Use of Colistin-Polymyxin B-Cellobiose Agar for Isolation of Vibrio vulnificus from the Environment

JAMES D. OLIVER,*, KELLY GUTHRIE, JANET PREYER, ANITA WRIGHT, LINDA M. SIMPSON, RONALD SIEBELING, and J. GLENN MORRIS, JR.

Department of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina 28223; Department of Microbiology, Louisiana State University, Baton Rouge, Louisiana 70803; and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland 21201

Received 22 July 1991/Accepted 21 November 1991

Colistin-polymyxin B-cellobiose agar was employed for the isolation of Vibrio vulnificus from shellfish. Isolates were examined phenotypically and with a gene probe and monoclonal antibody specific for V. vulnificus. Results indicated that colistin-polymyxin B-cellobiose agar is superior to both sodium dodecyl sulfate-polymyxin B-sucrose and thiosulfate-citrate-bile salts-sucrose agar in its ability to select and differentiate this species from background vibrios.

Vibrio vulnificus produces human infections which materialize as either septicemia or cellulitis (for a recent review, see reference 11). Persons developing septicemia generally exhibit chronic underlying disorders and consistently consume the consumption of raw seafood, especially oysters, as the source of infection. This condition carries with it a high fatality rate of approximately 60%. Alternately, the bacterium can enter through a skin lesion incurred prior to or during exposure to seawater or shellfish. The fatality rate for wound infections is approximately 20%, with surgical debridement and/or amputation of the infected area often required.

Studies on the distribution of this organism in the environment have shown it to be ubiquitous (5-7, 12-14, 16, 17), with the highest numbers in shellfish. The identification of the organism from environmental sources, however, has proved difficult (12, 13). Many of the phenotypic traits are variable (4), and the media most often employed have proved generally inadequate for either its isolation or differentiation from other vibrios (1-3, 8, 18). Recently, a new medium for the isolation and differentiation of V. vulnificus was developed and subjected to laboratory testing by Masad and Oliver (9). Cellobiose-polymyxin B-colistin (CPC) agar proved very successful for this purpose. The present study was designed to test the usefulness of CPC agar for the isolation of V. vulnificus from environmental samples.

As molluscan shellfish appear to be the main source of infection, oysters and clams were taken from the coastal waters of North Carolina during August of 1988. Within 2 h, the shellfish were homogenized in artificial seawater or swabbed directly and plated onto CPC agar. Two additional vibrio-selective and -differential media, thiosulfate-citrate-bile salts-sucrose (TCBS; Difco Laboratories) and sodium dodecyl sulfate-polymyxin B-sucrose (SPS) agar (7) were also employed. CPC agar was incubated at 40°C overnight, whereas TCBS agar and SPS agar were incubated at the ambient temperature (ca. 22°C). Presumptive V. vulnificus isolates were selected for further analysis and maintained on heart infusion agar. These included 72 cellobiose-positive colonies from CPC, 36 sucrose-negative colonies from TCBS, and 13 halo-positive colonies from SPS.

Colonies were transferred to nitrocellulose filter paper and hybridized with a V. vulnificus hemolysin gene probe as previously described (10). In addition, each isolate was tested by slide agglutination employing latex beads armed with antiflagellar core monoclonal antibody (15). Results of these studies suggest that a greater percentage of cellobiose-positive isolates from CPC agar could be identified as V. vulnificus than could sucrose-negative or halo-positive colonies from TCBS or SPS, respectively (Table 1).

To compare identification by traditional taxonomic methods with results of the gene probe and monoclonal antibody analyses, 33 of the cellobiose-positive isolates were also subjected to a battery of biochemical, morphological, and physiological tests used to speciate the pathogenic vibrios (11, 13). Results were submitted to numerical taxonomic analysis with the program TAXAN-6, by using the Jaccard coefficient and unweighted average linkage clustering.

On the basis of the phenotypic analysis, 29 (88%) of the 33 isolates fell into three main clusters (Table 2). Cluster II contained 10 strains identified phenotypically as V. vulnificus, along with two reference V. vulnificus strains. Cluster I (15 strains) was taxonomically similar to the gastrointestinal pathogen V. fluvialis, while cluster III (4 isolates) showed phenotypic similarity to the nonpathogenic V. harveyi. Of the four nonclustering isolates, one was identified by both the gene probe and H agglutination as a strain of V. vulnificus; this isolate was subsequently determined to be a lactose-negative strain of the species.

Taxonomic data were compared with the results of the gene probe and anti-H latex agglutination (Table 2). Seven (70%) of the 10 isolates identified phenotypically as V. vulnificus were probe positive, and eight (80%) were agglutinated. The single lactose-negative V. vulnificus isolate which clustered independently from the other 10 strains was detected by both the gene probe and H agglutination. All but one of the probe-positive cluster II strains was agglutinated, while two of the antibody-positive strains were probe negative. None of the remaining 22 cellobiose-positive strains studied was probe positive. In contrast, 13 of the remaining 22 isolates agglutinated in the anti-H latex reagent, but 11 of these were in cluster I.

SPS agar was originally formulated to select for V. vulnificus and V. cholerae and to differentiate these two species from heterologous vibrios by their production of alkaline

* Corresponding author.
sulfatase (7). On the basis of our study, SPS agar lacks selectivity for *V. vulnificus*, since none of the 13 sulfatase-positive isolates was identified as this species. TCBS agar proved a better medium for the isolation of *V. vulnificus*; of the sucrose-negative colonies tested, 11 and 24% were identified as *V. vulnificus* by the hemolysin gene probe and flagellar antibody, respectively. Unfortunately, TCBS agar does not permit differentiation of *V. vulnificus* from other sucrose-negative vibrios.

In contrast, CPC agar was clearly superior to TCBS agar in selecting for *V. vulnificus*. Of the 72 cellulose-positive colonies retrieved from CPC agar, 19 and 47% were identified as *V. vulnificus* by the gene probe and anti-H latex agglutination, respectively. Of the 33 cellulose-positive colonies taken from CPC agar for taxonomic analysis, 10 (30%) were identified as *V. vulnificus* while 24 to 27% were so identified by the gene probe and H agglutination.

The correlation between the probe and H agglutination in identifying the *V. vulnificus* isolates was quite good, and in one case both methods detected a lactose-negative strain of *V. vulnificus* not identified through classical taxonomic analysis. The phenotypic study suggested that the anti-H latex reagent may agglutinate *V. fluvialis* isolates (Table 1, cluster I). However, such false-positive results may be more desirable than false-negative results when screening for *V. vulnificus*. In addition, cluster I isolates were all sucrose and arginine dihydrolase positive and thus could be easily differentiated from *V. vulnificus*. Probing for the hemolysin gene may be more specific, although probing may not be suitable for routine screening as it involves the use of radioactively labeled materials. An alkaline phosphatase-labeled probe has now been developed in our laboratories, however, and is currently being field tested.

### TABLE 1. Selectivity of *V. vulnificus* by CPC, TCBS, and SPS media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Colony type selected (no. of colonies)</th>
<th>% Colonies positive by:</th>
<th>Probe</th>
<th>Monoclonal antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPC</td>
<td>Cellobose* (72)</td>
<td>19</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>TCBS</td>
<td>Sucrose* (36)</td>
<td>11</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>SPS</td>
<td>Halo* (13)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Results of the present study demonstrate the value of CPC agar in the isolation of *V. vulnificus* from clams and oysters. Its use, along with that of the hemolysin gene probe or anti-flagellar core monoclonal antibody for identification, may prove to be superior for the study of this important human pathogen in the natural environment.

We thank Remel Co. for providing the CPC agar employed during this study and Rita Colwell for providing TAXAN-6.

### REFERENCES


