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**:435, 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 DL-phenylalanine (reagent grade) is sterilized by autoclaving. Solutions (10%) of H_2SO_4 and $FeCl_3$ 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 H_2SO_4 solution and, after shaking, 2 drops or 0.1 ml of the $FeCl_3$ 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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POLYVINYLPYRROLIDONE 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

TABLE 1. *Measurement of intercellular fluid volume in packed-cell pellets of Scenedesmus obliquus**

Pellet volume	PVP recovered from pellet	Calculated PVP space	Intercellular volume†
μliters	*μg*	*μliters*	%
5.6	33	3.85	68.8
10.6	60	7.00	66.0
16.0	96	11.2	69.9
21.0	123	14.4	68.5
26.9	159	18.6	69.2
33.5	189	22.1	66.0

* Polyvinylpyrrolidone (PVP) was added to the algal culture to give a final concentration of 8.57×10^3 μg per ml. Each pellet represented the algae centrifuged from 3 ml of culture medium. All values represent the average of duplicate determinations.

† Obtained from: PVP space × 100 ÷ pellet volume.

nontoxic, water soluble, nonmetabolizable, and of large enough molecular size to prevent its penetration into the cells. Finally, an accurate assay had to be obtained with the amount of indicator dissolved in the intercellular fluid of packed-cell pellets of 10 to 40 μliters total volume. The conventional "inulin space" method, detailed by Ross and Mokotoff (J. Biol. Chem. **190**:659, 1951), proved unsatisfactory because of the large amounts of interfering substances present in the culture medium and released from the cells during hot-water extraction. Polyvinylpyrrolidone (PVP) type NP-K30 (Antara Chemicals, New York, N.Y.) seemed to meet most of the requirements listed above. Its average molecular weight (40,000) precluded its penetration of algal cell walls.

A method for the analysis of μg amounts of PVP (Levy and Fergus, Anal. Chem. **25**:1408, 1953) was adapted to give the following procedure. (i) A 0.5-ml amount of PVP stock solution (6.0×10^4 μg per ml) was thoroughly mixed with a known volume of algal culture in a capillary-tipped centrifuge tube (graduated in 1-μliter units from 0 to 50 μliters). (ii) The mixture was centrifuged to give a uniformly packed pellet. (iii) The supernatant fluid was removed with suction without disturbing the pellet, and the walls were thoroughly washed with distilled water to remove residual PVP. (iv) The pellet was then resuspended in a measured volume of water and recentrifuged. (v) The cell-free 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 μg per 10 ml). (vi) A 0.006 N potassium triodide solution (2 ml) was added to 10 ml of diluted sample, and the absorption at 500 mμ 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.

TABLE 2. *Polyvinylpyrrolidone (PVP) analyses conducted in the presence of different amounts of sea water**

Sea water concn of culture media (v/v)	Amount of PVP per sample (μg)		
	60	120	240
%			
0	0.455	0.675	1.13
10	0.458	0.680	1.13
80	0.455	0.685	1.13

* To eliminate the interference, 0.33% (v/v) sea water was added to the reaction mixture in all cases. Data are optical densities of reaction mixtures read at 500 mμ.

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 mμ 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.

Polyvinylpyrollidone 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.