Isolation of Serratia marcescens on Deoxyribonuclease-Toluidine Blue-Cephalothin Agar

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Received for publication 13 August 1972

Serratia marcescens was isolated on a new medium—commercial deoxyribonuclease agar with the addition of cephalothin (1,000 µg/ml) and Toluidine Blue (1,000 µg/ml). It was detected in water samples even though it comprised only 0.1 to 0.0001% of the total bacterial population.

The number of hospital-acquired infections due to Serratia marcescens has increased during the last decade. To understand better the ecology and epidemiology of these infections, we have designed a selective medium which simplifies the isolation and identification of this pathogen. Most strains of S. marcescens produce extracellular deoxyribonuclease (3, 4) and are resistant to cephalothin (2). We took advantage of these two properties in designing our medium. Strains (161) of S. marcescens previously described (1) were tested on deoxyribonuclease-Toluidine Blue agar with various concentrations of tetracycline, penicillin G, polymyxin B, colistin, cephalothin, and colistin-cephalothin mixture. Only two strains were inhibited at 1,000 µg of cephalothin/ml, so this concentration was chosen for the final medium.

The isolation medium, called deoxyribonuclease-Toluidine Blue-cephalothin (DTC) agar, was prepared as follows: 21 g of deoxyribonuclease agar (BBL), 2.5 g of agar-agar (Difco), 0.05 g of toluidine blue 0, and 500 ml of distilled water were mixed on a mechanical stirrer until the dye went into solution; the flask with a Teflon stirring bar was autoclaved at 121 C for 15 min and cooled to 50 C; 5 ml of 100,000 µg of sterile cephalothin/ml (obtained from the local pharmacy as Keflin, sterile injectible, Eli Lilly and Co., Indianapolis, Ind.) was added; and the medium was poured into sterile dishes. Strains of S. marcescens formed typical colonies on DCT agar and changed it from blue to red for several millimeters around the colony (3, 4).

Table 1 shows that S. marcescens was easily isolated on DTC agar from environmental water samples taken within 10 miles of Tuscaloosa, Ala. A 100-ml amount of water was filtered through a 0.22-µm G. E. Nucleopore filter (Arthur H. Thomas Co., Philadelphia).

| Sample                  | No. of bacteria per 100 ml of | Aerobic heterotrophs* | S. marcescens |
|-------------------------|--------------------------------|-----------------------|---------------|
| Lake                    |                                |                       |               |
| 1                       | >10⁶                           | ~63                   |               |
| 2                       | 8 x 10⁴                        | ~120                  |               |
| 3                       | 10⁵                            | 0                     |               |
| 4                       | 10⁶                            | 72                    |               |
| 5                       | 4 x 10⁵                        | 23                    |               |
| 6                       | 3 x 10⁵                        | ~200                  |               |
| 7                       | 2 x 10⁵                        | 0                     |               |
| 8                       | 9 x 10⁴                        | 0                     |               |
| 9                       | 10⁴                            | ~150                  |               |
| 10                      | 2 x 10⁴                        | 61                    |               |
| 11                      | >10⁶                           | 83                    |               |
| Stream                  |                                |                       |               |
| 1                       | 10⁵                            | ~100                  |               |
| 2                       | 10⁶                            | 0                     |               |
| 3                       | 2 x 10⁵                        | 0                     |               |
| 4                       | 9 x 10⁴                        | 1                     |               |
| 5                       | 10⁵                            | 0                     |               |
| Black Warrior River     | 10⁴                            | 0                     |               |
| Stagnant pool           |                                |                       |               |
| 1                       | 10⁴                            | 0                     |               |
| 2                       | >10⁶                           | 77                    |               |

*Defined to be number of bacteria which formed visible colonies on Trypticase soy agar after 4 days at 25 C.

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Pa.) and placed onto the agar. About 61% of the samples were positive for *S. marcescens*, which indicates that it is widely distributed in environmental water of Tuscaloosa County. The medium was highly selective against other bacteria. Typical colonies of *S. marcescens*, identified as described previously (1), were usually the only ones which developed on the filter after 24 hr at 37 C. Occasionally, colonies of *Pseudomonas* were present, but these were easily differentiated because of their weak (or negative) deoxyribonuclease reaction, colonial morphology (usually green), or positive oxidase test.

We also used the new medium to determine the percentage of healthy college students who carried *S. marcescens* in their guts; however, none of the 63 students tested were positive. Feces seeded in the laboratory with *S. marcescens* were always positive, so our results reflect the absence of *S. marcescens* in the sample population rather than a deficiency of the medium.

A few strains of *S. marcescens* will not grow on DTC agar, but we estimate that the number of these “false negatives” will be less than 3%. A bacterium which could cause a “false positive” is *Enterobacter liquefaciens* since 16 of the 20 strains we tested grew on DCT agar and were deoxyribonuclease positive (weakly at 37 C but strongly at 23 C). These two organisms are easily differentiated biochemically; *E. liquefaciens* ferments arabinose and raffinose, but *S. marcescens* ferments neither. The new medium should also prove useful in isolating *E. liquefaciens*, and may help clarify the taxonomic and ecological relationships between these closely related species which some workers consider to be synonymous.

We thank A. N. Holland III and Lois Faye Jones for suggesting which antibiotics would be selective for *S. marcescens* and Betty Davis, Enterobacteriologic Unit, Center for Disease Control, for the strains of *E. liquefaciens*. This research was supported by research grant CC 00592 from the Center for Disease Control, Atlanta, Ga.

**ADDENDUM IN PROOF**

Three other selective media for *S. marcescens* have come to my attention: deoxyribonuclease agar with Toluidine Blue, egg yolk, and 100 μg of cephalothin/ml (M. Goldin, J. G. Shaffer, and E. Brown., Bacteriol. Proc. p. 96, 1969); deoxyribonuclease agar with Toluidine Blue, 30 μg of cephalothin/ml, and 30 μg of colistin/ml (J. C. Cate., Proc. 7th Intern. Congr. Chemother., 1971); and a basal salts medium with 0.5% erythritol as the sole carbon source (L. J. Slotnick and M. Dougherty. Appl. Microbiol. 24: 292-293, 1972).

**LITERATURE CITED**

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