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Research Note

Selectivity and Specificity of a Chromogenic Medium for Detecting *Vibrio parahaemolyticus*†

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ABSTRACT

The thiosulfate–citrate–bile salts–sucrose agar (TCBS) used in the most-probable-number method for detecting *Vibrio parahaemolyticus* cannot differentiate growth of *V. parahaemolyticus* from *Vibrio vulnificus* or *Vibrio mimicus.* This study examined the selectivity and specificity of Bio-Chrome Vibrio medium (BCVM), a chromogenic medium that detects *V. parahaemolyticus* on the basis of the formation of distinct purple colonies on the medium. A panel consisting of 221 strains of bacteria, including 179 *Vibrio* spp. and 42 non-*Vibrio* spp., were examined for their ability to grow and produce colored colonies on BCVM. Growth of *Salmonella, Shigella, Escherichia coli, Enterobacter cloacae, Yersinia enterocolitica,* and *Aeromonas* was inhibited by both BCVM and TCBS. All 148 strains of *V. parahaemolyticus* grew on BCVM, and 145 of them produced purple colonies. The remaining 31 *Vibrio* spp., except one strain of *Vibrio fluvialis,* were either unable to grow or produced blue-green or white colonies on BCVM. Bio-Chrome Vibrio medium was capable of differentiating *V. parahaemolyticus* from other species, including *V. vulnificus* and *V. mimicus.* Further studies are needed to evaluate the sensitivity and specificity of BCVM for detecting *V. parahaemolyticus* in foods.

Contaminated seafood causes about 113,000 cases of food poisoning each year in the United States *(10).* Among these cases, raw shellfish accounts for more than 90% of seafood poisoning cases. *Vibrio parahaemolyticus* is a human pathogen that occurs naturally in estuary and marine environments *(4, 7).* This organism is frequently found in molluscan shellfish and is the main causative agent of acute gastroenteritis associated with raw oyster consumption *(6, 11, 13).* The infection can also cause life-threatening septicemia in persons having underlying medical conditions such as liver disease or immune disorders. *V. parahaemolyticus* has been isolated from a variety of seafood, including codfish, sardine, mackerel, and octopus, and is recognized as an important seafoodborne pathogen throughout the world *(3, 6, 8, 9).*

The occurrence of *V. parahaemolyticus* in seafood, especially in shellfish, is an important public health concern. It is estimated that 20 million Americans consume raw shellfish, making raw shellfish the biggest seafood hazard in the United States *(12).* Four major outbreaks of *V. parahaemolyticus* infections involving more than 700 cases of illness associated with raw oyster consumption occurred between 1997 and 1998 in the United States *(1, 2).* Effective postharvest processes for eliminating *V. parahaemolyticus* in contaminated oysters, as well as a more specific method for detecting this organism, are needed to minimize the health hazard associated with raw oyster consumption.

One of the commonly used methods for detecting *V. parahaemolyticus* in food is the most-probable-number (MPN) procedure using thiosulfate–citrate–bile salts–sucrose agar (TCBS) *(14).* However, a major problem with the MPN method is that TCBS cannot differentiate *V. parahaemolyticus* from some *Vibrio* species, including some strains of *Vibrio vulnificus* or *Vibrio mimicus.* Both *V. vulnificus* and *V. mimicus* can grow and appear as green or blue-green colonies similar to those produced by *V. parahaemolyticus* on TCBS. Consequently, several presumptive positive colonies formed on a TCBS plate need to be confirmed as *V. parahaemolyticus* by biochemical tests. The confirmation procedure is very labor-intensive and time-consuming. Therefore, final results might not be available for 5 to 8 days.

Recently, a commercial chromogenic medium (Bio-Chrome Vibrio medium, BioMedix, Pomona, Calif.) was developed for detecting *V. parahaemolyticus.* The medium contains a chromogenic substrate that is specific for *V. parahaemolyticus* and detects the organism on the basis of the formation of distinct purple colonies on growth. Growth of other *Vibrio* species, such as *V. vulnificus, V. mimicus,* and *V. cholerae,* on the medium is differentiated by the formation of blue-green colonies. Although this newly developed chromogenic medium apparently provides an opportunity to reduce labor and time involved in *V. parahae-*

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TABLE 1. *Non*-Vibrio *bacteria used to examine the selectivity of Bio-Chrome Vibrio medium for detecting* V. parahaemolyticus

Bacterial culture[a]	Strain
Salmonella Choleraesuis	ATCC 14028
Salmonella Lansing	SEA 2571
Salmonella Arizonae	SEA 2587
Salmonella Havana	SEA 2589
Salmonella Enteritidis	SEA 2595
Salmonella Oranienburg	SEA 2570
Salmonella Stanley	SEA 2577
Salmonella Houten	SEA 2586, SEA 2594
Salmonella Meleagridis	SEA 2588
Shigella sonnei	ATCC 25931, SEA 6650
S. flexneri	SEA 6651, SEA 6652, SEA 6653
Enterobacter cloacae	ATCC 23355
Aeromonas hydrophila	ATCC 7965, SPRC 536, SPRC 5145, SPRC 5180, SPRC 5183, SPRC 5231
A. veronii	SPRC 511, SPRC 5120
A. sobria	SPRC 5271
A. caviae	SPRC 5378
Escherichia coli	ATCC 25922, SEA 621, SEA 625, SEA 627, SEA 628, SEA 6211, SEA 6216, SEA 6223, SEA 6227, SEA 6313
Yersinia enterocolitica	SEA 122, SEA 1230, SEA 1267, SEA 1268, SEA 1269, SEA 1271

[a] All strains were from the culture collection of Pacific Regional Laboratory Northwest of the U.S. Food and Drug Administration (Bothell, Wash.).

molyticus detection, its application in routine analyses for detecting *V. parahaemolyticus* needs to be investigated. This study examined the selectivity and specificity of this chromogenic medium for detecting *V. parahaemolyticus.*

MATERIALS AND METHODS

Bacterial cultures. Forty-two strains of non-*Vibrio* bacteria (Table 1), including *Salmonella* (Choleraesuis, Lansing, Arizonae, Havana, Enteritidis, Oranienburg, Stanley, Houten, and Meleagridis), *Shigella* (*sonnei* and *flexneri*), *Aeromonas* (*hydrophila, veronii, sobria,* and *caviae*), *Enterobacter cloacae, Escherichia coli, and Yersinia enterocolitica,* and 179 strains of *Vibrio* species (*parahaemolyticus, vulnificus, cholerae, mimicus, hollisae, alginolyticus, fluvialis,* and *furnissii*) were used in the study. All cultures, except for 97 strains of *V. parahaemolyticus* and 3 strains of *V. cholerae* acquired from the Canadian Food Inspection Agency Burnaby Laboratory (Burnaby, British Columbia), were the collection of the U.S. Food and Drug Administration Pacific Regional Laboratory Northwest (Bothell, Wash.).

Selectivity and specificity of Bio-Chrome Vibrio medium for *V. parahaemolyticus* detection. Strains of non-*Vibrio* spp. were individually enriched twice in tryptic soy broth (TSB; Difco Laboratories, Becton Dickinson, Sparks, Md.) at 37°C overnight. Each of the enriched cultures was streaked onto both Bio-Chrome Vibrio medium (BCVM; BioMedix) and TCBS (Difco, Becton Dickinson) plates to test for its ability to grow on either medium. Strains of *Vibrio* spp. were used to examine the specificity of BCVM for *V. parahaemolyticus* detection. Each *Vibrio* strain was enriched twice overnight at 37°C in TSB supplemented with 1.5% NaCl. The enriched cultures were streaked onto BCVM and TCBS plates. All plates were incubated at 35 to 37°C for 18 to 24 h and examined for growth and formation of colored colonies.

TABLE 2. *Specificity of Bio-Chrome Vibrio medium (BCVM) and thiosulfate–citrate–bile salts–sucrose agar (TCBS) for detecting* V. parahaemolyticus

Vibrio spp.[a]	No. of strains	BCVM	TCBS
parahaemolyticus	145	Purple	Green
	3	White	Green
vulnificus	7	Blue-green	Green
	2	No growth	Green
	1	Blue-green	Yellow
mimicus	2	Blue-green	Green
cholerae	13	Blue-green	Yellow
alginolyticus	2	White	Yellow
furnissii	1	White	Yellow
hollisae	2	No growth	No growth
fluvialis	1	Purple	Yellow

[a] All strains, except 97 *V. parahaemolyticus* and 3 *V. cholerae* acquired from the Canadian Food Inspection Agency Burnaby Laboratory (Burnaby, British Columbia), were from the culture collection of Pacific Regional Laboratory Northwest of the U.S. Food and Drug Administration (Bothell, Wash.).

RESULTS

Selectivity of BCVM and TCBS for *V. parahaemolyticus* detection. Growth of *Salmonella, Shigella, Enterobacter, Aeromonas, E. coli,* and *Yersinia* were completely inhibited by TCBS (data not shown). Similar results were obtained with BCVM. None of the strains tested, except for one strain of *A. hydrophila,* were able to grow on BCVM. Growth of the *A. hydrophila* on BCVM resulted in formation of white colonies, which could easily be distinguished from the purple colonies formed by *V. parahaemolyticus.* These results indicated that selectivity of BCVM for *Vibrio* spp. was identical to that of TCBS. Both media contain selective agents that could inhibit growth of many gram-negative non-*Vibrio* bacteria.

Specificity of BCVM for *V. parahaemolyticus* detection. The specificity of BCVM for detecting *V. parahaemolyticus* was examined with 179 strains of *Vibrio* spp., including 148 *V. parahaemolyticus.* Growth of these bacteria and their abilities to form colored colonies on TCBS and BCVM plates are reported in Table 2. All 148 strains of *V. parahaemolyticus* grew on BCVM, and 145 of them produced purple colonies. No purple colonies were produced on BCVM by the remaining 31 *Vibrio* spp., except one strain of *V. fluvialis.* Growth of *V. cholerae, V. mimicus,* and *V. vulnificus* on BCVM resulted in the formation of blue-green colonies that were easily distinguishable from the growth of *V. parahaemolyticus.* Other *Vibrio* spp., including *V. alginolyticus, V. furnissii,* and *V. hollisae,* were either unable to grow or produced white colonies on BCVM.

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DISCUSSION

The selectivity of BCVM for detecting *Vibrio* spp. was comparable to that of TCBS. Both media exhibited strong inhibitory effects against growth of many bacteria but allowed the growth of *Vibrio* spp. (except *V. hollisae*). However, BCVM was more specific than TCBS for *V. parahaemolyticus* detection. Growth of *V. vulnificus* and *V. mimicus* on TCBS that could not be distinguished from growth of *V. parahaemolyticus* could easily be identified on a BCVM plate by the formation of blue-green colonies by *V. vulnificus* and *V. mimicus.*

The chromogenic substrate contained in BCVM allowing *V. parahaemolyticus* to produce distinct purple colonies seemed to be a unique characteristic of this medium. Our study found that 98% of the 148 *V. parahaemolyticus* strains tested were able to produce purple colonies on BCVM. These results are similar to a study reported by Hara-Kudo et al. *(5)*, who tested a similar chromogenic medium (CHROMagar Vibrio agar) for detecting *V. parahaemolyticus.* Among the 96 stains of various types of bacteria tested in that study, all 68 strains of *V. parahaemolyticus* formed purple colonies, whereas *V. mimicus* and *V. vulnificus* produced green colonies on the agar plate. However, it is not known whether BCVM and CHROMagar Vibrio agar both contain the same type of substrate for *V. parahaemolyticus.*

The formation of white colonies by a very small portion (2%) of *V. parahaemolyticus* and purple colonies by one strain of *V. fluvialis* on BCVM indicated that BCVM was not a perfect medium for *V. parahaemolyticus* detection. However, BCVM offers an advantage over TCBS for *V. parahaemolyticus* detection by being able to distinguish growth of *V. vulnificus* and *V. mimicus.* It is not clear whether all strains of *V. fluvialis* will produce purple colonies on BCVM until more strains are tested. On the basis of the limited information obtained from this study, TCBS appears to be the medium of choice for *V. parahaemolyticus* detection if large numbers of *V. fluvialis* are expected in samples. On the contrary, BCVM will work better than TCBS for *V. parahaemolyticus* detection when *V. vulnificus* or *V. mimicus* is a concern.

BCVM was more specific than TCBS for *V. parahaemolyticus* detection. It could differentiate growth of *V. parahaemolyticus* from that of *V. vulnificus* and *V. mimicus.* This chromogenic medium could be used to reduce time and labor involved in the MPN method for *V. parahaemolyticus* detection by reducing numbers of biochemical confirmation tests. Further studies are needed to determine the sensitivity and specificity of BCVM for detecting *V. parahaemolyticus* in foods.

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