(3) A return tube from the glass dome leads back to the top of the chamber. Air displaced from the chromatography tank by the incoming fluid escapes through the hole in the glass plate into the dome and passes via the return tube to the chamber, thus forming a completely closed system.

Added rigidity is afforded by mounting the apparatus on a "tufnol" plate, to which the glass chamber, stopcock, and rotary switch are firmly secured.

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REFERENCE

Bush, I. E. (1952). *Biochem. J.*, **50**, 370.

Practical Laboratory Tests for the Identification of *Proteus* Strains

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The rapid identification of *Proteus* strains is of importance since these relatively non-pathogenic micro-organisms are easily confused with other lactose - negative, pathogenic bacteria such as *Salmonellae*.

In many laboratories *Proteus* species are identified by their urease activity, usually by inoculating bacterial cells into a medium containing urea plus an indicator and noting the colour change after four to eight hours (Rustigian and Stuart, 1943). Since urea is decomposed by autoclaving, the medium must be sterilized by Seitz filtration. This complicates the technique and increases the possibility of contamination.

Proom and Woiwod (1951) proposed the use of a method based on the ability of certain *Proteus* species to form volatile amines. The procedure, however, is rather complicated and could hardly be used for routine diagnostic purposes.

More recently Singer and Volcani (1955) have described a simple and rapid method, based on the decomposition of tryptophan by *Proteus* cells to form a compound giving a brown colour with ferric chloride.

The present paper describes two modified techniques, one for detecting urease activity, and the other a simple spot test for the detection of volatile amines. The two modified methods are compared with the tryptophan-ferric chloride test of Singer and Volcani (1955).

Reagents

Procedure A.—The following are required:

Urea Solution.—Dissolve 1.0 g. urea and 0.1 g. phenolphthalein in 10 ml. 96% ethyl alcohol.

Saline Solution.—Make up 8.5 g. sodium chloride in 100 ml. distilled water.

Dilute Hydrochloric Acid Solution.—Add 1 ml. concentrated hydrochloric acid to 100 ml. distilled water.

Sodium Hydroxide Solution.—Add 0.4 g. sodium hydroxide to 100 ml. distilled water.

Procedure B.—The following are required:

2:4 Dinitro-fluoro-benzene Reagent (D.N.F.B.).—Add 0.65 ml. dinitro-fluoro-benzene to 50 ml. acetone.

Potassium Hydroxide Solution.—Dissolve 40 g. potassium hydroxide in 100 ml. distilled water.

Technique

Procedure A: Modified Urease Test.—Sodium hydroxide solution is added drop by drop to the urea solution until the indicator becomes pink. Diluted hydrochloric acid is then added till the solution just becomes colourless. Whatman No. 3 filter paper is impregnated with the colourless urea solution, dried at room temperature, and cut into strips (15×5 mm.) which are kept in a dry test-tube.

The bacterial strain to be tested is grown on a liquid or a solid medium. If a liquid medium is used the cells are harvested by centrifugation (2,500 r.p.m.) and the supernatant discarded. Bacteria grown on solid media are scraped off with a bacteriological needle and suspended in 0.1 ml. saline solution in a small test-tube (9×100 mm.). After immersing the urea-impregnated filter paper strip in the bacterial suspension the test-tube is placed in a 37° C. water-bath and examined after one to two hours. A red coloration of the filter paper strip indicates the presence of urease in the bacterial cell.

Procedure B: Detection of Volatile Amines by D.N.F.B.—The strain to be tested is grown in nutrient broth (Difco) for 16 to 20 hours. Potassium hydroxide solution is now added to make the medium alkaline.

A filter paper previously impregnated with 2:4 dinitro-fluoro-benzene reagent (D.N.F.B.) is placed over the mouth of the test-tube and held down tightly by a rubber band. The tube is then placed in a boiling water-bath for 15 minutes. Care must be taken that the solution in the test-tube does not come into contact with the impregnated filter paper strip. The upper part of the test-tube should be absolutely dry so that only volatile substances reach the filter paper. A yellow coloration of the impregnated filter paper indicates the presence of volatile amines.

If the reaction is doubtful a drop of concentrated hydrochloric acid is placed on the filter paper and if the colour persists the reaction for volatile amines is positive.

Results

Table I shows that out of all the *Enterobacteriaceae* tested only *Proteus* and *Providencia* strains gave

TABLE I
BIOCHEMICAL ACTIVITIES OF VARIOUS
ENTEROBACTERIACEAE

Bacteria Tested	Total No. of Strains	No. of Strains					
		Urease		D.N.F.B.		Tryptophan-FeCl ₃	
		Positive	Negative	Positive	Negative	Positive	Negative
<i>Proteus vulgaris</i>	46	46		46		46	
" <i>mirabilis</i>	10	10		10		10	
" <i>morganii</i>	10	10		10		10	
" <i>rettgeri</i>	16	16		2	14	16	
<i>Providencia</i> strains ..	22		22	6	16	22	
<i>Salmonella</i> ..	7		7		7		7
<i>Shigella</i> ..	7		7		7		7
<i>Escherichia coli</i> ..	7		7		7		7

positive tryptophan-ferric chloride reactions. This is in agreement with the findings of other authors (Singer and Volcani, 1955; Falkow, 1957; Thibault and Le Minor, 1957).

The urease filter paper strip test described was positive with all *Proteus* species, but negative with *Providencia* strains. This modified urease test proved to be useful also for other bacteria, such as *Corynebacterium pseudodiphtheriticum*, which are known to split urea (cf. Breed, Murray, and Hitchens, 1948). Bacteria grown on various solid media like blood agar, MacConkey, or Kligler iron agar medium, were also used. In some cases when growth from alkaline media was used the filter paper strip became pink immediately after its immersion into the bacterial suspension, but this pink colour faded on further incubation if the bacterial cells were unable to hydrolyse urea. It is thus essential to check the results after one to two hours to avoid false positive results.

The modification described has the advantage that a pure culture is not required as contamination will not interfere with the result of the test.

The method for the detection of volatile amines is based on the quantitative D.N.F.B. assay method described elsewhere (Bachrach, Segal, and Rozansky, 1958). The modified method was positive with *Proteus vulgaris*, *mirabilis*, and *morganii*, whereas *Proteus rettgeri* and *Providencia* strains gave variable results as already described by Proom (1955). *Streptococcus faecalis* also gave a positive D.N.F.B. result, but the amine responsible for this reaction is phenylethylamine (Bachrach, Gery, Sterk, and Rozansky, 1958), and not isoamyl and isobutyl amines which are produced by *Proteus* (Ekladius, King, and Sutton, 1957).

Summary

Two simple and rapid methods for the identification of *Proteus* strains are described. One is a modified urease test employing a filter paper strip impregnated with urea and indicator.

The second method is based on the detection of volatile amines by a filter paper impregnated with 2:4 dinitro-fluoro-benzene.

Results are compared with those of the tryptophan-ferric chloride test.

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REFERENCES

- Bachrach, U., Segal, M., and Rozansky, R. (1958). *Proc. Soc. exp. Biol. (N.Y.)*, **97**, 874.
— Gery, I., Sterk, V. V., and Rozansky, R. (1958). *Bull. Res. Coun. Israel*, **7E**, 158.
Breed, R. S., Murray, E. G. D., and Hitchens, A. Parker (1948). *Bergey's Manual of Determinative Bacteriology*, 6th ed., p. 617. The Williams and Wilkins Co., Baltimore.
Ekladius, L., King, H. K., and Sutton, C. R. (1957). *J. gen. Microbiol.*, **17**, 602.
Falkow, S. (1957). *Amer. J. clin. Path.*, **28**, 99.
Proom, H. (1955). *J. gen. Microbiol.*, **13**, 170.
— and Woiod, A. J. (1951). *Ibid.*, **5**, 930.
Rustigian, R., and Stuart, C. A. (1943). *Proc. Soc. exp. Biol. (N.Y.)*, **53**, 241.
Singer, J., and Volcani, B. E. (1955). *J. Bact.*, **69**, 303.
Thibault, F., and Le Minor, L. (1957). *Ann. Inst. Pasteur*, **92**, 551.