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Extracellular Deoxyribonucleases in Members of the Family Enterobacteriaceae

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The group differentiation of Enterobacteriaceae by biochemical tests that has been designed by Edwards and Ewing (*Identification of Enterobacteriaceae*, Burgess Publishing Co., Minneapolis, 1962) has provided a means for identification of a variety of gram-negative bacilli isolated from various clinical specimens in the diagnostic bacteriology laboratory. *Serratia* strains are occasionally isolated from clinical material such as urine, sputa, and wounds. The majority of these strains, in our experience, have not been pigment producers, and have sometimes been difficult to characterize rapidly because of similarities to other members of the Enterobacteriaceae which ferment lactose late or not at all. The production by the majority of *Serratia* strains of an extracellular deoxyribonuclease provides an ancillary biochemical test for the identification of this organism, and it may also serve as a screening test when selecting for *Serratia* strains in an epidemiological survey.

Although deoxyribonucleases have been found in extracts of a variety of microorganisms (Lehman, Progr. Nucleic Acid Res. **2**:83, 1963; Laskowski, *In* P. D. Boyer, H. A. Lardy, and K. Myrback [ed.], *The Enzymes*, vol. 5, Academic Press, Inc., New York, 1961, p. 145), extracellular deoxyribonucleases have thus far been reported from only a small number of species, such as *Staphylococcus aureus* (Cunningham et al., J. Am. Chem. Soc. **78**:4642, 1956), group A streptococci (Wannamaker, J. Exptl. Med. **107**:797, 1958), *Corynebacterium diphtheriae* (Messinova et al., Federation Proc. **22**:T1033, 1963), and *S. marcescens* (Eaves and Jeffries, J. Bacteriol. **85**:273, 1963).

An agar plate method for determining the extracellular hydrolytic activity of microorganisms on deoxyribonucleic acid (DNA) or ribonucleic acid has been described (Jeffries, Holtman, and Guse, J. Bacteriol. **73**:590, 1957); it involves the incorporation of nucleic acids in the agar media. Such a deoxyribonuclease test medium is commercially available (Difco), and was used in this study. A number of strains of gram-negative species were grown on this medium for 18 hr at 37 C. Deoxyribonuclease activity was assayed by lightly flooding the plates with 1 M HCl, which caused the DNA in the agar to form a diffuse, cloudy precipitate throughout the plate. Around those colonies which produced an extracellular deoxyribonuclease, a distinct, clear zone was observed as the result of cleavage of the DNA to smaller nonprecipitable oligonucleotide fragments. A survey for this enzymatic activity among the Enterobacteriaceae and among certain other groups of gram-negative bacilli isolated from clinical material in the hospital diagnostic laboratory is presented in Table 1.

TABLE 1. *Survey of gram-negative bacteria for presence of deoxyribonuclease*

Group	Strains tested	Deoxyribonuclease*		
		+	±	−
Escherichia coli	182	—	—	182
Proteus	117	1	8	108
Klebsiella	77	—	—	77
Serratia	58	56	2	—
Pseudomonas	59	—	3	56
Aerobacter	26	—	—	26
Citrobacter	10	—	—	10
Herellea	8	—	—	8
Alcaligenes	7	1	—	6
Providence	6	—	—	6
Salmonella	7	—	—	7
Mima	4	—	—	4
Arizona	1	—	—	1
Shigella	1	—	—	1
Aeromonas	1	—	—	1

* Symbols: + = zone of clearing greater than 2 mm from edge of colony; ± = zone of clearing 1 to 2 mm from edge of colony; − = no clearing.

All 58 strains of *Serratia* tested (including 20 strains kindly provided by W. H. Ewing, Communicable Disease Center, Atlanta, Ga.) showed the presence of this extracellular deoxyribonuclease. In most cases, the zones of clearing extended at least 3 mm from the colony edge.

The only other gram-negative organisms to show a large zone of clearing were one strain of *Proteus* and one of *Alcaligenes*. Several other *Proteus* strains showed small equivocal zones, but 92% of the *Proteus* strains showed absolutely no activity by this method. None of the strains of *Escherichia coli*, *Klebsiella*, or *Aerobacter* showed evidence of an extracellular deoxyribonuclease.

The presence of an extracellular deoxyribonuclease, under the particular assay conditions employed, in all strains of *Serratia* tested but only in a rare strain of other gram-negative bacilli provides an additional simple laboratory test for the identification of this organism.

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Regulatory Mechanisms in the Biosynthesis of Isoleucine and Valine

II. Identification of Two Operator Genes

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Volume 89, no. 3, p. 659, col. 1, line 2: change "sensitivity to acid" to "insensitivity to acid."

Regulatory Mechanisms in the Biosynthesis of Isoleucine and Valine

III. Map Order of the Structural Genes and Operator Genes

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Volume 89, no. 3, p. 662, col. 1, line 14 of "Results": change AB1514 to AB2070. Line 19 of "Results": change AB1206 to AB1005. Table 1: for strain AB1255 add *str-17* or *9* or *8*; add the genotype for strain AB2070: *ilvE12*, *metE46*, *try-3*, *his-4*, *thi-1*, *gal-2*, *lac-1* or *4*, *mal-1*, *mtl-1*, *str-8* or *9*, *T6r-3*, *pro-2*, *ara-9*.

Rhythmic Response of *Serratia marcescens* to Elevated Temperature

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Volume 89, no. 3, page 791, col. 2, line 12 of "Materials and Methods": Change "K_2HPO_4, 7.8 g" to "K_2HPO_4, 3.9 g."