Combined Medium to Determine Deoxyribonuclease Activity and Phenylalanine Deamination by Enterobacteriaceae

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Received for publication 19 September 1969

A combination medium has been developed to aid in the identification of Serratia and urease-negative Providencia recovered from clinical specimens.

Production of an extracellular deoxyribonuclease in agar media by Serratia marcescens has been demonstrated by a number of investigators. Jeffries and associates (4) observed the effect of varying pH and temperature on the ability of various organisms to hydrolyze nucleic acids. Oberhofer and Hajkowski (in press) and other workers (1, 3, 5, 6) have used the test for deoxyribonuclease activity to characterize rapidly the nonchromogenic strains of Serratia from other members of the Enterobacteriaceae which are biochemically similar. The test is based on the ability of the organisms to hydrolyze nucleic acids into smaller, nonprecipitable fragments.

The deamination of phenylalanine to phenylpyruvic acid by some Enterobacteriaceae is used for group differentiation of Proteus and Providencia species (2). The method requires inoculation of an agar slant containing phenylalanine and is used as the criterion for differentiating Providencia species from the urea-splitting Proteus rettgeri.

A combination of the agar plate method containing deoxyribonucleic acid (DNA) described by Jeffries et al. (4) and the phenylalanine agar method was used to test the activity of bacteria against these substances. Commercially available DNA (Difco) was added to Difco phenylalanine agar base. The medium has the following composition (grams/liter): DNA, 2.0; \( \alpha \)-L-phenylalanine, 2.0; yeast extract, 3.0; NaCl, 5.0; Na\(_2\)HPO\(_4\), 1.0; agar, 12.0. The final pH of the medium is 7.1.

A number of strains of gram-negative species, with the exception of swarming Proteus, were band-inoculated on this medium in a petri dish and grown for 18 to 24 hr at 37 C. Both the deoxyribonuclease activity and deamination of phenylalanine were demonstrated by flooding the plates with aqueous 10% (w/v) ferric chloride.

The reagent was sufficiently acid to precipitate the DNA. Deoxyribonuclease activity was indicated by the clear zone of hydrolyzed DNA surrounding the streak (Fig. 1). Those organisms deaminating phenylalanine were surrounded by a zone of green coloration.

VARIOUS MEMBERS OF THE Enterobacteriaceae and certain other groups of organisms isolated from clinical materials were screened to determine their activity on this medium (Table 1). Most organisms except Escherichia coli, Klebsiella-Enterobacter, and Pseudomonas species were stock cultures held at room temperature after isolation and identification by conventional methods (2, 3).
TABLE 1. Examination of gram-negative bacteria for the presence of deoxyribonuclease and phenylalanine deaminase

| Organism          | No. tested | Deoxyribonuclease | Phenylalanine deaminase |
|-------------------|------------|-------------------|-------------------------|
|                   |            | +     | ±      | -      | +     | ±      | -      |
| Serratia          | 126        | 126   | 2      | 6      | 126   | 2      | 6      |
| Alcaligenes       | 8          | 8     | -      | -      | 8     | -      | -      |
| Aeromonas         | 2          | 2     | -      | -      | 2     | -      | -      |
| Proteus vulgaris  | 12         | 4     | 8      | 12     | 2     | 6      | 3      |
| P. morganii       | 32         | 32    | 23     | 6      | 3     | -      | -      |
| Providencia       | 21         | 21    | 21     | 21     | -      | -      | -      |
| Escherichia coli  | 79         | 79    | 79     | 79     | -      | -      | -      |
| Klebsiella-Enterobacter | 72 | 72 | 72 | 72 | - | - | - |
| Herellea          | 25         | 25    | 25     | 25     | -      | -      | -      |
| Citrobacter       | 6          | 6     | 6      | 6      | -      | -      | -      |
| Salmonella        | 5          | 5     | 5      | 5      | -      | -      | -      |
| Pseudomonas       | 32         | 32    | 32     | 32     | -      | -      | -      |

* Symbols: +, clear zone exceeding 3 mm around the streak; ±, clear zone less than 3 mm around the streak; −, no zone.

* Symbols: +, color around streak intense green; ±, color around streak perceptible; −, no color.

The genera giving strongly positive and moderately positive deoxyribonuclease results were Serratia, Alcaligenes, Aeromonas, and Proteus. All 126 isolates of Serratia tested showed strong deoxyribonuclease activity and no deaminase production. The zones of clearing surrounding these organisms extended 3 to 5 mm from the streak. Four strains of P. vulgaris, two of Alcaligenes, and two of Aeromonas gave weakly positive results for deoxyribonuclease. The width of the zones for these organisms was 1 to 2 mm.

Of the 95 strains of Proteus and Providencia, 92 showed the presence of phenylpyruvic acid on this medium. Three strains of P. morganii were negative and six gave a weak deaminase reaction.

Use of this combined medium to test gram-negative bacilli for the production of extracellular deoxyribonuclease and the ability to deaminate phenylalanine provides a simple, one-step laboratory test. The medium can be used as a part of the routine biochemical testing to assist in the identification of organisms isolated from clinical specimens.

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