Rapid Identification of *Serratia marcescens* by Coagglutination

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All 20 O serotypes of *Serratia marcescens* produce a common, soluble antigen. Crude antigen was obtained by ammonium sulfate precipitation of spent growth medium, antiserum was produced in rabbits, and a coagglutination test for rapid identification of *S. marcescens* was developed. A total of 701 clinical isolates of gram-negative bacilli were examined, and a 100% correlation between biochemical identification of *S. marcescens* and identification by coagglutination was found.

Roig et al. (6) have shown that *Serratia marcescens* produces two extracellular antigens which can be detected by the immunoprecipitation test of Elek (3). One antigen was identified as the smooth lipopolysaccharide specific for each serotype. The other was identified as an antigen carried by all strains of the genus *Serratia* but not by other gram-negative bacteria. In this report, the preparation of an antiserum which contains antibodies against this *Serratia*-common antigen is described, and evidence that by coagglutination it is possible to rapidly detect isolates of *Serratia marcescens* is presented.

Cultures of *S. marcescens* serotype O:5 CDC-4015-73, kindly supplied by B. R. Davies (Centers for Disease Control, Atlanta, Ga.) were grown for 18 h at 35°C in shaker flasks containing 800 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Cells were removed by centrifugation, and ammonium sulfate was added to the supernatant to a final concentration of 60% (wt/vol). After 12 h at 4°C, the precipitate was recovered by centrifugation and dialyzed against distilled water until no more ammonium sulfate was detected in the dialysis fluid.

Rabbits were immunized with the precipitate. A total of 2 mg of lyophilized antigen per ml of incomplete Freund adjuvant was injected subcutaneously in doses of 1 ml twice a week for 4 weeks, and the animals were bled 1 week after the last injection.

To determine whether the antiserum con-

tained appropriate antibodies, antiserum agar plates were prepared as described by Craven et al. (1). Similar plates were prepared with serum from normal rabbits. The agar plates were spot inoculated with one representative strain of each of the 20 serotypes (2, 5), incubated overnight at 35°C, and examined for halos of precipitation. The results showed that all *Serratia* serotypes developed a halo of precipitation only in the plates containing the immune serum.

Similar results were obtained by coagglutination. *Staphylococcus aureus* cells (Cowan I, ATCC 12598) were prepared as described by Kronwall (4). A 1-ml amount of a 10% (vol/vol) cell suspension was coated with antibodies by incubation for 3 h at room temperature with 0.2 ml of undiluted serum from rabbits immunized with antigen prepared from a supernatant of *S. marcescens* O:5 (CDC-4015-73). After three washings with phosphate-buffered saline (pH 7.2), the cells were suspended in 2 ml of the buffer. The 20 O serotype *S. marcescens* strains were grown at 35°C on Trypticase soy agar, and colonies were emulsified in a drop of the sensitized staphylococcal suspension on a slide and rocked for 1 min. All 20 O serotype strains agglutinated the staphylococci.

To assess the practical implications of the above findings, we screened 701 gram-negative bacteria by coagglutination by suspending them from triple sugar iron slants (Difco Laboratories, Detroit, Mich.) inoculated with single colonies from isolation plates during the process of biochemical identification (Table 1). Of 701 isolates, only 39 yielded positive reactions with this coagglutination reagent, a result in complete agreement with the biochemical analyses of the isolated bacteria. Significantly, none of the *Serratia* strains was serotype 5 which was used for

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TABLE 1. Bacteria tested

Microorganisms	No. of strains
<i>Escherichia coli</i>	319
<i>Proteus</i> spp.....	103
<i>Enterobacter</i> spp.....	74
<i>Klebsiella</i> spp.....	70
<i>Serratia marcescens</i>	39
<i>Pseudomonas aeruginosa</i>	41
<i>Salmonella</i> spp.....	20
<i>Acinetobacter calcoaceticus</i>	14
<i>Citrobacter</i> spp.	9
<i>Shigella sonnei</i>	8
<i>Yersinia enterocolitica</i>	3
<i>Campylobacter fetus</i>	1

the preparation of the *S. marcescens* antigen.

Many identification tests cannot be performed by small laboratories. Therefore, there is a need for a simple, rapid method for the identification of the species that does not involve an extensive range of biochemical tests. *S. marcescens* strains often grow better at a temperature below 37°C; they may not be pigmented and require additional examinations for the production of DNase and lipase. Our results show that all *S. marcescens* serotypes produce a common anti-

gen. Thus, detection of this antigen by coagglutination offers a simple, inexpensive, and rapid method for the identification of *S. marcescens*.

We thank B. R. Davis and P. A. D. Grimont for providing the *Serratia* reference strains used in this study and T. L. Pitt for his helpful discussions during the preparation of this manuscript.

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