Hydrolysis of casein: a differential aid for the identification of *Serratia marcescens*

WILLIAM A. SALISBURY AND JOSEPH J. LIKOS

From the Veterans Administration Hospital, Phoenix, Arizona, USA

SYNOPSIS Through the hydrolysis of casein, a cultural characteristic has been established in the preliminary identification of *Serratia marcescens*. Compared against 438 strains of the families, *Pseudomonadaceae* and *Enterobacteriaceae*, this organism has a reproducible capability to decompose casein, while most other Gram-negative bacilli failed to produce any hydrolysis, or, at the most, hazy zones only. Routine use has shown this to be a valuable differential aid in the identification of *Serratia marcescens*.

The separation and identification of the Gram-negative bacteria within the genera *Pseudomonas*, *Aeromonas*, *Serratia*, and *Proteus* in a busy hospital microbiology laboratory can be a difficult and time-consuming chore. Many methods have been described to assist in the separation and identification of this group of bacteria. We should like to add an observation which has proved quite successful in the isolation of the nonpigmented strains of *Serratia marcescens* (Clayton and von Graevenitz, 1966; Wilfert, Barrett, Ewing, Finland, and Kass, 1970). This organism has demonstrated an easily recognizable and reproducible capability to hydrolyze casein in a defatted milk medium.

Serratia marcescens has become a significant organism (Davis, Foltz, and Blakemore, 1970; Sanders, Luby, Johanson, Barnett, and Sanford, 1970; Black, Hodgson, and McKechnie, 1971) in our nosocomial infections and hospital epidemiology. Utilizing the work of Brown and Scott Foster (1970), we have been using a defatted milk agar (Hasting, 1903; Gordon and Smith, 1955; Ajello *et al*, 1963) in our routine as a diagnostic aid in the identification of *Pseudomonas aeruginosa* by its hydrolysis of casein and pigmentation. Preliminary examination of these milk agar plates reveals a nonpigmented colony with a recognizable zone of hydrolysis that is distinctly different than *Pseudomonas* and is confirmed to be *Serratia marcescens* by its characteristic DNase production (Elston and Elston, 1968; Black *et al*, 1971) decarboxylase activity (Ewing, Davis, and Edwards, 1960), sensitivity profiling (Bauer, Kirby, Sherris, and Turck, 1966), and colony characteristics on other

media. This reproducible colony characteristic has successfully withstood comparison against 438 strains of the families, *Enterobacteriaceae* and *Pseudomonadaceae*. Our study, as listed in Table I, has demonstrated that three bacterial species of the above families produce hydrolysis of milk protein as follows: *Serratia marcescens* (97%), *Pseudomonas aeruginosa* (99%), and *Proteus mirabilis* (94%).

Materials and Methods

The selection of a single and inexpensive milk agar is one that a laboratory of any size could utilize in their bacteriological screening. Gordon and Smith (1955) and Ajello, Georg, Kaplan, and Kaufman (1963) used the following casein medium in the separation of *Nocardia* and *Streptomyces* species. We have incorporated this medium in our procedure.

CASEIN MEDIUM (AJELLO *et al*, 1963; HASTINGS, 1903)

Prepare separately:

- 1 Skim milk (dehydrated or instant non-fat milk) 10 g
- Distilled water 100 ml
- 2 Distilled water 100 ml
- Agar 2 g

Autoclave at 120°C for 20 minutes. Cool both solutions to about 45°C. Mix and pour into sterile petri dishes.

All organisms were seeded on the skim milk agar by point inoculation. For screening procedures, six to eight isolates may be cultured on each plate. The cultures were incubated for 24 hours at 35 to 37°C

Received for publication 3 October 1972.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 1 *Serratia marcescens* at 24 hours.

Fig. 2 *Serratia marcescens* at 48 hours.

Fig. 3 *Pseudomonas aeruginosa* at 24 hours.

Fig. 4 *Pseudomonas aeruginosa* at 48 hours.

Fig. 5 Large clear transparent zone is *Pseudomonas aeruginosa* at 72 hours.

Small clear transparent zone is *Serratia marcescens* at 72 hours.

Small hazy zone is *Proteus mirabilis* at 72 hours.

Organism	No. Tested	24-Hr Reading at 35-37°C Average diameter of clear zone (a) (millimetres)	48-Hr Reading at Room Temperature Average diameter of clear zone (a) (millimetres)	Remarks
<i>Alk. faecalis</i>	10	0	0	Two appeared with hazy zones
<i>Citrobacter-Esch.Gr.</i>	3	0	0	—
<i>Ent. aerogenes</i>	19	0	0	—
<i>Ent. cloacae</i>	14	0	3(SH)	Hazy zone only
<i>Ent. hafnia</i>	8	0	3(SH)	Hazy zone only
<i>Ent. liquefaciens</i>	2	0	0	—
<i>Esch. coli</i>	58	0	0	—
<i>Herellea vaginicola</i>	16	0	0	—
<i>Klebsiella pneumoniae</i>	49	0	0	—
<i>Proteus mirabilis</i>	53	2(SH) ¹	4(SH)	Three produced no clearing
<i>Proteus morganii</i>	8	0	0	—
<i>Proteus rettgeri</i>	13	0	0	—
<i>Proteus vulgaris</i>	6	3(SH)	6(RH)	Two produced only hazy zones
<i>Providencia alcalifaciens</i>	2	0	0	—
<i>Pseudomonas aeruginosa</i>	99	6(RH) ²	13(RH)	One produced no clearing
<i>Salmonella, group A</i>	2	0	0	—
<i>Serratia marcescens</i>	74	5(RH)	12(RH)	Two produced no clearing
<i>Shigella-sonnei, subgroup D</i>	2	0	0	—

Table I Results of casein hydrolysis among members of the families Enterobacteriaceae and Pseudomonadaceae

¹SH = slow hydrolysis, ²RH = rapid hydrolysis

and then examined for evidence of casein hydrolysis. Plates were left at room temperature for another 24 hours, when the second inspection was made. Figures 1, 2, 3, 4, and 5 show typical examples. Cultures were not incubated at 35 to 37°C for the entire 48 hours because of the rapid spreading and overgrowth of a number of the isolates.

Discussion

Table I shows the comparison between different members of the families, *Enterobacteriaceae* and *Pseudomonadaceae*, presenting their ability to decompose casein in a short period of time. Routine use has proven valuable in confirming the presence of *Pseudomonas aeruginosa* and *Serratia marcescens* with clear transparent zones while most other organisms failed to produce any hydrolysis or at the most, hazy zones only. Few aberrant results were obtained in the bacterial strains isolated. We conclude from this study that the hydrolysis of casein is a valuable differential aid in the identification of *Serratia marcescens*.

The authors wish to express their thanks to Charles Dillman for his technical assistance and to Mr D. E. Deuel for preparation of the photographic material.

References

- Ajello, L., Georg, L. K., Kaplan, W., and Kaufman, L. (1963). *Laboratory Manual for Medical Mycology* (Public Health Service Publication, no. 994). Communicable Diseases Center, Atlanta, Georgia.
- Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. clin. Path.*, **45**, 493-496.
- Black, W. A., Hodgson, R., and McKechnie, A. (1971). Evaluation of three methods using deoxyribonuclease production as a screening test for *Serratia marcescens*. *J. clin. Path.*, **24**, 313-316.
- Brown, M. R. W., and Scott Foster, J. H. (1970). A simple diagnostic milk medium for *Pseudomonas aeruginosa*. *J. clin. Path.*, **23**, 172-177.
- Clayton, E., and von Graevenitz, A. (1966). Nonpigmented *Serratia marcescens*. *J. Amer. med. Ass.*, **197**, 1059-1064.
- Davis, J. T., Foltz, E., and Blakemore, W. S., MD's. (1970). *Serratia marcescens*. *J. Amer. med. Ass.*, **214**, 2190-2192.
- Elston, H. R., and Elston, J. H. (1968). Further use of deoxyribonuclease in a screening test for *Serratia*. *J. clin. Path.*, **21**, 210-212.
- Ewing, W. H., Davis, B. R., and Edwards, P. R. (1960). The decarboxylase reaction of *Enterobacteriaceae* and their value in taxonomy. *Publ. Hlth. Lab.*, **18**, 77-83.
- Gordon, R. E., and Smith, M. M. (1955). Prepared group of characters for the separation of *Streptomyces* and *Nocardia*. *J. Bact.*, **69**, 147-150.
- Hastings, E. G. (1903). Milkagar as a medium for demonstrating the production of proteolytic enzymes. *Science*, **17**, 372.
- Sanders, C. V., Jr., Luby, J. P., Johanson, W. G. Jr., Barnett, J. A., and Sanford, J. P. (1970). *Serratia marcescens* infections from inhalation therapy medications: Nosocomial outbreak. *Ann. intern. Med.*, **73**, 15-21.
- Wilfert, J. N., Barrett, F. F., Ewing, W. H., Finland, M., and Kass, E. H. (1970). *Serratia marcescens*: biochemical, serological, and epidemiological characteristics and antibiotic susceptibility of strains isolated at Boston City Hospital. *Appl. Microbiol.*, **19**, 345-352.