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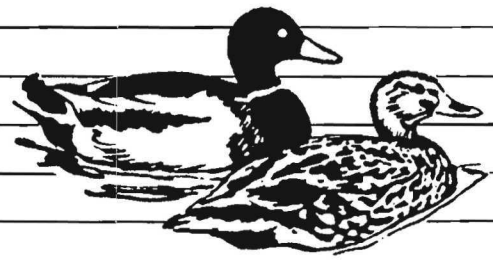

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An Alternative Bacteriological Medium for Isolation of *Aeromonas*

Aeromonas species have been associated with infections in cold- and warm-blooded animals, including humans. *Aeromonas* species are gram-negative, facultatively anaerobic, typically oxidase positive, ubiquitous aquatic bacteria. During the past decade, motile aeromonads have been the third-most prevalent bacterial pathogen of cultured channel catfish (*Ictalurus punctatus*). Infections caused by *Aeromonas* in humans are attributed to the motile aeromonads, which are divided into three general phenotypic groups equivalent to the species *A. hydrophila*, *A. sobria*, and *A. caviae*.

Because most strains of *Aeromonas* grow on media selective for lactose fermenters, they may be overlooked as commensals in mixed populations of heterotrophs. However, several media have been developed for detecting aeromonads from fishes, humans, and the environment. In view of the potential losses to the commercial- and sportfishing industries and the serious implications for human health, definitive isolation of *Aeromonas* may be of value to developing therapeutic strategies. This study served to compare the efficacy of two solid media for the selection of *Aeromonas* species.

Starch–Glutamate–Ampicillin–Penicillin (SGAP-10C) Was Compared With Rimler–Shotts (RS) Medium

The starch–glutamate–ampicillin–penicillin (SGAP-10C) medium, initially developed for the recovery of aeromonads from water, was compared with Rimler–Shotts (RS) medium, developed as a medium with differential potential for the rapid isolation and presumptive identification of *A. hydrophila* from fishes (Tables 1 and 2). To improve selectivity for aeromonads, the base of SGAP-10C had been modified by the addition of ampicillin (20 mg/L) to reduce the numbers of pseudomonads, and the addition of 10 µg/L of C from glucose to permit recuperation of stressed aeromonads. The ingredients of RS were compounded to achieve a maximal acidic (maltose fermentation) or basic reaction (decarboxylation of lysine or ornithine, or both). Many fish diagnostic laboratories use RS medium.

The selective and differential capacities of the media were assessed by occurrence, phenotype, and

growth of 99 isolates representing 15 genera of bacteria. Strains of bacteria were obtained from kidney, brain, and liver tissues of infected channel catfish, from the American Type Culture Collection (Rockville, Maryland), and from laboratory stocks. Bacteria were maintained at 4°C on tryptic soy agar (TSA) before use.

Each organism was subcultured onto blood agar plates (TSA with 5% sheep's blood) for 24 h at 4°C before identification of standard morphological and biochemical characteristics with tubed media as well as a rapid identification system (Minitek Differentiation System, Beckton Dickinson, Cockeysville, Maryland). Each isolate was grown on TSA at 25°C for 24 h before streaking onto the test media. After incubation of the plates at 25°C for 24 h for RS and 48 h for SGAP-10C, bacterial growth, colonial morphology, and differential characteristics were noted.

Recovery Rates of *Aeromonas* spp. on Both Media Tested Were Similarly High

The recovery rate was 97% with RS and 95% with SGAP-10C. With the other genera, the inhibition rates were 20% on RS and 80% on SGAP-10C (Table 3). Growth of *Aeromonas* spp. was heavy on both media incubated at 24°C. *Aeromonas* colonies were larger on SGAP-10C than on RS. Heavy growth appeared on SGAP-10C at 48 h and on RS at 24 h. At 37°C, growth was weak on SGAP-10C but heavy on RS. On both media, colonies were round, smooth, and raised and had entire edges.

Three primary colony types of *Aeromonas* were observed on RS and on SGAP-10C. On RS, colony color was yellow, greenish-yellow, or green with black centers, and the media was sometimes hydrolyzed or turned opaque. On SGAP-10C, colonies generally appeared buff, the media was hydrolyzed or turned opaque or pink, and the surrounding media turned from the original orange to dark red. *Pseudomonas* spp. growth was pink on SGAP-10C, and the media turned pink. On RS, more than 60% of the isolates were greenish-yellow, and 100% of these were pink with red media on SGAP-10C.

Our results showed that the rate with which RS inhibited gram-negative bacteria was 54% less than the rate of SGAP (Table 3), presumably because of the lack of inhibitors of gram-negative species. The SGAP-10C medium is more selective than media that contain yeast extract or peptone as the carbon sources (e.g., RS). Rimler–Shotts medium supported the growth of *Plesiomonas*, *Pseudomonas*, and *Edwardsiella*, sometimes found in the organs of infected fishes. The lack of NaCl in SGAP-10C inhibits the growth of halophilic vibrio.

Abundant growth of *Aeromonas* on SGAP-10C occurs at 48 h after inoculation, and initial cultures will probably be pure. Good growth occurs on RS at 24 h, but additional streaking may be needed for isolation. Therefore, for isolation of *Aeromonas* colonies with either media, the process takes 48 h.

SGAP-10C Medium Offers an Additional Choice for the Laboratory Selective Recovery of *Aeromonas* spp.

Motile aeromonads frequently occur with other bacteria. This co-occurrence confuses the etiological significance in the disease process. Selective media facilitate isolation of bacteria in pure culture and expedite drug-susceptibility testing and disease management. With the recognition of the increasing incidence of *Aeromonas* spp. as pathogens, additional choices of selective or differential media for aeromonads are welcome.

The SGAP-10C medium seems to be an efficient selective medium for *Aeromonas*. Because densities of *A. hydrophila* in water have correlated with the prevalence of infected fishes, and SGAP-10C is effective at recovering bacteria from aquatic sources, membrane filtration of pond water onto SGAP-10C could be a predictor of high numbers of *Aeromonas* species. The use of SGAP-10C at 24°C and 48 h may offer an additional choice of a selective medium for the laboratory recovery of *Aeromonas* species in fishes and food, and in environmental, veterinary, and clinical microbiology.

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Table 1. Composition of SGAP-10C (starch–glutamate–ampicillin–penicillin) medium. After the glucose and antibiotics were filtered with a 0.22-μm filter, they were added to the heat-sterilized and cooled agar mixture.

Ingredients	Quantity
L(+) sodium glutamate	10.0 g
Soluble starch	20.0 g
KH_2PO_4	2.0 g
$MgSO_4$	0.5 g
Phenol red	0.36 g
Agar	15.0 g
Sodium G penicillin	1,000 IU
Ampicillin	0.02 g
D-glucose	25 μg
Water	1,000 mL

Table 2. Composition of Rimler–Shotts medium. Components were dissolved by stirring, pH adjusted to 7.0, and boiled for 1 minute.

Components	Quantity
L-lysine-hydrochloride	5.0 g
L-ornithine-hydrochloride	6.5 g
Maltose	3.5 g
Sodium thiosulfate	6.8 g
L-cysteine-hydrochloride	0.3 g
Bromothymol blue	0.03 g
Ferric ammonium citrate	0.8 g
Sodium deoxycholate	1.0 g
Novobiocin	0.005 g
Yeast extract	3.0 g
Sodium chloride	.0 g
Agar	13.5 g
Water (q/s)	1,000 mL

Table 3. The ability of selected bacteria to grow on Rimler–Shotts (RS) and starch–glutamate–ampicillin–penicillin (SGAP-10C) media.

Bacterial species	Strains tested	Growth[a] (% and no.) of strains on RS	Growth[a] (% and no.) of strains on SGAP-10C
Aeromonas hydrophila	16	100 (16)	94 (15)
A. sobria	33	100 (33)	100 (33)
A. caviae	3	100 (3)	67 (2)
Aeromonas ATCC strains	10	80 (8)	90 (9)
Edwardsiella ictaluri	7	100 (7)	0 (0)
E. tarda	2	100 (2)	0 (0)
Pseudomonas spp.	6	100 (6)	50 (3)
Plesiomonas shigelloides	4	75 (3)	0 (0)
Acinetobacter sp.	1	100 (1)	0 (0)
Klebsiella spp.	2	100 (2)	100 (2)
Proteus vulgaris	1	100 (1)	100 (1)
Citrobacter freundii	1	100 (1)	0 (0)
Pantoea agglomerans	1	0 (0)	0 (0)
Staphylococcus spp.	2	0 (0)	0 (0)
Escherichia coli	1	100 (1)	0 (0)
Mycobacterium spp.	2	0 (0)	0 (0)
Bacillus spp.	5	60 (3)	0 (0)
Micrococcus luteus	1	100 (1)	0 (0)
Neisseria subflava	1	100 (1)	0 (0)

[a]Bacteria were incubated at 24°C for 24 h on RS and for 48 h on SGAP-10C. First number is percent; *n* is in parentheses.