Reports and Letters

Submerged Culture of Micrococcus Lysodeikticus for Large-Scale Producton of Cells

Published methods for the production of large amounts of cells of *Micrococcus lysodeikticus* (*1*) for the isolation of bacterial catalase have consisted of surface culture techniques. For obvious reasons these methods are not very satisfactory or convenient. We have, therefore, explored the possibility of growing this organism as a submerged culture in a large volume of liquid medium (10 liters or more) and harvesting the cells by centrifugation with the Sharples centrifuge (*2*). Our initial efforts were unsuccessful until we discovered the requirement for a high *p*H by this organism. This report outlines the essential details of the culturing techniques we have adopted for growing and harvesting *M. lysodeikticus*. We expect to publish a more detailed report later.

The organism was obtained from the American type culture collection, No. 4698. The stock culture is maintained on agar slants at room temperature by transfers approximately once every 7 or 8 days. The viability of these organisms is remarkably stable at room temperature. The agar medium consists of the following: 1 percent yeast extract (Anheuser-Busch), 2 percent dextrose, 0.5*M* K_2HPO_4 (8.7 grams per liter), and 1 percent of a salt solution containing the following: 4 percent $MgSO_4 \cdot 7H_2O$, 0.2 percent NaCl, 0.2 percent $FeSO_4 \cdot 7H_2O$, and 0.16 percent $MnSO_4$. The *p*H of the medium is brought up to approximately 8.0 with KOH (1 milliliter of 50-percent KOH per liter) before the 2-percent agar is added. The same medium is also used to grow the inoculum culture for the submerged cultures.

The liquid medium of the submerged cultures is made up from three solutions as follows. (i) Solution A: into a 12-liter flask are placed 9.1 liters of distilled water, 100 milliliters of the aforementioned solution, and 85 grams of $NaHCO_3$ (final molarity in medium 0.1*M*). The surface of the liquid is sprayed with Dow Corning antifoam A spray. The flask is then autoclaved with a cotton plug for 1 hour at 15 pounds pressure. (ii) Solution B: into a 1-liter flask are placed 100 grams of yeast extract and 400 milliliters of water. (iii) Solution C: into a 500-milliliter flask are placed 200 grams of glucose and 300 milliliters of water. The two cotton stoppered flasks are autoclaved for 20 minutes at 15 pounds pressure. After cooling, solutions B and C are added to solution A aseptically.

The inoculum is prepared either from surface cultures or from submerged cultures (with the afore-mentioned liquid medium) grown by the shake flask technique in 250-milliliter erlenmeyer flasks. We prefer the former method because it permits us to detect by visual inspection any possible contaminants in the inoculum. For growing the surface cultures, we have used Corning No. 4422 culture flasks that contain 1 liter of the agar medium. Approximately 48 hours after the agar medium has been inoculated, the cultures are harvested by washing the surface with sterile distilled water. If the cultures are not contaminated in this process, two successive harvests can be made from the same flask. The contents of two flasks are added to one submerged culture flask.

After inoculation, the liquid culture medium is vigorously aerated by sucking air through the liquid. The aeration system consists of an air exhaust and an air intake line which pass through a rubber stopper in the flask. The intake air is filtered through sterile cotton and dispersed in the medium by means of a sintered glass plug. The entire aeration apparatus is autoclaved separately. The cells are harvested from the liquid medium after 48 hours of growth at room temperature by means of the Sharples centrifuge.

The average yields of cells from the submerged cultures have been approximately 3.5 grams (dry weight) per liter of medium. A few experiments have been made with the shake flask technique and on one occasion the yield was 9 grams per liter. The total yield of the latter was small because of the limited number of flasks that could be handled conveniently. We have not yet attempted to determine the reason for the difference in yield by the two methods. However, the two most likely reasons are (i) larger initial inoculations by the shake-flask method and (ii) better agitation of the bacteria in the shake flasks. It was pointed out to us that growth of aerobic organisms in large-scale liquid cultures is more vigorous if the cultures are simultaneously agitated and aerated (*3*). It is of interest to note that either the potassium or the sodium salt of bicarbonate may be used and that the molarity of the buffer can be as high as 0.2*M* without causing any detectable effect on the growth of the organism. However, 0.1*M* bicarbonate is adequate to keep the *p*H above 8.0 for 48 to 72 hours. The high *p*H is reached by the simple procedure of boiling off CO_2 in the autoclaving procedure.

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References and Notes

1. D. Herbert and J. Pinsent, *Biochem. J. London* 43, 193 (1948).
2. I wish to acknowledge the technical assistance of Elizabeth Gaudy in this study. This investigation was supported by research grant C-2550 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.
3. L. A. Underkofler, private communication.

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Plea for the Extension of Biological Abstractions

In any comparison of the biological and physical sciences one is struck by an apparent dearth of great theoreticians and radical turning points in the former, particularly within the present century. This is not to make the absurd assertion that the biological sciences have not had their great moments, but such moments have usually sprung from what immediately preceded. The progress has been amazing—but staid. There have been no grand explosions, one might almost say, since Pasteur and Darwin. The mathematical sciences understandably lend themselves to the purely speculative. However, there is more than such inherent difference. Those in the physical sciences, although undoubtedly awed by sheer magnitude, dare to ruminate upon the whole. That tremendous abstraction, life, is seldom considered in like manner except by philosophers and theologians, both of whom actually deal with man and his opinions of himself—endeavors generally conceded to be valuable although transcendental and unscientific. The biologist, deeply immersed in lymph, sap, or metabolites, seldom troubles himself with sweeping generalizations. It has been suggested that it is a vestige of the Middle Ages, a dear memento of the egocentric universe, a last refuge for man and his dignity. However, even ignoring the supposed scriptural ban does not appreciably facilitate