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An Alternative Bacteriological Medium for the Isolation of *Aeromonas* spp.

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ABSTRACT: Two solid bacteriologic media were compared for cultivating *Aeromonas* spp. from piscine sources: the Rimler-Shotts (RS) medium and a starch-glutamate-ampicillin-penicillin-based medium (SGAP-10C) used for the recovery of *Aeromonas* spp. from water samples. The selective and differential capacities of the media were assessed March through October 1992 by recovery rate and phenotype of 99 isolates representing 15 genera of bacteria. Recovery frequency of *Aeromonas* spp. ($n = 62$) was similar at 97% on RS and 95% on SGAP-10C. The SGAP-10C medium proved to be more specific than RS toward *Aeromonas* species ($P \leq 0.005$). Use of SGAP-10C at 24 C for 48 hr offers a better choice for the laboratory recovery of *Aeromonas* spp. from clinical fish specimens.

Key words: *Aeromonas* spp., *Ictalurus punctatus*, SGAP-10C, Rimler-Shotts medium, diagnostic, medium, selective, disease.

Aeromonas spp. are Gram-negative, facultatively anaerobic, typically oxidase positive, ubiquitous aquatic bacteria (Hazen, 1983). *Aeromonas* spp. have been associated with infections in cold- and warm-blooded animals and in humans (Khardori and Fainstein, 1988). During the past decade, motile aeromonads have been the third-most prevalent bacterial pathogen of cultured channel catfish (*Ictalurus punctatus*) (Durborow et al., 1991). Infections caused by *Aeromonas* spp. in humans generally are attributed to the motile aeromonads as a group, and clinical laboratories generally do not speciate them (Khardori and Fainstein, 1988).

Aeromonas spp. share some properties with *Plesiomonas* spp. and *Vibrio* spp. of its family Vibrionaceae, and with members of the family of Enterobacteriaceae. The motile aeromonads comprise 14 DNA hybridization groups and currently are divided into three general phenotypic groups equivalent to the species *A. hydrophila*, *A. sobria*, and *A. caviae* (Popoff, 1984).

Because most strains of *Aeromonas* spp. grow on media selective for lactose fermenters, they may be overlooked as commensals in mixed populations of heterotrophs. Media have been developed for detecting aeromonads from fishes (Shotts and Rimler, 1973; McCoy and Pilcher, 1974), humans (von Graevenitz and Bucher, 1983), and the environment (Huguet and Ribas, 1991). In view of the potential losses to the commercial and sport fishing industries and the serious implications for human health, definitive isolation and identification of *Aeromonas* spp. is necessary to develop therapeutic strategies. Our objective was to compare the efficacy of two solid media for the selection of *Aeromonas* spp. A medium for recovery of aeromonads from water, starch-glutamate-ampicillin-penicillin (SGAP-10C) (Huguet and Ribas, 1991), was compared with Rimler-Shotts (RS) medium (Shotts and Rimler, 1973), commonly used in fish diagnostic laboratories for the cultivation of *Aeromonas* spp.

The SGAP-10C medium (Huguet and Ribas, 1991) was initially developed for the recovery of aeromonads in preference to pseudomonads, and Rimler-Shotts medium (Shotts and Rimler, 1973) was developed for the presumptive identification of *A. hydrophila* from fishes. All chemicals used in this study (March through October of 1992) to make the media were obtained from Sigma Chemical Company, St. Louis, Missouri (USA).

Bacteria from kidney, brain, and liver tissues of channel catfish (Amos, 1985) were provided by the Alabama Fish Farming Center, Greensboro, Alabama (USA). Strains of *Aeromonas* spp. from the American Type Culture Collection (Rockville, Maryland, USA) included *A. hydrophila*

ATCC 7966 and 14715, *A. veroni* ATCC 35624, *A. caviae* ATCC 15468, *A. schubertii* ATCC 43700, *A. jandaei* ATCC 49568 and ATCC 49572, *A. trota* ATCC 49657 and ATCC 49661, *A. veroni-sobria* ATCC 9071, *A. sobria* ATCC 43979, CDC Group 77B ATCC 35941, CDC Group 501 ATCC Group 43946, and *A. eucrenophila* ATCC 23309. Strains from other genera (Table 1) were obtained from laboratory stocks. Bacteria were maintained at 4 C on tryptic soy agar (TSA) (Difco, Detroit, Michigan, USA) before use.

Each organism was subcultured onto blood agar plates (TSA with 5% sheep's blood) (Carolina Biological Supply Company, Burlington, North Carolina, USA) for 24 hr at 4 C before identification of standard morphological and biochemical characteristics with media in tubes and a rapid identification system (Minitek Differentiation System, Beckton Dickinson, Cockeysville, Maryland). Species identification was made according to Popoff (1984), except for incubation at 25 C rather than 30 C. Supplemental primary tests (Table 2) also were run by the methods of Janda et al. (1984).

Each isolate was grown on TSA at 24 C for 24 hr prior to streaking onto the test media. After incubation of the plates at 24 C for 24 hr for RS and 48 hr for SGAP-10C, bacterial growth, colonial morphology, and differential characteristics were noted. A chi-square analysis of independence (Zar, 1984) was performed on frequency of recovery and inhibition.

The recovery frequency of *Aeromonas* spp. on both media tested was similarly high, with 97% with RS, and 95% with SGAP-10C (Table 1). The inhibition rates of other genera tested were 20% on RS (one minus 12 of 15 genera) and 80% on SGAP-10C (one minus three of 15 genera), where chi-square (df = 1) = 8.53, $P \leq 0.005$ (Table 1).

Growth of *Aeromonas* spp. was greatest on both media when they were incubated at 24 C. *Aeromonas* spp. colonies were larger on SGAP-10C (approximately 3 mm) than on RS (approximately 1.5 mm). At 37 C, growth of *Aeromonas* spp. was heavier on RS than on SGAP-10C as judged by the larger colony widths on RS and the smaller colonies and occasional absence of growth on SGAP-10C. Because maximal growth appeared on SGAP-10C at 48 hr and on RS at 24 hr, observations were made after those incubation periods. On both media, colonies were round, smooth, and raised, and had entire edges.

Three primary colony types of *Aeromonas* spp. were observed on RS and on SGAP-10C. On RS, colony color was yellow, greenish-yellow, or green with black centers, and the media sometimes were hydrolyzed or turned opaque. On SGAP-10C, the colonies were buff-colored, yellow, or pink and the surrounding media sometimes hydrolyzed, became opaque, or changed from the original orange to dark red. On RS, 65% of the *Aeromonas* spp. appeared greenish-yellow which is evidence for the presence of decarboxylases

TABLE 1. The ability of selected bacteria to grow on Rimler Shotts (RS) and SGAP-10C media.[a]

Bacterial species	Number of strains tested	Number of strains which grew on	
		RS	SGAP-10C
Aeromonas hydrophila	16	16	15
A. sobria	33	33	33
A. caviae	3	3	2
Aeromonas ATCC strains	10	8	9
Edwardsiella ictaluri	7	7	0
E. tarda	2	2	0
Pseudomonas spp.	6	6	3
Plesiomonas shigelloides	4	3	0
Acinetobacter sp.	1	1	0
Klebsiella spp.	2	2	2
Proteus vulgaris	1	1	1
Citrobacter freundii	1	1	0
Pantoea agglomerans	1	0	0
Staphylococcus spp.	2	0	0
Escherichia coli	1	1	0
Mycobacterium spp.	2	0	0
Bacillus spp.	5	3	0
Micrococcus luteus	1	1	0
Neisseria subflava	1	1	0

[a] Bacteria were incubated at 24 C for 24 hr on RS and for 48 hr on SGAP-10C.

TABLE 2. Primary biochemical characteristics for the identification of *Aeromonas* spp.

Bacterium	Esculin hydrolysis	Gas from glucose	Voges-Proskauer	Salicin fermentation	Arginine dihydrolase
Aeromonas hydrophila	+	+	+	+	+
A. sobria	−	+	v[a]	−	−
A. caviae	+	−	−	+	+

[a] v, variable.

(Shotts and Rimler, 1973). On SGAP-10C, all *Aeromonas* spp. colonies were pink and the surrounding media turned to a red color, evidence for a basic reaction. On SGAP-10C, colonies of *Pseudomonas* spp. were the color of fuchsia as was the surrounding medium.

The SGAP-10C medium is a defined medium that is based on glutamate-starch-penicillin medium (GSP), selective for *Pseudomonas* spp. and *Aeromonas* spp. (Kielwein, 1969). This medium is more selective than media containing yeast extract or peptone as the carbon sources. The lack of NaCl inhibits the growth of halophilic vibrio. To improve selectivity for aeromonads, GSP was modified by the addition of ampicillin (20 mg/l) to reduce the pseudomonad numbers, and the addition of 10 μg/l of C (25 μg) from glucose to permit recuperation of stressed aeromonads (Huguet and Ribas, 1991).

Rimler-Shotts medium was developed for the rapid isolation and presumptive identification of *A. hydrophila* (Shotts and Rimler, 1973). The ingredients were compounded to achieve a maximal acidic (maltose fermentation) or basic reaction (decarboxylation of lysine or ornithine). Many fish diagnostic laboratories use RS. Based on our results, the frequency with which RS inhibited bacteria other than *Aeromonas* spp. was 20% and the frequency on SGAP-10C was 80% (Table 1); this was a difference of 60%, and significant ($P \leq 0.005$). We believe this lower probability of inhibition by RS was due to the low level of inhibitors of Gram-negative species. Rimler-Shotts medium supported the growth of *Plesiomonas* spp., *Pseudomonas* spp., and *Edwardsiella* spp. All of these can be found in the organs of infected fishes (Durborow et al., 1991).

Other bacteria frequently are isolated from fish with motile aeromonads. This cooccurrence 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 (Janda et al., 1984), additional choices of selective or differential media for aeromonads are needed.

Abundant growth of *Aeromonas* spp. on SGAP-10C occurred at 48 hr after inoculation and, based on our trials in diagnostic situations, growth of only aeromonads occurred. Good growth occurred on RS at 24 hr, but additional streaking may be needed for isolation. Therefore, for isolation of *Aeromonas* spp. colonies with either media, the process takes 48 hr.

The starch-glutamate-ampicillin-penicillin medium appears to be an efficient selective medium for *Aeromonas* spp. Because densities of *A. hydrophila* in reservoirs have been correlated with the prevalence of infected fishes (Hazen, 1983) and SGAP-10C is effective at recovering bacteria from aquatic sources (Huguet and Ribas, 1991), membrane filtration of pond water onto SGAP-10C could be used to indicate high numbers of *Aeromonas* spp. This medium may offer a better choice for the laboratory selective recovery of *Aeromonas* spp. from fishes, and for environmental, veterinary, and clinical microbiology.

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