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Tween 80 Medium for Differentiating Nonpigmented *Serratia* from Other *Enterobacteriaceae*

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The ability of *Serratia* to hydrolyze Tween 80 can be utilized to distinguish this genus from other *Enterobacteriaceae*.

Recent recognition of *Serratia marcescens* in endocarditis and other diseases (2, 8, 14) has renewed interest in this microorganism. Previously, prodigiosin synthesis or red pigmentation had been useful in its identification; however, nonpigmented isolates are common (4, 14), and pigmented isolates often lose this phenotypic characteristic (10). Therefore, identification of *S. marcescens* in the clinical laboratory requires standard biochemical procedures (4). Deoxyribonuclease test media (5, 9) and corn oil lipase medium (3) have been useful, but these are nonspecific or have practical limitations. Tysset et al. (13) reported that Tween 80 was hydrolyzed by marine isolates of *Serratia*. The purpose of our study was to ascertain whether, among the *Enterobacteriaceae*, Tween 80 hydrolysis is specific for the genus *Serratia*.

Free fatty acid is released upon the hydrolysis of Tween 80. In the presence of Ca^{2+}, calcium oleate is precipitated, forming an opaque zone under and around the colony. The composition of our test medium, a modification of that of Sierra (11), is as follows: 3.3% (wt/vol) tryptose blood agar base (Difco Laboratories), 0.4% (vol/vol) Tween 80 (J. T. Baker Chemical Co.), and 0.015% (wt/vol) $CaCl_2$. Unless used shortly after preparation, plates are stored in sealed containers to prevent desiccation and remain satisfactory at least for 2 weeks. Plates are examined 24 and 48 h after incubation at 37°C. A positive reaction, normally observed within 24 h after inoculation, is a distinct precipitate in the agar (Fig. 1). The test also can be carried out successfully at lower temperatures.

The usefulness of the medium was demonstrated by testing 275 diverse clinical and food isolates as well as reference strains of *Enterobacteriaceae*, including most of the recognized species (Table 1). Initially, 62 representatives of 12 other bacterial groups were examined, but since results were variable, even within species, further studies were limited to *Enterobacteriaceae*. Among members of this family, only *Serratia* yielded positive reactions.

The differentiation of *Serratia* from other *Enterobacteriaceae* was examined in the following experiments. Two test groups consisting of *S. marcescens*, *Escherichia coli*, *Enterobacter aerogenes*, and *Citrobacter freundii* (group 1)

TABLE 1. *Enterobacteriaceae tested*

Microorganism	No. of strains
Citrobacter freundii	15
C. intermedius (C. diversus)	3
Edwardsiella tarda	2
Enterobacter aerogenes	4
E. cloacae	24
Escherichia coli	33
Erwinia amylovora	1
E. carotovoria	1
E. herbicola (Enterobacter agglomerans)	3
Hafnia alvei (Enterobacter hafniae)	14
Klebsiella ozaenae	1
K. pneumoniae	13
Proteus inconstans (Providencia)	3
P. mirabilia	6
P. morganii	2
P. rettgeri	1
P. vulgaris	2
Salmonella arizonae (Arizona hinshawii)	3
S. cholerae-suis	3
S. enteriditis (various serotypes)	30
S. paratyphi-A	2
S. schottmuelleri	2
S. spp.	9
S. typhimurium	2
S. typhi	5
Serratia marcescens	76[a]
Shigella boydii	1
S. dysenteriae	4
S. flexneri	4
S. sonnei	3
Yersinia pestis	2
Y. pseudotuberculosis	2
Total	275

[a] Only *S. marcescens* is recognized in Bergey's *Manual* of Determinative Bacteriology, 8th ed, although 49 specimens were identified as *S. marcescens*, 19 as *S. liquefaciens*, 2 as *S. rubidaea*, and 6 as *Serratia* sp. using the method of Ewing and Martin (6).

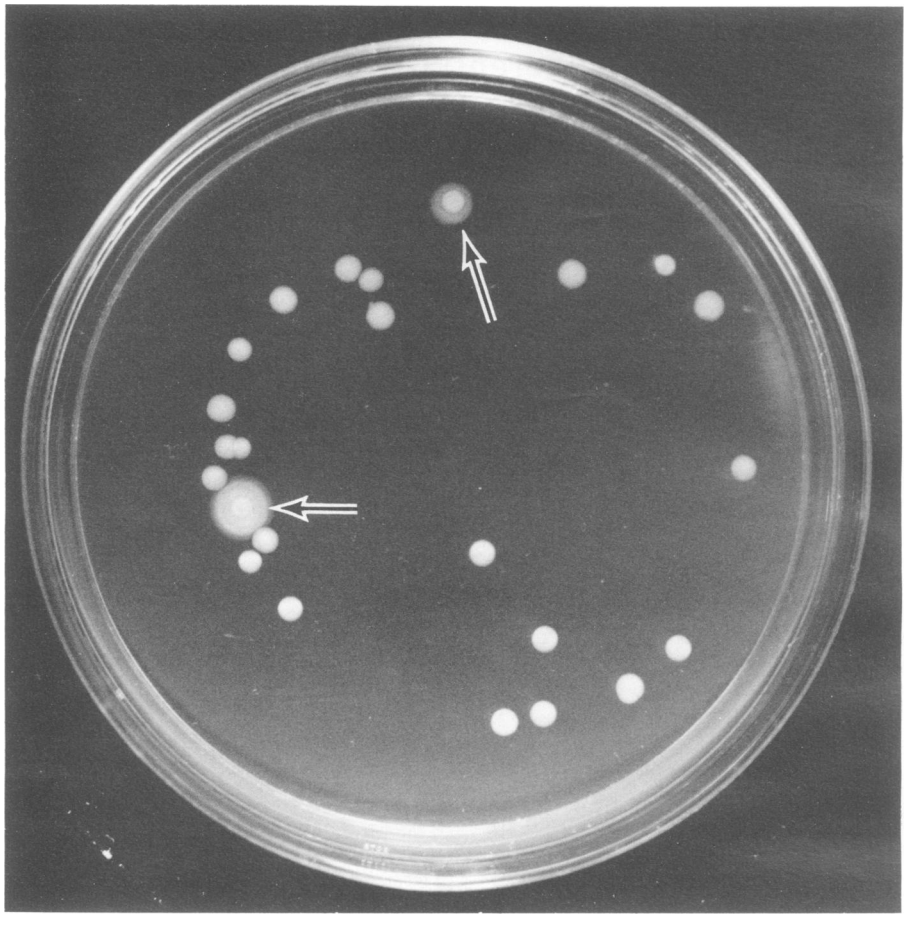

FIG. 1. *Colonies of S. marcescens with zones of precipitate (arrows) among colonies of S. enteriditis, H. alvei, and K. pneumoniae.*

and *S. marcescens*, *Salmonella enteriditis*, *Klebsiella pneumoniae*, and *Hafnia alvei* (group 2) were grown individually in 5 ml of Trypticase soy broth at 35°C for 18 h. In the first experiment, 1 ml of each of the four organisms in each group was pooled and diluted in a 10-fold series. Plates of the test medium were then inoculated with portions from each dilution tube. In the second experiment, 0.5 ml of each pooled group was inoculated into fresh broth and incubated for 18 h. These cultures were then treated as above. For both experiments, plates with well-isolated colonies (20 to 50) were selected (Fig. 1), and all isolates displaying positive or negative reactions were identified. As expected, only *Serratia* produced a zone of precipitate, and no false-negative reactions were detected.

The report of Davis and Ewing (3) is interesting in that lipase activity, tested on complex corn oil agar medium, was specific for *Serratia*, *E. liquefaciens* (now *Serratia*), and *Proteus* species, although some negative reactions among this group were noted. The enzyme that hydrolyzes water-soluble Tween 80 is not a true lipase (15), but an esterase, and should be treated separately. Microorganisms that have lipases do not necessarily split Tween 80 (5, 12). It appears that the test for Tween 80 hydrolysis is more specific and sensitive for *Serratia* than the lipase test, but substantiation will require the examination of many additional strains. The use of Tween 80/$CaCl_2$ agar medium can greatly simplify the identification of *Serratia*.

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