Investigations

ISOLATION OF ANTIBIOTIC-PRODUCING ACTINOMYCETES FROM SOIL

The study of actinomycetes in the introductory course is often ignored, perhaps because teachers do not realize how easy actinomycetes are to isolate. This and testing for antibiotic production can be done readily in the high school or college microbiology laboratory without any unusual equipment. We present here an experimental method that consistently yields good results and emphasizes that actinomycetes are very important antibiotic producers occurring in large numbers in the soil.

Procedure. Primary isolation of actinomycetes is done by sprinkling a minute quantity of good garden soil over the surface of a water agar plate (fig. 1), inverting the plate, and incubating it at room temperature in the dark for about seven days. To be certain that the soil is thoroughly dry, store some in a clean, uncovered container for a week or so before using. We prepare a 1.5% (weight/volume) water agar solution by dissolving fine, granular agar in distilled water. The pH is adjusted to 7.3, and the solution is sterilized in a pressure cooker or autoclave at 15 psi for 15 minutes.

When colonies appear on this medium they can be picked and transferred to ISP 2 (Pridham et al. 1957) medium, which promotes vigorous growth and heavy sporulation of actinomycetes. ISP 2 is composed of the following (all available from Difco, Inc., Detroit, Mich. 48201): 4.0 g bacto-yeast extract; 10.0 g bacto-malt extract; 4.0 g bacto-dextrose; and 1 liter distilled water. Adjust to pH 7.3, then add 20.0 g bacto-agar. Heat to boiling to dissolve the agar, dispense to culture tubes, and sterilize by autoclaving at 15 psi for 15 minutes.

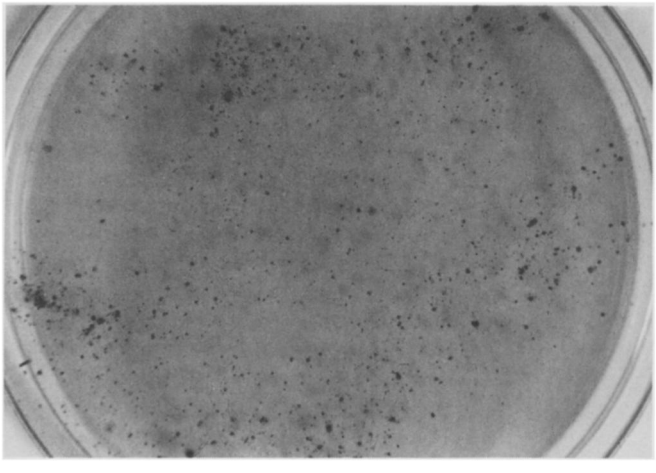

Fig. 1. Primary isolation of actinomycetes is done by sprinkling a minute quantity of good garden soil over the surface of a water agar plate.

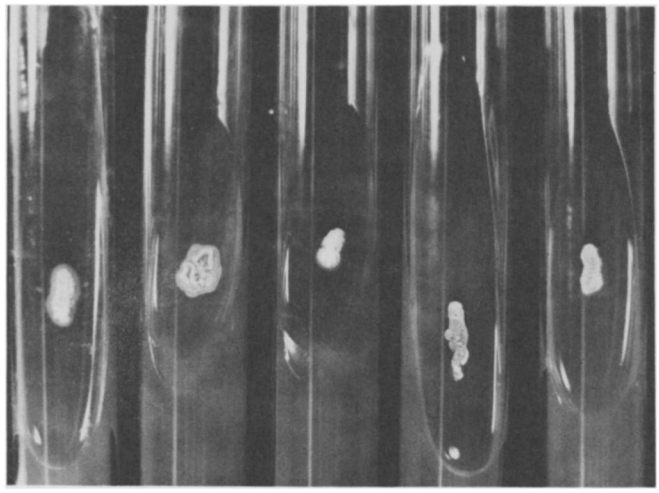

Fig. 2. Isolated actinomycete colonies after incubation for 2-3 days on ISP 2 medium.

Most of the colonies on the water agar plates will be *Streptomyces* ssp., which have abundant aerial mycelia and conidia. Colonies can be tentatively identified through the low-power objective of a compound microscope. Mark their location on the bottom valve of a dissecting microscope. To pick a colony, lightly touch its center with a sterile, cooled, sharp dissecting needle; then transfer adhering spores to a sterile slant of ISP 2 medium. Incubate the tubes for 2-3 days at room temperature in the dark (fig 2). Occasionally, another bacterium or a mold will be transferred to the slants by accident. These tubes can be discarded. Methods for characterization of *Streptomyces* ssp. have been detailed by Shirling and Gottlieb (1966).

To test for antibiotic production, make a water suspension of the actinomycete spores and streak an agar plate with these and with inoculum from cultures of gram-positive and gram-negative test bacteria as well. *Escherichia coli, Proteus vulgaris,* and *Staphylococcus aureus* are good assay organisms. Poor or no growth of these true bacteria indicates susceptibility to one or more substances produced by the actinomycete.

A water suspension of the spore can be made easily. Pour a 9-ml sterile water blank into the actinomycete slant, and with a sterile, cooled transfer loop scrape the spores off the slant and into the water. Use the same loop to make one streak across the center of an

ISP 2 plate. Incubate the inoculated plate for 5–7 days. Crabtree and Hinsdill (1974) recommend antibiotic assay soft agar in place of ISP 2 medium. Its ingredients are 5.0 g tryptone; 5.0 g peptone; 3.0 g yeast extract; 1.0 g beef extract; 2.0 g glucose; 7.0 g agar; and 1 liter water. This substrate is not only richer but allows more rapid diffusion of antibiotics if an organism is producing them. Invert and incubate the plate at room temperature in the dark for 5–7 days. After that, streak representative gram-positive and gram-negative test bacteria at right angles to the actinomycete streak. Not all actinomycetes will produce antibiotics that inhibit bacterial growth, but some do. Again, poor or no growth of the test bacteria indicates

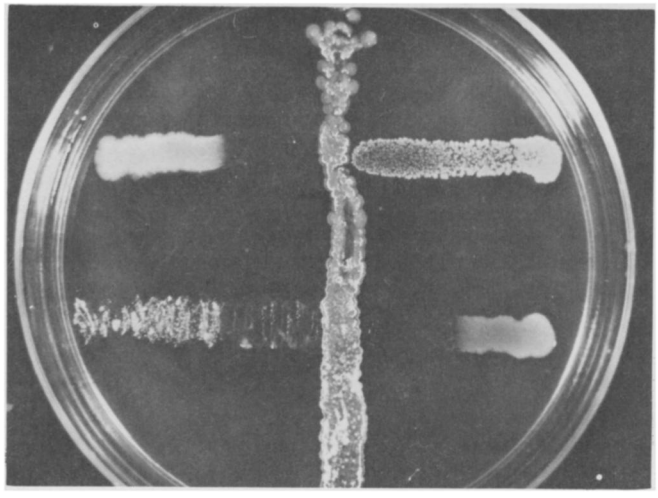

Fig. 3. Test for antibiotic production. Upper right, *Sarcina lutea* (resistant); lower right, *Escherichia coli* (susceptible); upper left, *Proteus vulgaris* (susceptible); lower left, *Staphylococcus aureus* (susceptible).

that they are susceptible to some diffusible substances produced by the actinomycetes (fig. 3). If kept refrigerated, the spore suspension often stays viable for 15–18 months and can be used in subsequent experiments.

References

CRABTREE, K. T., and R. D. HINSDILL. 1974. *Fundamental experiments in microbiology.* W. B. Saunders Co., Philadelphia. (P. 338, media 2 and 3.)

PRIDHAM, T. G., P. ANDERSON, C. FOLEY, L. A. LINDENFELSER, C. W. HESSELTINE, and R. G. BENEDICT. 1957. A selection of media for maintenance and taxonomic study of *Streptomycetes. Antibiotics Annual* 1956-57:947.

SHIRLING, E. B., and D. GOTTLIEB. 1966. Methods for characterization of *Streptomycetes* species. *International Journal of Systematic Bacteriology* 16:313.

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Why Inquiry?

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these students still felt as confident in their ability to plan experiments, make observations, and draw conclusions as the traditional group which was provided with directions. The discovery group learned without teacher-initiated protocols.

In analyzing the responses of the two groups, the following facts are quite clear: (i) the traditional group showed a reliance on the teacher for learning; and (ii) knowledge alone seemed to be the factor in determining the degree of involvement and achievement. By their responses, the students in the traditional group indicated that they understood science to be a body of immutable conclusions about science. In contrast, the discovery group understood science to be the process of discovery. Statement 64 shows that 21% of this group came to this understanding. None of the students in the traditional group agreed with the statement.

Active Student Involvement

The results of this study clearly delineate two approaches to science education. With one approach, the student will discover facts on his own; he will learn to draw conclusions from careful observations; and he will achieve an understanding of science while enjoying his learning experience. This approach places the student in a learning situation that makes the process of science a real experience while enhancing critical thinking.

The second approach presents knowledge as true and permanent. Students rely on the teacher for the information and regard factual knowledge as the sole determiner of their achievement. Since it is clearly teacher-oriented, situations which require critical thinking are relegated to the teacher, not the student. In essence the student is the passive observer.

If the goal of science education is the active involvement of the student in the process of science, the choice is clear.

About the Cover

The sketch of the Rhesus macaque on the cover of this issue was done by Douglas L. Cramer while serving as staff artist at the Oregon Regional Primate Research Center. Cramer is currently assistant professor in anthropology at Rutgers University and lecturer in anatomy at New York University Medical School. A four-and-a-half hour tour of the Primate Research Center has been scheduled for the NABT convention in Portland later this month. The Center conducts frontier studies in biomedical research through the use of primates.