A SIMPLE METHOD FOR THE ISOLATION OF PURE CULTURES FROM SINGLE BACTERIAL CELLS.

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The use of "pure line" strains of bacteria is required in many problems encountered in bacteriological research. A number of methods have been devised for obtaining cultures which represent the progeny derived from a single cell. Most of these methods possess at least one of the following disadvantages: (1) intricate apparatus, (2) complicated technique, (3) loss of time, due to the frequent failure of a single cell to grow when transplanted.

During studies on variants occurring in streptococcus cultures, a simple method was devised by which pure line strains can be obtained with comparative ease. This method represents a modification of the Hansen[1] method for the isolation of pure cultures of yeast. Although Hansen's method has been employed extensively in the fermentation industries for a number of years, its most advantageous features have not been incorporated in any of the previous bacteriological methods. The essential features of the method as adapted to the isolation of single cell cultures of bacteria are described below.

Apparatus and Materials.

Cover-Glass.—A large 43 × 50 mm. cover-glass, 0.13 mm. thick, is ruled into squares of 4 sq. mm. area by etching with a fine needle and hydrofluoric acid through paraffin. The system of numbering the squares is illustrated in Fig. 1. *Ring.*—The cover-glass is supported on a metal ring. Convenient dimensions proved to be 35 mm. diameter, 3 mm. height and 1 mm. thickness. The ring can be cut

[1] Hansen, E. C., *Meddelelser Carlsberg Lab.*, 1886, ii, No. 4, 152.

from brass tubing. The metal side arms are attached for convenience in handling. *Glass Slide.*—The chamber is formed by attaching the ring to a large slide 50 × 75 × 1 mm. The assembled apparatus is shown in Fig. 2.

Inoculating Spatula.—The spatula used for inoculation is formed from a glass rod 4 mm. in diameter. One end is drawn out in a flame to 2 mm. in diameter, and the tip flattened and bent at a convenient angle.

Culture Medium.—Any clear solid medium suitable for the cultivation of the test organism may be used provided it is made as free as possible from all extraneous particulate matter. Freedom from particles in the culture medium is essential to the success of the method.

Technique.

All glassware should be scrupulously clean before use. Rings, glass slides and cover-slips are sterilized in separate Petri dishes by dry heat. Aseptic precautions are followed throughout the technique. The assembling of the different parts is done under the protection of the lifted Petri dish cover. The metal ring is dipped in hot paraffin and then placed in contact with the unetched side of the cover-slip. The paraffin is allowed to harden. The surface of the cover-glass within the ring is flooded with melted agar sufficient to cover the entire area. In order that the film formed may be the thinnest possible, a warm pipette is held at right angles to the surface and all the recoverable agar is withdrawn and discarded. After this delicate film of agar has hardened it is inoculated as follows: A loopful of a young broth culture is transferred to 5 cc. of infusion broth and thoroughly mixed. The sterile glass spatula is dipped into the dilute bacterial suspension and the excess drained off against the side of the tube. The spatula carrying the minimum inoculum is lightly stroked over the entire surface of the film. The inoculated cover-glass and attached ring are inverted and sealed to the large glass slide which has been previously ringed with paraffin.

The preparation is then examined under the microscope with a high dry objective. After a thorough search, squares containing only one organism are selected and the location of each plotted on a diagram with reference to the index number. The location of the

cell within the selected square is also recorded on the diagram. The orientation is further facilitated by the use of a mechanical stage. Usually six or seven squares, each containing a single organism, are plotted to insure against any failure of growth.

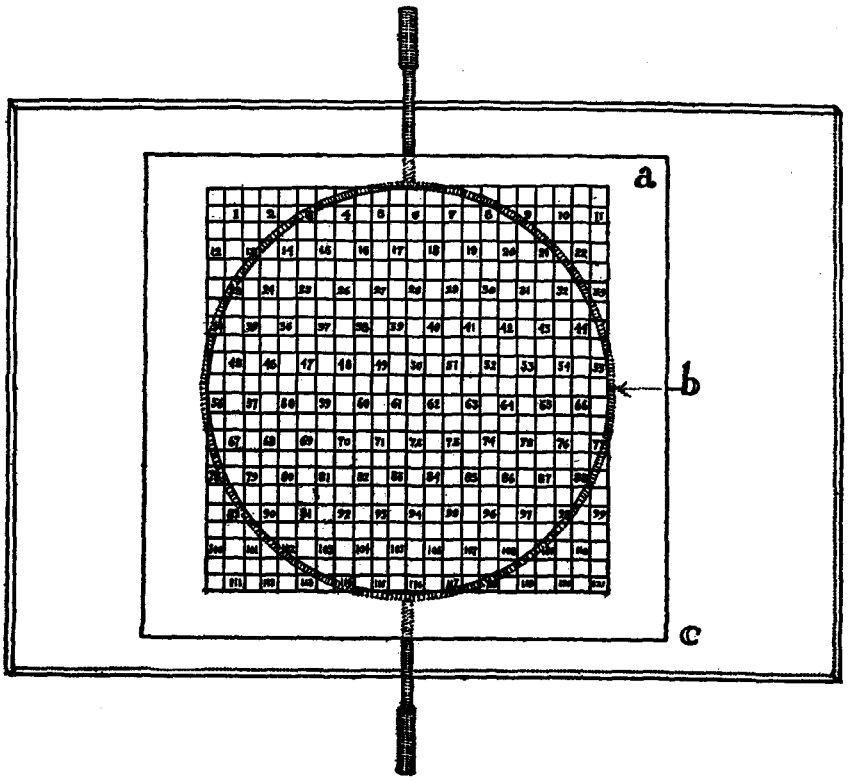

FIG. 1. Top view of apparatus. (*a*) Cover-glass; note that all squares not numbered are adjacent to numbered ones. (*b*) Brass ring and side arms. (*c*) Glass slide.

The entire apparatus can now be removed from the microscope stage and incubated at 37°C. until colony formation is well developed. At the end of the incubation period the length of which is determined by the growth rate of the particular organism, the preparation is again examined microscopically. With the aid of the diagram and the stage readings, the fields previously selected are located and the

individual square is again searched to make certain that it contains only a single colony. As each individual square is checked, its position on the cover-glass is indicated by an ink dot made with a fine pointed pen while the preparation is under low power magnification. The ring carrying the cover-glass is then removed and by means of a fixed reading lens the colony indicated by the dot is transferred with a fine platinum needle to a suitable culture medium.

The principal sources of error in the method are practically eliminated by the selection of cover-glasses of the proper thickness and by the use of a culture medium which is wholly translucent and free as possible from all microscopic particles. If the agar is spread in a very thin film, little difficulty is experienced in the selection of single bacterial cells, when the illumination is adjusted. The moisture of

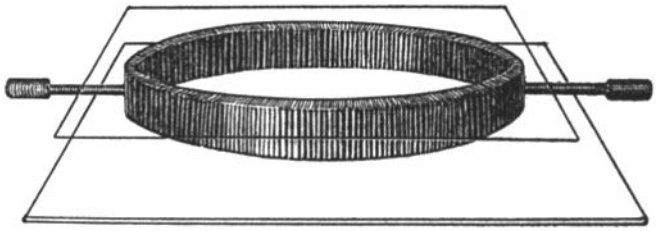

FIG. 2. Side view of apparatus.

the shallow chamber is maintained by the agar medium, which makes it possible to omit the excess fluid usually added in moist chamber preparations The excess moisture is not only unnecessary but is undesirable since the coalescence of drops of condensed water befog the field of vision and tend to cause the migration of bacterial cells.

COMMENT.

The procedure as outlined has been used in the study of streptococci and pneumococci but it is equally adapted to the isolation of other aerobic organisms and by slight modification might be made useful in the study of anaerobic types.

The method is simple in operation and no special technique is required to obtain the results desired. That several selected colonies can be isolated from the same preparation is most advantageous,

since the chance of successful transplant is thereby correspondingly increased. Moreover, the fact that a number of different colonies can be kept under observation on the same preparation, makes the method particularly adaptable to studies on variation in colony structure. In a successful preparation, it is often possible to obtain a dozen colonies, each of which is known to be derived from a single cell. This is a distinct advantage in the study of the origin of variants which differ in colony structure.

SUMMARY.

A method is described for the isolation of pure cultures from single bacterial cells, which is simple in operation and which requires no complicated apparatus. In addition to its simplicity, the method possesses the further advantage that several selected colonies can be isolated from the same preparation.