Precipitate Produced by *Serratia marcescens* on MacConkey Agar: Useful Diagnostic Test

WILLIAM A. BLACK†

Department of Microbiology, University Hospital, London, Ontario, Canada

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The production of a precipitate by *Serratia marcescens* on Oxoid MacConkey agar has proven useful as a laboratory diagnostic test. This phenomenon is specific for *Serratia* within the *Enterobacteriaceae*, although precipitate production is also given by *Acinetobacter anitratus* and some *Pseudomonas*, *Alcaligenes*, and *Aeromonas* species. Precipitate production seems to be specific for certain batches of MacConkey agar, and is probably related to a specific property of some batches of bile salts.

The ability of a laboratory to consistently isolate and accurately identify *Serratia marcescens* has become increasingly important for a variety of reasons. Thus, for example, the incrimination of *Serratia* in infections derived from contaminated nebulizers (7) and other patient care devices (4, 5) has made its detection necessary during routine environmental surveys for sources of potential nosocomial infections. Also, after reports of the isolation of *S. marcescens* from a wide variety of clinical infections, the possibility of this organism occurring as a significant pathogen in almost any type of patient specimen must be considered. Finally, since *S. marcescens* is often resistant to multiple antimicrobial agents, including gentamicin (6, 8, 13), its presence in an infected site is a potential cause of therapeutic antibiotic failure, and it becomes imperative that this organism be isolated and its antimicrobial susceptibility tested even when it is occurring in small numbers in mixed cultures.

The observation, therefore, that *S. marcescens* produced a precipitate (Fig. 1) on the batch of MacConkey agar in use in this laboratory was considered potentially important, and further study was indicated. This report contains the findings of the resulting investigation into the extent of the occurrence of a precipitate among various batches of MacConkey medium, the reproducibility of precipitate production and its specificity for *S. marcescens* and, finally, the nature of the reaction itself.

MATERIALS AND METHODS

Batch specificity of MacConkey agar. The precipitate was originally noted with *S. marcescens* on a

batch of Oxoid MacConkey salt-free medium (Oxoid CM7b, batch 003 18001) during routine quality control testing. This was a fortuitous observation, since only one other batch of CM7b from a total of six tested gave a similar precipitate. This is shown in Table 1, where it can also be seen that a precipitate was produced on all seven batches of Oxoid MacConkey CM7, a medium which differs from the salt-free CM7b only in the fact that it contains sodium chloride (5 g/liter). Three batches of Oxoid MacConkey no. 2 (CM109), which is also salt containing but which contains a different bile salt from that of CM7 and CM7b, gave negative results. Eight further batches of MacConkey medium from four other producers also gave negative results.

Specificity of the reaction for *Serratia*. During the course of 5 months, 2,204 consecutive gram-negative clinical isolates were tested by using a replica-plating technique (Fig. 2) and the batch of MacConkey medium on which the reaction had been first seen. For completeness, 48 stock cultures of less common organisms were also tested. The final identification of all organisms was made by using a battery of 33 biochemical substrates in the replica-plate series and additional conventional tests for nonfermenters or where otherwise indicated.

RESULTS

Of 131 strains of *S. marcescens*, 129 were positive and gave a strongly positive (+++) reaction (Table 2). Two stock cultures of *S. liquefaciens* and one stock culture of *S. rubidaea* gave a negative (–) and a moderately positive (+/–) result, respectively. Precipitates were not produced by any of the 383 strains of the *Klebsiella-Enterobacter* group tested and, apart from a single strain of *Proteus mirabilis*, the other species giving a precipitate could be easily differentiated from *S. marcescens* on the basis of the oxidase and oxidation-fermentation tests. Moreover, species other than *S. marcescens* produced less precipitate, and it was often only

† Present Address: Department of Health, Division of Laboratories, Box 34020, Station D, Vancouver, B.C. V6J 4M3, Canada.

visible by transmitted light. This latter technique, which was never necessary for *S. marcescens*, also produced a halo effect, due to clearing of the medium around some organisms, which was unrelated to the surface precipitate itself.

Investigation of the phenomenon: (i) special properties of *Serratia*. It was felt initially that such a specific reaction might be attributable to DNase production but, although a -/+ precipitate was produced by all test strains of *Pseudomonas maltophilia*, a DNase-positive organism, slightly stronger reactions were given by *P. cepacia* which is DNase negative. Also, no precipitate was given with several test strains of *Staphylococcus aureus*, which is DNase positive and which grows well on this particular brand of crystal violet-free MacConkey agar. Finally, a

precipitate was produced by one DNase-negative strain of *S. marcescens* and, as shown in Table 2, by other organisms which are DNase negative.

(ii) Effect of pH. The effect of pH on precipitate production was tested by using three batches of CM7b which had given, respectively, a marked precipitate, no precipitate, and a slight precipitate on preliminary testing at the normal

TABLE 1. Occurrence of precipitate on 24 batches of MacConkey medium from 5 suppliers

Supplier ^a	Code	No. tested	Reaction ^b
Oxoid	CM7b ^{c,d}	6	1+++ 1+ 4-
Oxoid	CM7 ^c	7	4+++ 3+
Oxoid	CM109	3	3-
Difco	0075-01	3	3-
GIBCO	1401850	1	1-
GIBCO	1401851 ^c	1	1-
BBL	11387 H8292-1705-02	1	1-
	11393 H8292-1707-02 ^c	1	1-
Pfizer	312A	1	1-

^a Suppliers were: Oxoid Ltd., London; Difco Laboratories, Detroit, Mich.; Grand Island Biological Co. (GIBCO), Grand Island, N.Y.; BBL Microbiology Systems, Cockeysville, Md.; and Pfizer Inc., New York, N.Y.

^b Values indicate number of batches considered strongly positive (+++), positive (+), or completely negative (-), based on the quantity of precipitate produced.

^c Without crystal violet.

^d Salt free.

FIG. 1. Demonstration of precipitate produced by *S. marcescens* on Oxoid MacConkey agar.

FIG. 2. Appearance, using a replica-plating technique, of *S. marcescens* (large zone of precipitation) and *A. anitratus* (small zone of precipitation).

TABLE 2. Frequency and intensity of production of precipitate by 2,252 gram-negative bacilli on an optimal batch of MacConkey medium

Organism	No. tested	No. positive	Reaction ^a
<i>Serratia marcescens</i>	131	129	+++
<i>S. liquefaciens</i>	2	0	—
<i>S. rubidaea</i>	1	1	+/-
<i>Pseudomonas aeruginosa</i>	328	155	+/-
<i>P. cepacia</i>	7	7	+/-
<i>P. maltophilia</i>	3	3	-/+
<i>P. fluorescens</i>	4	0	—
<i>Acinetobacter anitratus</i>	24	10	+/-
<i>Alcaligenes</i> species	12	3	+/-
<i>Aeromonas</i> species	4	3	+/-
<i>Proteus mirabilis</i>	238	1	+/-
<i>Indole</i> and <i>Proteus</i>	92	0	—
<i>Escherichia coli</i>	885	0	—
<i>Klebsiella pneumoniae</i>	259	0	—
<i>Enterobacter cloacae</i>	75	0	—
<i>E. aerogenes</i>	41	0	—
Other <i>Enterobacter</i> isolates	8	0	—
<i>Citrobacter</i> species	53	0	—
<i>Salmonella</i> species	9	0	—
<i>Shigella</i> species	16	0	—
<i>Arizona hinshawii</i>	3	0	—
<i>Providencia stuartii</i>	51	0	—
<i>Yersinia enterocolitica</i>	3	0	—
<i>Y. pseudotuberculosis</i>	1	0	—
<i>Edwardsiella tarda</i>	2	0	—
Total	2,252		

^a Intensity of reaction, based on the quantity of precipitate produced, was either very strongly positive (+++), moderately positive (+/-), slightly positive (-/+), or completely negative (—).

pH of 7.4. With the first two, adjustment of the final pH between 8.2 and 6.5 had no effect on precipitate production by *S. marcescens*. With the latter batch, the reaction was enhanced by a pH of 8.2 and diminished by a pH of 6.5. It appears, therefore, that although an alkaline pH will influence a marginal batch of medium, even large changes in pH will have no effect on either strongly positive or completely negative batches.

(iii) **Effect of bile salts.** Several portions of MacConkey medium were prepared in the lab by using Oxoid constituents throughout, and they contained either no bile salts or Oxoid bile salts L55 (batch number 119986) in concentrations ranging from 2.5 to 20.0 g/liter. The normal concentration of L55 in CM7 and CM7b is 5 g/liter. No precipitate was produced by *S. marcescens* on any of these media, suggesting that if the reaction is related to the presence of bile salts, as seems likely, then it must be specific to certain batches of L55. This would explain the

variation noted among batches of CM7b (Table 1).

DISCUSSION

The isolation and laboratory identification of *S. marcescens* can often be tedious, and it is now recognized that red-pigmented strains constitute only a small fraction of the total number of clinical isolates of the species. The more frequently encountered nonpigmented strains usually present as nonlactose fermenters and are essentially indistinguishable from other closely related species on the usual differential primary plating media. Recognizing this difficulty and the clinical importance of identifying *Serratia*, many special diagnostic and selective media for this organism have been described. DNase test medium (1), for example, has been modified by the addition of dyes (9, 11) and made even more selective by the addition of cephalothin (2). Other media for the isolation of *S. marcescens* include erythritol agar (10), caprylate thallous agar (12), and Tween 80 agar (3). Each of these formulations has its uses, but there are obvious limitations to the number of plates which can be employed in the routine diagnostic laboratory.

The production of a specific precipitate on MacConkey medium by *S. marcescens* suggested, therefore, that this important organism could be initially detected without making any alterations or additions to normal laboratory diagnostic protocols. As has been shown, the reaction has indeed proven useful in distinguishing *S. marcescens* from other closely related members of the *Enterobacteriaceae*. All other organisms which gave a precipitate belonged to different families and were readily distinguished by oxidation-fermentation and oxidase tests.

Optimum production of precipitate was given by Oxoid CM7 and by some batches of Oxoid CM7b, and it seems likely that the phenomenon is related to a property of some batches of Oxoid bile salt L55.

The use of this reaction as a screening test should not preclude the employment of a full range of confirmatory tests on suspect organisms. It will, however, lead one to suspect possible *S. marcescens* much more rapidly. In addition, it will minimize unnecessary work on organisms which do not produce the precipitate. In both regards, one of the most valuable applications of the screening test has been in environmental surveys in the hospital, where a wide variety of unusual gram-negative bacteria may be encountered. The production of a precipitate by *S. marcescens* on appropriate Oxoid MacConkey agar has been used as a screening test in both the clinical diagnostic and epidemiology

sections of this laboratory for over 2 years with excellent results.

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