10²                            | 78         | 60                        | 94     | 63   | 101    | 32   |         |      |
|        | 10¹                            | 60         | 66                        | 89     | 75   | 86     | 40   |         |      |
| 4750   | 10⁴                            | 93         | 69                        | 113    | 103  | 111    | 125  |         |      |
|        | 10³                            | 90         | 66                        | 105    | 89   | 97     | 66   |         |      |
|        | 10²                            | 104        | 72                        | 125    | 87   | 140    | 101  |         |      |
|        | 10¹                            | 77         | 81                        | 109    | 72   | 119    | 76   |         |      |
| 9187   | 10⁴                            | 73         | 70                        | 62     | 60   | 78     | 88   |         |      |
|        | 10³                            | 86         | 67                        | 98     | 58   | 98     | 77   |         |      |
|        | 10²                            | 76         | 46                        | 99     | 59   | 104    | 66   |         |      |
|        | 10¹                            | 83         | 68                        | 96     | 52   | 101    | 47   |         |      |

* Values expressed as per cent recovery.

¹ Not done.

medium. At an initial concentration of 10⁴ cells/ml, however, recovery from fish ranged from 66 to 109% for the former and from 36 to 94% for the latter.

**DISCUSSION**

The modified isolation medium offers substantial advantages to the researcher over and above its efficient recovery of *V. parahaemolyticus*. Starch hydrolysis is a characteristic exhibited by all strains of *V. parahaemolyticus* but by only a few competitive species, e.g., *Pseudomonas* species. Furthermore, hydrolytic zones exhibited by *Vibrio* are much larger than the very small zones surrounding most competitors. In addition, competitors usually require more than 18 hr of incubation to become visible. Consequently, over 80% of colonies exhibiting characteristic zones of starch hydrolysis, when suspensions of *V. parahaemolyticus* in fish homogenate were plated on experimental isolation medium, were verified. In contrast, the differential system of BTB-Teepol and TCBS agar depends upon the fermentative production of acid from sucrose. The latter characteristic is slowly positive with many strains, and the appearance of yellow colonies often depends upon the degree of crowding. Therefore, both fermenting and nonfermenting colonies must be picked. As a result, 60% or less of colonies picked from these media were verified. In short, more manipulations are required by the fermentative media than by the experimental isolation medium. The same is true for the original salt-starch-agar formulation, although the reason is not evident at present.

Flooding a plate of the modified isolation medium with Lugol's iodine solution, after a representative number of colonies has been picked for verification, markedly improves visibility and countability of *V. parahaemolyticus*. Iodine is most useful when the plate count is low and colonies are well isolated.

The excellent growth of *Vibrio* inoculated into the modified isolation medium without agar suggests that this formulation could be used with success as an enrichment broth. Further, a multiple-tube dilution series using the broth would allow quantification by the most-probable-
number procedure of \textit{V. parahaemolyticus} in seawater or seafood homogenates.

An application of the membrane filter technique for detecting \textit{V. parahaemolyticus} and \textit{V. comma} based upon the use of TCBS agar was recently described (9). In the present study, recovery of \textit{V. parahaemolyticus} based upon the use of TCBS compared unfavorably with the modified isolation medium. It is possible that substitution of the latter medium for TCBS in the membrane technique would improve recovery significantly and perhaps allow quantification.

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