Improved Surface Culturing of Mycobacterium tuberculosis

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Received for publication 11 May 1967

In studies on surface components of virulent mycobacteria, there is a continuous need for relatively large quantities of these organisms as produced in surface culture in Roux bottles. Achieving effective surface growth depends upon adequately seeding the surface of the liquid medium. A practice which may well have been employed even by Koch has been the transfer of a pellicle of "veil growth" from a mature surface culture into a fresh flask, and lowering the pellicle gently onto the surface of the liquid medium (H. S. Willis and M. M. Cummings, Diagnostic and Experimental Methods in Tuberculosis, p. 111, Charles C Thomas, Publisher, Springfield, Ill., 1952).

The "donor" veil may be obtained according to a method described by H. Zinsser (Microbiology, p. 512, 13th ed., Meredith Publishing Co.,

FIG. 1. Seeding by vigorous agitation, bottle at left; seeding by gentle tilting, bottle at right.

A scheme which avoids the tedium of vein transfer, the long delay in spreading of the pellicle, and growing of the initial surface culture has been described by R. J. Dubos and G. Middlebrook (Am. Rev. Tuberc. 56:334, 1947). Bottles containing an appropriate liquid medium are inoculated with a 6- to 10-day-old culture of mycobacteria in Tween-albumin medium and are incubated several days in a horizontal position. The bottles are then gently tilted to an upright position and lowered again, which serves to disturb the depth culture and float some of it to effect surface seeding. The bottles are then incubated for the appropriate number of weeks to mature.

The latter method has occasionally given us inconsistent results owing to inadequate surface seeding. When the bottles are retitled at several intervals during the incubation and the surface culture is thus redistributed, then somewhat more satisfactory growth is achieved.

Consideration of the physical principles governing surface seeding led us to predict that more effective seeding would be achieved if the early depth growth in Roux bottles is violently disturbed and aerated, to simulate mineral flotation practices in which hydrophobic particles of solids collect at an air-water interface and are floated to the water surface. This prediction was confirmed. After 4 to 6 days of incubation, the Roux bottle is raised, the film of depth culture adhering to the glass surface is freed by gentle sloshing of the liquid, and the bottle is held vertically and is vigorously agitated by a rotary wrist motion so that air is entrapped in the liquid and much foam is produced. Care is taken that the liquid is not thrown up to the cotton plug. The bottle is then laid back on the shelf, and the collapsed foam which collects at the edge of the liquid is coaxed away from the wall by gently tilting the bottle. The surface seeding achieved in this manner is impressive and immediately observable, most of the depth growth being collected by the foam and floated onto the surface as a collapsed scum. Rapid surface growth then follows.

The accompanying photographs illustrate the density of veil growth observable 10 days after surface seeding by vigorous agitation as compared with seeding by gentle tilting (Fig. 1). When the latter flask was disturbed several times during a 30-day incubation period, the density of veil growth eventually appeared to equal that of the vigorously disturbed culture.

In a typical experiment, 116 Roux bottles, each containing 100 ml of Wong-Weinzirl medium (S. Wong and J. Weinzirl, Am. Rev. Tuberc. 33:577, 1936) were inoculated with 2-ml portions of a 7-day culture of H37Rv, incubated at 37°C for 6 days, and surface-seeded by vigorous agitation. The growth was harvested 22 days later by autoclaving for 30 min at 78 to 80°C. A 352-g amount of washed, rubber-dammed moist bacilli was obtained, which yielded 93.6 g of vacuum-dried product.

W. C. Morse, U.S. Army Medical Research and Nutrition Laboratories, Fitzsimons General Hospital, has kindly informed us that he has adopted the method described here as a routine procedure, with excellent results.

This investigation was supported by Public Health Service grant AI-06538 from the National Institute of Allergy and Infectious Diseases.

The excellent technical assistance of Judith Warren is acknowledged with thanks.