L-Arabinose-Ornithine-Irgasan Medium for Differentiating *Serratia* Species

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A semisolid medium (designated *Serratia* differentiation medium) containing L-arabinose, ornithine, and selective inhibitor was used to differentiate three clinically encountered *Serratia* species. The inhibitor, Irgasan DP-300, was incorporated to eliminate false-positive reactions from most remaining *Enterobacteriaceae*. The suspected *Serratia* colony was inoculated as a stab into the medium. *Serratia marcescens* was indicated by a change in color from olive to purple following 18 h of incubation, whereas *S. rubidaea* (not listed in *Bergey's Manual of Determinative Bacteriology*) was indicated by a change to bright yellow. *S. liquefaciens* (described in *Bergey's Manual of Determinative Bacteriology* [8th ed., 1974] as *Enterobacter liquefaciens*) produced a small purple band at the top of the medium and a yellow or yellow-green butt. Absence of growth and color change following incubation indicates that the suspected colony is a non-*Serratia*. Thirty-six *Serratia* strains and 97 other *Enterobacteriaceae* and *Pseudomonadaceae* strains were tested. Two strains of the non-*Serratia* *Enterobacteriaceae* (one each of *Citrobacter freundii* and *Proteus morganii*) and two strains of *Pseudomonas aeruginosa* produced a color change in the medium. All of the *Serratia* strains tested were correctly identified using this medium, while 96% of the other species tested were inhibited.

Bergey's Manual of Determinative Bacteriology (5) recognizes only one species, *Serratia marcescens*, in the genus *Serratia*. However, Ewing (1) and Ewing et al. (2) recognize two additional species—*S. liquefaciens* and *S. rubidaea*. These species are classified by their pigment, growth characteristics, and biochemical activities.

Recently, selective media for the isolation of *Serratia* have been described (6, 7), but there is no medium which both differentiates the three *Serratia* species and inhibits most other microorganisms, some of which may be misidentified as *Serratia*.

A medium containing erythritol and Irgasan DP-300 (4,2',4'-trichloro-2-hydroxydiphenyl ether; Ciba-Geigy, Summit, N.J.) has been used in isolation of *Serratia* sp. from raw sewage (4, 6). Isolates recovered using this medium must be tested biochemically to identify species. *Serratia* differentiation (SD) medium was developed for species identification of *Serratia* obtained on primary isolation media. This medium combines two well-known biochemical tests (L-arabinose fermentation and ornithine decarboxylase activity), Irgasan as a selective inhibitor, and bromothymol blue and phenol red indicators to differentiate three *Serratia* species (Table 1).

The ability of the medium to correctly identify *Serratia* was tested, utilizing 36 *Serratia* strains. Additionally, 12 *Serratia* were tested with both SD medium and the API 20E biochemical test (Analytab Products, Inc., Plainview, N. Y.) to determine the reliability of the medium in correctly identifying *Serratia* when prepared and used by individuals other than the investigators.

The medium's effectiveness in inhibiting the non-*Serratia* was tested, utilizing 97 strains of clinically encountered *Enterobacteriaceae* and *Pseudomonadaceae*.

MATERIALS AND METHODS

Bacterial cultures. Twenty-seven strains representing three *Serratia* species were obtained from Long Island Jewish Hillside Medical Center, New Hyde Park, N.Y.; Center for Disease Control, Atlanta, Ga.; St. Mary's Nazareth Hospital, Chicago, Ill.; and McDonnell Douglas Life Sciences, St. Louis, Mo. Nine additional strains obtained from the American Type Culture Collection brought the total to 36 *Serratia* strains. In testing the effectiveness of the medium in inhibiting multiplication of the non-*Serratia*, we used 97 strains representing the species listed in Table 2. *Enterobacteriaceae* were identified biochemically by means of the API 20E biochemical profile. The biochemical identification of *Serratia* required additional L-arabinose and deoxyribonuclease media. For the recognition of other bacteria, i.e., nonfermenting or oxi-

dase-producing gram-negative organisms and the gram-positive organisms, the tests applied were in accordance with those listed in the *Manual of Clinical*

TABLE 1. *Biochemical reactions utilized in SD medium*

Species	L-Arabinose fermentation	Ornithine decarboxylase	SD medium color change
<i>S. marcescens</i>	—	+	Purple throughout
<i>S. liquefaciens</i>	+	+	Green-yellow butt, purple band
<i>S. rubidaea</i>	+	—	Yellow throughout

Microbiology (3). Results of identification were verified by laboratory personnel other than the investigators. Periodic retesting was performed to assure that no contamination had occurred during maintenance transfer of organisms. The great majority of the donated strains were isolated originally from clinical specimens. During the study, strains were maintained by inoculation of commercially prepared blood agar plates (Remel Media, Lenexa, Kans.).

SD medium preparation. The medium was prepared as a base solution (solution A) to which inhibitor and indicator were added and a carbohydrate solution (solution B).

Medium base (solution A) was prepared as follows: yeast extract (Baltimore Biological Laboratories [BBL], Cockeysville, Md.), 1 g; L-ornithine (Sigma, St. Louis, Mo.), 1 g; NaCl, 0.5 g; and agar (BBL), 0.4 g, were added to 90 ml of distilled water and heated to boiling. One milliliter of stock inhibitor and 1 ml of

TABLE 2. *Reactions of 133 cultures tested with SD medium*

Species name	Strains tested	Reaction in SD medium
<i>Serratia marcescens</i>	13	Purple
<i>S. liquefaciens</i>	12	Green-yellow butt, purple band
<i>S. rubidaea</i>	11	Yellow
<i>Escherichia coli</i>	5	No growth
<i>Klebsiella pneumoniae</i>	6	No growth
<i>K. ozaenae</i>	2	No growth
<i>Enterobacter aerogenes</i>	4	No growth
<i>E. hafniae</i> (<i>Hafnia alvei</i>) ^a	4	No growth
<i>E. cloacae</i>	4	No growth
<i>Proteus mirabilis</i>	2	No growth
<i>P. vulgaris</i>	2	No growth
<i>P. rettgeri</i>	2	No growth
<i>P. morgani</i>	2	Growth, one strain (purple); no growth, one strain
<i>Citrobacter freundii</i>	6	Growth, one strain (yellow); no growth, five strains
<i>Pseudomonas aeruginosa</i>	10	Growth, one strain (purple); no growth, nine strains
<i>P. diminuta</i>	3	No growth
<i>P. maltophilia</i>	3	No growth
<i>P. stutzeri</i>	2	No growth
<i>P. putrefaciens</i>	1	No growth
<i>P. fluorescens</i>	3	No growth
<i>Providencia alcalifaciens</i> (<i>Proteus inconstans</i>) ^a	2	No growth
<i>P. stuartii</i> (<i>Proteus inconstans</i>) ^a	2	No growth
<i>Acinetobacter calcoaceticus</i> var. <i>anitratus</i>	4	No growth
<i>Shigella dysenteriae</i>	1	No growth
<i>S. flexneri</i>	1	No growth
<i>S. boydii</i>	1	No growth
<i>S. sonnei</i>	2	No growth
<i>Salmonella arizonae</i> (<i>Arizona hinshawii</i>) ^a	4	No growth
<i>Salmonella</i> —various serotypes	5	No growth
<i>Salmonella choleraesuis</i>	3	No growth
<i>S. typhi</i>	1	No growth
<i>Staphylococcus aureus</i>	3	No growth
Group D enterococcus	2	No growth
<i>Bacillus subtilis</i> var. <i>niger</i>	2	No growth
<i>Micrococcus rubens</i>	1	No growth
<i>M. luteus</i>	2	No growth

^a Name in *Bergey's Manual of Determinative Bacteriology* (5).

stock indicator were added, and the pH was adjusted to 6.7 with 1 N NaOH.

Stock inhibitor (solution 1) was prepared by dissolving 0.1 g of Irgasan DP-300 in 10 ml of 1 N NaOH. The mixture was heated gently until the DP-300 dissolved and was then transferred to a 100-ml volumetric flask and brought to volume with distilled water.

Stock indicator (solution 2) was prepared by dissolving 0.2 g of bromothymol blue (Fisher Scientific, Pittsburgh, Pa.) and 0.1 g of phenol red (Matheson, Coleman and Bell, Cincinnati, Ohio) in 10 ml of 1 N NaOH and 50 ml of distilled water. The mixture was stirred for 1 h, transferred to a 100-ml volumetric flask, and brought to volume with distilled water.

Solutions 1 and 2 were stored at room temperature and discarded after 4 weeks.

Carbohydrate (solution B) was prepared by dissolving 1 g of L-arabinose (Sigma) in 10 ml of distilled water.

Solutions A (92 ml) and B (10 ml) were autoclaved separately at 121°C for 15 min, cooled to 48°C in a water bath, and mixed aseptically to yield approximately 100 ml of the final medium. The slight loss in volume was due to evaporation during autoclaving.

Two-milliliter portions of the medium were aseptically pipetted into sterile disposable tubes (Falcon) which were then capped tightly and stored at 4°C until used. No medium was used after 4 weeks of storage.

Procedures. Single colony isolates of the test organisms were inoculated as a straight stab through the center of the tubed medium. Tubes were incubated at 37°C for 18 h, and reactions were recorded. A total of 36 *Serratia* and 97 non-*Serratia* were tested.

Additionally, a comparative study was performed to assess the reliability of the medium in correctly identifying the three *Serratia* species. Twelve organisms were submitted as unknowns to an impartial investigator. The unknowns, submitted on numbered blood agar plates, included two non-*Serratia* (*K. pneumoniae* and *E. aerogenes*) and ten nonpigmented strains of *Serratia*. The ten strains included two *S. marcescens*, three *S. liquefaciens*, and five *S. rubidaea*.

The investigator inoculated the SD medium with the blood agar isolates and simultaneously performed API 20E biochemical tests.

Following an 18-h incubation at 37°C, the test results were recorded for each of the unknowns. API identification results and SD medium results were compared to the test key, and the performance of both methods was evaluated.

RESULTS AND DISCUSSION

Each of the 13 strains of *S. marcescens* inoculated on SD medium produced a purple color reaction, whereas the 11 *S. rubidaea* produced a bright yellow color. All 12 strains of *S. lique-*

faciens produced a green or green-yellow reaction in the butt, with a small purple band evident at the top of the medium (Table 2). Ninety-three of the 97 non-*Serratia* tested were totally inhibited. Four of the 97 non-*Serratia* tested produced a reaction in the medium. They included one strain each of *Citrobacter freundii*, which produced a bright yellow reaction, *Proteus morganii*, and two strains of *Pseudomonas aeruginosa*, each producing a purple color reaction. None of the 19 strains of *Klebsiella-Enterobacter* tested produced a reaction in this medium.

Results of the comparative study of the SD medium and the API biochemical test method demonstrate the medium's effectiveness for identification of the clinically encountered *Serratia*. An impartial investigator correctly identified the 10 *Serratia* strains using SD medium and was able to choose the 2 non-*Serratia* from the 12 unknowns by noting the absence of growth. Three of the ten *Serratia* were misidentified by the API method.

The SD medium combines two biochemical parameters, L-arabinose fermentation and ornithine decarboxylase activity, to produce an easily readable color reaction for differentiation of three clinically encountered *Serratia*. Furthermore, the medium is inhibitory to most remaining organisms.

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