

## Bacto-TB Hydrolysis Reagent (Tween 80) for Identification of *Serratia*

MATEI WEINER

Department of Pathology and Clinical Laboratories, The Methodist Hospital of Brooklyn, Brooklyn, New York 11215

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Among 21 species of *Enterobacteriaceae*, only *Serratia* hydrolyzed the commercially available Bacto-TB hydrolysis reagent (Tween 80). The procedure allowed accurate differentiation of *Serratia* within 24 h.

It is recognized that *Serratia* species can cause urinary tract infections, pneumonia, septicemia, endocarditis, etc. (1, 2, 4). It is important, therefore, to identify *Serratia* in clinical isolates. This can be achieved with the aid of commercially available test systems, such as R/b, Enterotube, and API, or of conventional systems with which, in most instances, a deoxyribonuclease plate is required.

Recently, Lovell and Bibel (3) reported that organisms of the genus *Serratia* can be differentiated from other *Enterobacteriaceae* by their ability to hydrolyze Tween 80 incorporated into tryptose blood agar base.

The purpose of this communication is to report our experience in identifying *Serratia* by means of commercially available Bacto-TB hydrolysis reagent (catalog no. 3192-56-5, Difco Laboratories). The reagent is a sterile phosphate-buffered solution of polysorbate (Tween 80) and neutral red (Difco Technical Information no. 1440, 1975).

The test was performed by suspending various species of *Enterobacteriaceae* in 1 ml of sterile distilled water in small test tubes (13 by 75 mm) and adding 0.1 ml of Bacto-TB hydrolysis reagent to each tube. The tubes were incubated at 35°C. A positive reaction was indicated by a change of the indicator from the original amber to pink-red. Depending on the size of the inoculum, the reaction became positive within 4 to 18 h of incubation. With inocula consisting of a loopful of bacteria, the reaction became positive after overnight incubation. Inocula containing 10<sup>8</sup> colony-forming units per ml gave a positive reaction in 4 h.

A total of 210 strains of various species of *Enterobacteriaceae* were tested. Table 1 shows, in agreement with the findings of Lovell and Bibel (3), the accuracy with which *Serratia* can be differentiated from all other members of *Enterobacteriaceae* on the basis of Tween 80 hydrolysis reagent.

TABLE 1. Reaction of *Enterobacteriaceae* in TB hydrolysis reagent

Species	No. of cultures tested	No. of positive cultures
<i>Citrobacter diversus</i> ( <i>intermedius</i> )	2	0
<i>C. freundii</i>	7	0
<i>Edwardsiella tarda</i>	1	0
<i>Enterobacter aerogenes</i>	22	0
<i>E. agglomerans</i>	2	0
<i>E. cloacae</i>	16	0
<i>E. hafniae</i>	6	0
<i>Escherichia coli</i>	43	0
<i>Klebsiella pneumoniae</i>	29	0
<i>Proteus mirabilis</i>	16	0
<i>P. morganii</i>	6	0
<i>P. rettgeri</i>	4	0
<i>P. vulgaris</i>	3	0
<i>Providencia</i>	6	0
<i>Salmonella arizonae</i>	1	0
<i>S. enteritidis</i>	4	0
<i>S. schottmuelleri</i> ( <i>paratyphi B</i> )	1	0
<i>Serratia marcescens</i> <sup>a</sup>	39	39
<i>Shigella sonnei</i>	5	0
<i>S. flexneri</i>	2	0
<i>Yersinia enterocolitica</i>	1	0

<sup>a</sup> Among 39 *Serratia* isolates, 26 were identified as *S. marcescens*, 12 as *S. liquefaciens*, and 1 as *S. rubidaea*, but only *S. marcescens* is recognized in *Bergey's Manual of Determinative Bacteriology*, 8th ed.

The test is simple to perform and economical because one vial of reagent (which has a shelf life of 1 year) is sufficient for 50 tests.

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