NOTES

A VERSATILE MEDIUM FOR THE CULTIVATION OF ERYSIPELOTHRIX RHUSIOPATHIAE

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Versatility is a desirable, though often forfeited, characteristic of a bacteriological medium. This factor is especially wanting in the media designed for the cultivation of *Erysipelothrix rhusiopathiae* as indicated by the numerous preparations described and employed by various investigators who have worked with this organism.

It is the object of this note to describe a medium for the cultivation of *E. rhusiopathiae* which, in addition to being simple to prepare, relatively inexpensive, and biologically nourishing, appears to embody this desirable characteristic of versatility.

When the following preparation is adjusted to pH 7.6 with 0.5 N NaOH prior to autoclaving at 121 C for 15 min, a small inoculum will result in heavy growth of the organism after 24 hr of incubation at 37 C: Tryptose broth (Difco), 2.6 g; nutrient broth (Difco), 0.8 g; distilled water to 100.0 ml.

This "nutrient-tryptose broth" can be solidified by the addition of 1.5 to 2.0 per cent agar or used as a semisolid medium through the addition of 0.15 to 0.2 per cent agar.

Observations indicate this to be an excellent medium for the isolation of *E. rhusiopathiae* from blood and tissues, as a means of obtaining a large number of cells, for the carrying of a culture over a period of time, as a medium for making plate counts of *E. rhusiopathiae*, and as a medium for the reconstitution of lyophilized stock cultures. For general use the broth form appears to be the most suitable; however, for the prolonged maintenance of a culture the semisolid appears to be the form of choice.

The morphological, biochemical, and pathogenic characteristics of this organism were not altered perceptibly through the use of this medium. Both the rough and smooth forms grew equally well.

Other applications of this medium to *E. rhusiopathiae* will appear in forthcoming publications.

A SIMPLE METHOD FOR ISOLATING INDIVIDUAL MICROBES[1]

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Many methods have been proposed for single cell isolation with or without gadgetry (Hildebrand, Botan. Rev., **16**, 181, 1950). Another procedure is described now, not for any novelty in its principles, but to encourage its wider application in teaching and research.

The micrurgical use of an oil chamber has been developed and popularized by de Fonbrune (*Technique de micromanipulation*, Masson Cie, Paris, 1949) primarily as an adjunct to the pneumatic micromanipulator, but the advantages of this chamber for routine purposes need only a trial to be appreciated. For present purposes, the oil chamber merely needs to consist of a clean cover glass or microscope slide, ruled on the reverse in 4 mm squares with india ink. The face

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of the slide is sterilized in a flame, cooled, and coated with paraffin oil (White Mineral Oil, USP) to a depth of about 0.5 mm. De Fonbrune advises against attempting to sterilize the oil, and my own experience on this advice has been satisfactory. A capillary pipette is drawn by hand from 4 mm glass tubing to a terminal diameter of about 0.1 mm and controlled by mouth by a connecting rubber tube. It is filled partially with the microbic suspension diluted in its growth medium to a density of 10^6 to 10^7 per ml. A drop of 10^{-7} to 10^{-6} ml is deposited then at the center of each square under the oil. This soon spreads to a diameter of 0.1 to 0.2 mm as it adheres to the glass; the flattening provides excellent optical conditions for the search of each drop by phase contrast, dark field, or low aperture microscopy. The drops that have been verified to contain precisely one cell then are recorded. Immediate recovery of the individual microbes often can be accomplished by repeated flushing of the drops in and out of the capillary pipette. More consistent recovery of clones derived from single cells, approaching 100 per cent with enteric bacteria, is achieved by adding about 10^{-5} ml additional fluid medium to each drop and incubating the slides in a container of oil, e.g., a staining dish, until a large clone develops that can be transferred wherever required by a capillary pipette. The slide can be held safely in a vertical position if the drops are not too large. Uninoculated drops and isolations from known mixtures should be followed, of course, as controls.

A more elaborate chamber for prolonged incubation consists of a rectangular well, built on a slide and filled with oil, over which a cover glass preparation may be inverted. Capillary pipettes drawn from quartz tubing also have proven useful, for they can be sterilized quickly by flaming after a brief rinse to remove surplus solids. The oil "chamber" may be recommended also for any studies that require protracted examination of small culture volumes: cell lineages, motility, agglutination, chemotaxis, and the like. More complicated single cell analyses are done better with micromanipulatory aids, but the present method may find routine applications in any laboratory, whenever doubt arises as to the adequacy of conventional plating methods, to answer questions of clonal purity.

AN INDUCED 6,8[1] FORM FROM SALMONELLA PAPUANA[2]

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In 1948 Bruner and Edwards (J. Bact., **55**, 449) reported on the intertransformability of *Salmonella anatum* and *Salmonella newington*. The changes involved were concerned with induction of form variation in the O antigens of these serologic types by growing them in high concentrations of absorbed serums. The transformations were between members of the subgroups of somatic group E. In studying induced form variation within the subgroups of somatic group C, the 6,8 form described in this note was obtained.

O sera 6,7 and 6,8 were prepared by injecting rabbits with boiled cultures of *Salmonella oslo* and *S. narashino*, respectively. To obtain single factor sera 7 and 8, the 6,7 serum was absorbed by *S. narashino* (6,8: a-enx) and the 6,8 serum by *S. oslo* (6,7: a-enx). The antigens used were 0.5 per cent phenolized suspensions of these microorganisms, and the technique employed in making the adsorptions has been outlined in detail by Edwards and Bruner (Ky. Agr. Expt. Station Cir. 54, 13, 1942). The homologous O titers for these absorbed serums were 1 to 500, and they were added to semisolid medium to make final concentrations of 1 to 15.

Cultures tested of the 6,7 subgroup were: *S. oslo*, *S. mission*, *S. lomita*, *S. concord*, *S. infantis*, and *S. papuana*. Within the 6,8 subgroup cultures

[1] In accordance with a recent decision of the Enterobacteriaceae Subcommittee of the International Association of Microbiologists, the O antigens of Salmonella types are designated here by arabic numerals.

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[3] Technical assistance by Wiene van Thiel.