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SURFACE GROWTH OF BACTERIA ON CELLOPHANE[1]

BY G. B. REED[2] AND D. G. MCKERCHER[3]

Abstract

A method is described for the cultivation of bacteria on the surface of sheets of cellophane spread over layers of absorbent material as cotton saturated with any desired fluid medium. The method has proved to be useful in preparing suspensions of bacteria reasonably free from contamination by the culture medium.

In the many undertakings that require the surface growth of bacteria in mass, as the preparation of certain antigens, agar is at times a disturbing factor. During the recent period of agar scarcity a procedure was developed that has more recently been used in order to avoid agar. The procedure consists in spreading a sheet of cellophane over a layer of absorbent material saturated with the desired fluid culture medium and cultivating the organisms on the surface of the cellophane.

The method is of most value where larger surfaces than provided by ordinary Petri dishes are required. Enamel baking dishes, 1 to 2 in. deep, with nearly perpendicular sides, with a slightly larger dish used as a cover, as a Petri dish, are satisfactory. For still larger areas ordinary plastic cafeteria trays* have proved convenient. Where only a small number are used each tray may be conveniently enclosed in a heavy paper envelope with an opening at one end. For larger numbers of trays it is convenient to equip a large rectangular autoclave with racks that will permit sliding one tray above another, without covers, in the manner of a chest of drawers. When the autoclave door is opened the trays may be withdrawn individually or pulled out part way, like drawers, for inoculation. Such an autoclave must be further equipped with a thermoregulator that will permit operation as an autoclave at 120° C. and later as a steam heated incubator.**

Various grades of cellophane, with the exception of "moisture proof" are satisfactory. "Plain cellophane,"*** No. 300, which is listed as 0.00088 in. thick or No. 450, 0.0012 in. thick are satisfactory. These may be obtained in sheets cut to size or in rolls cut to any desired width.

Various absorbent materials have been used to hold fluid media and support the layers of cellophane. Filter pulp, as supplied by Reeve Angel in sheets

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* *New trays should be soaked in water overnight and well steamed before use.*
** *Such thermoregulators and steam control valves may be obtained from the Bristol Company of Canada, Limited, Toronto.*
*** *Canadian Industries Ltd., Cellophane Division, Toronto.*

about 1 cm. thick, may be cut to fit the trays, enough fluid medium is added to completely saturate the pulp, the cellophane sheet added and firmly rolled down with a photographic print roller. Absorbent cotton, about half the thickness of the ordinary roll, is equally good when similarly treated. Bran, meal, or peat have also been used with good results. Enough fluid medium is added to the material to make a thick paste that is spread roughly in the tray, a sheet of cellophane is added and rolled smooth. It is not necessary to use clarified fluid medium as it filters through the cellophane.

In one large series of cultures the fluid medium consisted of 7% crude corn steep liquor in water without clarification. This was mixed with the following proportions of crushed peat, as used by Lochhead and Thexton (1), for the storage of bacteria, and asbestos fiber as used for insulation.

For 25 trays (15 × 20 in.)

Crude corn steep liquor	1200 ml.
Tap water	16,000 ml.
Crushed peat	2 kgm.
Asbestos fiber	4 kgm.

The acid corn steep and acid peat are neutralized by the alkaline asbestos to give a well buffered medium, after autoclaving, of pH 7.2 to 7.4.

The only difficulty in handling this material is to prevent curling of the cellophane in the autoclave. This can be avoided by preventing the formation of a vacuum as the autoclave cools. A water manometer should be attached to the autoclave. In most autoclaves this can be done very simply by introducing a T in the pipe leading to the pressure gauge and providing the side arm with a valve and a small glass manometer. In operation, as the autoclave cools and the pressure gauge approximates zero, the valve to the manometer may be cracked momentarily at short intervals until the manometer just registers zero. The air port on the autoclave is then opened; this prevents vacuum formation on further cooling. Incidently the procedure is of value in the preparation of coagulated serum or egg slants.

The cellophane surface may be inoculated in a variety of ways. A few drops of inoculum added with a pipette spreads readily with an angle glass rod or the angle rod may be wrapped with gauze and kept moist by adding inoculum drop by drop.

The period of growth and the method of harvesting will vary with the type of organism. Cells of more butyrous growths or the almost woolly growths of some species of *Bacillus*, before sporulation is complete, can be readily harvested by scraping the surface of the cellophane with a square of glass, as a lantern slide cover, and pushing the mass of cells from the edge of the glass into a beaker with a glass spatula. Cell masses in more watery growths, as most *Salmonella* species or spores of *Bacillus* after the vegetative rods have

autolyzed, may be more readily collected with a vacuum device. Such a device may be made of about $\frac{1}{4}$ in. stainless steel tubing in the form of a T with the cross arm 3 to 4 in. long carrying a very narrow slit, 0.2 to 0.5 mm. The long arm of the T, which serves as a handle, is connected by rubber tubing to a receiving vacuum bottle. It is readily possible by this method to collect vegetative cells or spores in the form of a thin paste with the order of 10^{10} to 10^{12} cells per ml.

Reference

1. LOCHHEAD, A. G. and THEXTON, R. H. Can. J. Research, C, 25 : 1. 1947.