

Medium for the Isolation of *Aeromonas hydrophila*

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A new differential medium, Rimler-Shotts, was tested with 109 isolates representing 13 genera of bacteria obtained from aquatic environments and animals. This medium was effective in presumptive identification of the strains of *Aeromonas hydrophila* examined, with 94% accuracy. Strains of *Citrobacter* which were hydrogen sulfide-variable could not be separated from *A. hydrophila*. This medium was designed to facilitate diagnosis of *A. hydrophila* infections in animals and humans.

The first isolations of microorganisms with characteristics typical for *Aeromonas* were obtained in the late 1800s. They were associated with diseases of aquatic animals and their environment, but are no longer available for comparison (3, 12, 18, 23). Some 50 years later, additional bacterial types were isolated which were referred to as the C27 group and were later tentatively placed into the genus *Aeromonas* (7).

In 1961, Ewing et al. suggested the adoption of the following species: (i) *Aeromonas hydrophila*, which includes organisms previously specified as *A. liquefaciens* and *A. punctata*; (ii) *A. salmonicida*; and (iii) *A. shigelloides*, previously referred to as the C27 group (6).

Since their first description, the motile members of this genus (*A. hydrophila* and *A. shigelloides*) have been reported with increasing frequency from disease conditions of both warm- and cold-blooded vertebrates (1, 5, 6, 8, 13, 16, 17). A number of methods and schemes for the isolation and identification of these organisms have been developed, usually requiring several days to a week to complete (5, 6, 8-10). Current interest in fish farming, the ecological impact, and increased awareness in both veterinary and human medicine have brought this group and, in particular, *A. hydrophila* to the forefront (13, 16, 17).

A. hydrophila is a gram-negative fermentative organism belonging to the order *Pseudomonadales*. It has many biochemical characteristics similar to some genera of the family *Enterobacteraceae*. Due to these similarities, *A. hydrophila* is often difficult to recognize, especially from mixed populations of gram-negative bacteria, and on chance isolation

may be mislabeled. It was with these problems in mind that the reported work was done. The purpose of this study was to develop a simple medium with a differential potential for the rapid isolation and identification of *A. hydrophila*.

METHODS AND MATERIALS

A medium referred to as Rimler-Shotts (RS), which is a modification from several media developed for enterobacteria, was prepared (11, 14, 15). It was composed of L-lysine-hydrochloride, 5 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, 5.0 g; agar, 13.5 g; and water in sufficient quantity to make 1 liter. Components were dissolved by stirring; pH was adjusted to 7.0; and the mixture was heated to a boil for 1 min, cooled to 45 C, and poured into plates. Prepared plates were refrigerated until needed.

Other media used in this study included MacConkey agar, triple sugar iron agar, Trypticase soy broth, maltose broth, Moeller ornithine broth, and Moeller lysine broth. All of these media were obtained from BBL and were prepared and used as recommended.

Prior to the inoculation of media, all organisms were grown in Trypticase soy broth and diluted 1:10 in sterile physiological saline. In instances where enumeration was desired, standard procedures were followed in making logarithmic dilutions with sterile physiologic saline (2). Fractions of these dilutions were plated in triplicate. The inoculum was then spread over the surface of the plates with a glass rod. All inoculated plates and tubes were incubated at 37 C and observed, and findings were recorded at 18, 20, and 24 h.

A wide variety of organisms was evaluated on RS medium. These organisms represented 109 isolates belonging to genera frequently associated with water

or fecal contamination, or both (Table 1). The identity of all organisms used in this study was verified by standard biochemical characterization (4).

RESULTS

It was found that four characteristic types of colonies were obtained when organisms were inoculated on RS medium. The first was yellow, which indicated maltose fermentation. The second was yellow with a black center, which indicated the same type of reaction as the first but with H₂S production. The third colonial type was one which produced shades of greenish-yellow to green, which indicated decarboxylation of lysine or ornithine, or both. A fourth type was green with a black center, indicating the same reaction as the third but with H₂S production. Growth inhibition occurred from time to time with members of the genus *Proteus* and *A. shigelloides*, although inhibitors were added to retard other species. A correlation of

colonial types associated with the genera studied is presented in Table 2.

Studies to determine the most ideal time for interpretation and further study of organisms grown on RS medium suggest that plates should be observed after the 20th h but no later than the 24th h. Plates observed after the 26th h demonstrated a slow reversion of the fermentative reaction (yellow) toward a basic reaction (green). Only in a minimal number of cases did the medium fail to react as anticipated. Exceptions included: 1 of 20 (5%) *A. hydrophila*, 1 of 13 (7.5%) *Salmonella* spp. (a strain of *S. typhi* which was only weakly lysine positive and ornithine negative), and 5 of 7 (71%) *Citrobacter* spp. (This was related to the H₂S production by the strains of *Citrobacter* spp. studied. It was found that three of the five strains in question only produced H₂S in colonially crowded areas, whereas only about half of the colonies from the remaining two strains produced H₂S.) Of the 109 strains of organisms studied, only 7 failed to react as anticipated (Table 3). Two of these cases, an isolate of *A. hydrophila* and an isolate of *S. typhi*, could not be considered as characteristic strains. RS medium produced characteristic, anticipated reactions, based on conventional biochemical studies, in all cases except for the H₂S-variable reactions encountered with *Citrobacter* spp. This medium was effective in identifying *A. hydrophila* without further study in 104 of 109 instances (95%). If all strains, aberrant and otherwise, are considered, the performance of RS medium in detecting *A. hydrophila* on initial culture and without further study was 94%.

The sensitivity of RS medium was studied in a comparison with MacConkey agar. MacConkey agar was chosen for comparison since it is capable of growing most, if not all, of the gram-negative organisms of the enteric and associated groups. No significant difference in the ability to propagate *A. hydrophila* was observed between these media when seven randomly selected strains of *A. hydrophila* were compared.

DISCUSSION

The ingredients used in this medium were selected and compounded so as to achieve a maximal acidic (maltose fermentation) or basic reaction (decarboxylation of lysine or ornithine, or both). Hydrogen sulfide production was primarily dependent upon the utilization of sodium thiosulfate or L-cysteine-hydrochloride, or both, with ferric ammonium citrate being uti-

TABLE 1. Organisms used for comparative study of reactions on Rimler-Shotts medium

Organism	No. of strains	Source
<i>Aeromonas hydrophila</i>	20	Fish, rhino, human, mink, water, bovine
<i>Escherichia coli</i>	14	Canine, feline, equine, bovine, porcine, racoon
<i>Shigella</i> type		
A	1	Human
C	1	Human
D	1	Human
B	2	Human, monkey
<i>Proteus mirabilis</i>	5	Canine, porcine, hamster
<i>Citrobacter</i> spp.	7	Duck, human, porcine, bovine
<i>Klebsiella</i> spp.	9	Human, feline, canine, bovine
<i>Enterobacter</i> spp.	10	Canine, porcine, equine, bovine, caprine
<i>Salmonella</i> spp.	10	Turkey, chicken, pigeon, bovine
<i>typhi</i>	1	Human
<i>cholerae</i>	1	Porcine
<i>pullorum</i>	1	Chicken
Arizona types		
10A-10B	2	Chicken
7	3	Turkey
24	1	Goose
5	1	Canine
<i>Herellea vaginicola</i>	1	Porcine
<i>Pseudomonas</i> spp.	8	Water, canine, bovine, human
<i>Edwardsiella tarda</i>	1	Snake
<i>Serratia marcescens</i>	1	Bovine
<i>P. rettgeri</i>	1	Porcine
<i>P. morgani</i>	1	Bovine
<i>P. vulgaris</i>	2	Bovine
<i>A. shigelloides</i>	4	Fish
	109	

genic members of the *Enterobacteraceae* for inoculation to differential media, and in subsequent identification.

This medium has been in routine use in the diagnostic laboratories at the College of Veterinary Medicine, University of Georgia, for the past year. During this time, the isolations of *A. hydrophila* have increased approximately 10-fold. The facility with which the medium can be prepared and used without autoclaving permits wide spread use in epidemiological, ecological, and epizootological investigations, as well as routine use in the rapid isolation and identification of *A. hydrophila* from aquatic and domestic animals, wildlife, and humans.

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