A RAPID TEST FOR PROTEUS SPECIES

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Rapid identification of Proteus species can represent a significant saving of time in the medical bacteriology laboratory. It is usually 2 days from receipt of the specimen to the reading of the differential slant. At this point, it is customary to inoculate one of the urea media (Edwards and Ewing, Identification of Enterobacteriaceae, Burgess Publishing Co., Minneapolis, 1955), read the result the following day, and then proceed with generic identification. Rapid urease tests have been described (Stuart, VanStratum, and Rustigian, J. Bacteriol. 49:135, 1945) and can be employed to identify Proteus species within 1 hr. The following procedure, modified from Shaw and Clarke (J. Gen. Microbiol. 13:155, 1955), may be employed to identify Proteus species from differential slants within 10 min, thereby speeding generic diagnosis by 1 day. The procedure is based on the presence of phenylalanine oxidase in Proteus species and the apparent absence of this enzyme from other enteric bacteria tested.

A 0.4% solution of D-phenylalanine (reagent grade) is sterilized by autoclaving. Solutions (10%) of H₂SO₄ and FeCl₃ are prepared in sterile distilled water; all three reagents are kept on the laboratory bench.

To carry out the test, approximately 1 ml of the sterile phenylalanine solution is added to a 10 by 75 mm Kahn tube or similar, small, sterile test tube; a large (4 mm) loopful of the organism to be tested is taken from the differential slant and emulsified in the amino acid solution. The suspension is incubated at 37°C for 10 min. To the colorless suspension are added 2 drops or 0.1 ml of the H₂SO₄ solution and, after shaking, 2 drops or 0.1 ml of the FeCl₃ solution. The appearance of a green color, without regard to intensity of color, constitutes a positive test (for phenylpyruvic acid) and characterizes the organism under test as a member of the genus Proteus.

Shaw and Clarke (J. Gen. Microbiol. 13:155, 1955) employed a 1-hr incubation period in a buffered solution of the amino acid and found that all of 118 cultures of Proteus and Providence group organisms were positive by the phenylalanine oxidase test and that 75 other cultures of Enterobacteriaceae were negative. The modification of the test proposed here has been evaluated over a 4-month period. Every Proteus species and Providence group species isolated, as well as numerous other isolates, have been tested with the following results: 68 Proteus and 2 Providence group species were positive; negative results were obtained with 76 Escherichia, 5 Paracolobactrum, 3 Salmonella, 4 Aerobacter, 1 Alcaligenes, 1 Shigella, and 34 Pseudomonas species.

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POLYVINYLPERYRROLIDONE AS AN INDICATOR FOR MEASURING INTERCELLULAR SPACE IN PACKED-CELL PELLETS

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In a study of salt uptake of unicellular green algae, a method was required for determining the intercellular space in a packed-cell pellet of centrifuged algal cells. It was necessary that the indicating substance be unaffected by the presence of algal cells, culture media, and organic compounds extracted from cells when heated to 100°C in aqueous mixtures. The indicator had to be
The supernatant fluid (1 ml) was diluted with 0.4 M citric acid to bring the PVP concentration within the range of linear absorbance (25 to 250 mg per 10 ml). (vi) A 0.006 N potassium triiodide solution (2 ml) was added to 10 ml of diluted sample, and the absorption at 500 nm was determined and related to a standard curve. When extraction of the cells was necessary, the resuspended pellet (iv) was heated to 80°C for 10 min prior to recentrifugation. The total pellet volume was read from the calibrated capillary tube. The volume of intercellular space was calculated from the PVP analysis. Table 1 illustrates the precision of this method. Another test demonstrated that PVP was not removed from solution during incubation with different quantities of living cells. A third test showed that neither the hot-water extraction procedure nor the cellular extract altered PVP analyses.

Application of this method to cultures containing varying concentrations of natural sea water showed a strong interference with PVP determination. Fortunately, this interference is completely saturated at 8% (v/v) natural sea water in the culture medium. No further increase in absorption at 500 nm was observed on addition of up to 1 ml of 90% sea water to 12 ml of reaction mixture (v and vi). In all future analyses and in standard curves relating optical density to PVP concentration, saturating levels of natural sea water were added to the citric acid buffer solution. No further correction was necessary. Table 2 confirms the effectiveness of this procedure.

Polyvinylpyrrolidone has proved to be a superior indicator for the determination of intercellular space in algal suspensions and should be applicable in work with other microorganisms.