Microbiology Laboratories And the Use of a Bacteria Bank

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THE IDEA OF maintaining a bacteria culture bank emanated from an NSF microbiology course I took at Drake University the summer of 1966. I discussed the concept with six of my colleagues at Alhambra High School (Phoenix, Ariz.), and we decided to apply it to the 4-6-week microbiology units we each include in our respective courses. After obtaining representative bacteria cultures to establish stock cultures, I contacted Norbert J. Konzal, science supervisor of Phoenix Union High School System, and offered to supply cultures, free of charge, to interested biology teachers from other schools in the district. Konzal notified the other schools to elicit response to my proposal, and with widespread requests the program was initiated.

Each school year, students who have shown aptitude for working with the cultures in their microbiology units are selected to assist in implementing the culture supply program. All students who take basic biology spend approximately six weeks learning about microorganisms through laboratory exercises based on the use and handling of microbes, mainly bacteria. Of primary importance, and therefore most heavily stressed, are the techniques involving proper precautions for safe handling of all equipment and materials. First, students learn safe and efficient methods of utilizing all common lab equipment, including the microscopes and bunsen burners. Then they apply their skills to specific lab assignments.

Building Basic Skills

Following are six examples of microbiology labs most frequently selected for the 4-6-week unit at Alhambra High School. These labs have, over a period of ten years, proved to be the most worthwhile in enabling students to acquire a basic understanding of bacteria and their relationship to animal and plant life.

1. *Ubiquity.* This lab is of primary importance because it demonstrates the omnipresence of bacteria. Students obtain random bacteria from their surroundings. With sterile swabs, they collect bacteria from desks, tables, door knobs, and so on and transfer the bacteria to sterile plates of nutrient agar. Or the students expose petri plates for a specified period of time in different locations. These procedures are followed by incubation of the cultures and, 24-48 hours later, observation.

2. *Gram staining.* The gram stain lab enables students to determine whether bacteria are positive or negative for the purpose of classification. Usually, bacteria of different sizes are employed, for example, a gram-positive large bacterium (*Bacillus megaterium*) and a gram-negative small bacteria (*Escherichia coli*).

3. *Relative size.* To observe the sizes of bacteria as compared to red blood cells, students place a bacterial smear of various bacteria on top of a previously prepared blood smear.

4. *Isolation of bacteria.* To learn techniques for handling bacteria, students mix bacteria of different color combinations—for example, *Sarcina lutea* (yellow) and *Bacillus subtilis* (white) or *Serratia marcescens* (red) and *B. megaterium* (white)—in a liquid medium. Students learn to isolate by streaking agar plates.

5. *Motility of bacteria.* This lab provides students with a basic understanding of how bacteria move. They grow various bacteria in a liquid medium for 18-24 hours, and then they deposit a small drop of bacteria culture on a cover slip and place a depression slide—with a ring of petroleum jelly around the depression—over the cover slip. After tapping the cover slip to seal it and inverting the slide, students can observe movement under a regular microscope at about 430X. For this purpose, we usually use *E. coli, B. megaterium,* or *Rhodospirillum rubrum.*

6. *Evaluating effectiveness.* One of the lab exercises students enjoy most involves testing the effectiveness of various name brand mouthwashes, toothpastes, and antiseptics. Generally, we use *E. coli* and *B. subtilis* in a liquid medium to determine which

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Table 1. Examples of bacteria used for high school lab exercises in microbiology.

Bacterium	*Shape*	*Gram stain*	*Color*
Serratia marcescens	bacillus (short rods)	-	red
Sarcina lutea	coccus (round)	+	yellow
Bacillus megaterium	bacillus (very large rods)	+	white*
B. subtilis	bacillus	+	white*
Escherichia coli	bacillus (short rods)	-	white
Rhodospirillum rubrum	spirillus (large)		pink
Chromobacterium violaceum	bacillus	+	violet

*spore-forming

products will inhibit growth of a gram-negative bacterium and which will inhibit growth of a gram-positive bacterium. The procedure consists of swabbing a petri plate of agar media so that the entire surface of the plate is covered. Students then dip a sterilized filter paper disk into the product to be evaluated and place the disk on the agar surface. (For greater economy, plates may be divided into four equal portions to allow simultaneous testing of four products.) Antibiotics are evaluated similarly, using commercially prepared antibiotic disks.

The Culture Supply Program

Hence, basic biology students acquire skills necessary for transferring bacteria, isolating pure cultures, making smears for microscopic viewing, and staining smears. From among the students interested in participation in the culture supply program, several are then chosen who have demonstrated the best mastery of the techniques involved in maintaining and transferring cultures. Obviously, the pupils selected have also demonstrated a high degree of maturity and trustworthiness.

Table 2. Basic equipment necessary for operation of a culture supply program.

Item	*Cost*
50 culture tubes[a]	$10.00
2 transfer loops	1.00
1 two-quart saucepan (for preparing media)	3.50
1 spoon	.50
1 hot plate	20.00
1 autoclave—pressure sterilizer	60.00
1 funnel	1.00
1 ring stand	10.00
1 refrigerator (used)[b]	50.00
1 bunsen burner	3.50
Total	$159.50

[a]For greater economy, we use test tubes stopped with cotton.

[b]Optional. With a refrigerator cultures need be renewed only every four or six weeks. Otherwise, cultures should be subcultured every week or so.

These students are then trained to prepare culture media—tryptic soy agar and nutrient agar for bacteria, Sabouraud dextrose agar and potato dextrose agar for fungi, and so on. After the media have been autoclaved for sterilization purposes, the students transfer, by inoculation, the bacteria from stock culture to new tubes of sterile media. After incubation at room temperature for 24–48 hours (depending on the bacteria) the cultures are refrigerated at approximately 4 °C. Relatively safe to handle and therefore commonly used bacteria are listed in table 1.

Students operating the culture supply program prepare all equipment, make and sterilize media, prepare slants, and clean used equipment, in addition to maintaining cultures. They keep a record of culture tubes going to and from the participating schools, and they make routine transfers every 4–6 weeks to maintain viable stock. They dispose of old cultures by autoclaving.

A project room adjoining the classroom is used for the program. The equipment necessary for maintaining the bacteria bank and the annual cost of operating such a program are itemized in tables 2 and 3.

Stock cultures of the bacteria listed in table 1 are purchased each September by the school maintaining the culture bank. After the initial outlay for stock culture, this cost could be eliminated or reduced if a teacher were on hand to maintain the cultures throughout the summer months. Otherwise, stock cultures are available from the Americal Type Culture Collection (12301 Parklawn Dr., Rockville, Md. 20852) which provides strains especially designed for teaching. Or, for schools operating on a stricter budget, stock cultures may be purchased from a commercial biological supply house. Tryptic soy agar is the medium most often utilized. The science department at Alhambra High School (the Phoenix school maintaining the bacteria bank) purchases the stock cultures annually out of the regular department supply budget. The schools receiving the cultures do not pay anything for this service.

Table 3. Annual cost of operating a culture supply program.

Item	*Cost*	*Total*
8 cultures	$4-$6 each	$32-$48
1/4 lb. tryptic soy agar	$18/lb.	$ 4.50
1/2 lb. cotton*	$2/lb.	$ 1.00
		$37.50-$53.50

*used to stopper test tubes in place of culture tubes

Thirteen schools, eleven of which are in the Phoenix Union High School District, receive the cultures. Although most of the schools request about 8 cultures each school year, several requisition 10-16 cultures because not all teachers in a given science department utilize the materials simultaneously. Cultures are usually prepared on demand for the various schools. Student assistants prepare and label cultures, check transfers to affirm growth, wrap and address packages, and then place them in the district mail. The district provides an interschool mail service at no cost to individual schools, and usually a teacher accompanies his request for cultures with an equal number of replacement tubes.

Most of the student assistants participate in the culture supply program in their junior and senior years. Therefore, one or two new students need to be chosen each September to replace the previous year's graduated seniors. A total of two or three students usually work two to three periods (55 minutes each) per week on the bacteria bank. Customarily, the teacher's preparation period is the time selected for this project so that help is available to the student assistants if they need it.

Given the basic equipment, commonly used by most science departments, operation of a culture supply program requires approximately $40 to $55 annually if there are no mailing costs involved. Establishment of a bacteria bank requires a teacher's time for ordering stock cultures, instituting procedures for operation of the bank, and training the student assistants to continue the program. I estimate time spent on our project at less than 10 hours a school year. For example, in a given month (September 1975) the program supplied over 45 cultures to various local schools with a total expenditure of less than 1½ hours of teacher time. Therefore, such a program can be initiated with a minimum of demand on a busy teacher's schedule.

Advantages of a Bacteria Bank

The advantages of a bacteria bank or culture supply program operated from one school are many:

1. A reduction of district, city, or area school expenditures. For example, if each of the 11 district schools in the Phoenix Union High School system ordered 8 cultures of bacteria at $6 each, the total cost to the district would be well over $500, whereas this district, because of the culture supply program, actually spends less than $40 per year on obtaining cultures. Obviously, this enables participating schools with limited science departmental budgets to acquire a larger variety of cultures than would otherwise be possible, and teachers in a given science department may order the same cultures as their colleagues but at different times throughout the year in accordance with their personal instructional patterns. Without a culture supply program such a procedure is out of the question with most school budgets.
2. Cultures are available throughout the school year. Delivery time through the bacteria bank normally requires only two to three days. Orders from a biological supply house usually require a waiting period of four to six weeks because it takes two to three weeks for the teacher's school and district offices to process his order and the supplier requires another week or two to deliver. Thus, process and delivery time through suppliers prevents ordering cultures after April 1 of a given school year for use during that year.
3. Cultures are fresh because they are made on demand.
4. Operation of a bacteria bank strengthens interdepartmental cooperation among the many science departments involved in the program.
5. The culture supply program involves two or three student assistants each year in a practical application of studied material.

Far from placing any sacrifice on learning, the fundamentally remunerative advantages of this highly successful method for bacteria culturing are directly proportionate to limited funds, equipment, materials, and space.

Call for Papers

Special sessions are planned to enable active members of NABT to present papers at the 1976 convention scheduled for Denver, Colo., next October. To be eligible, papers should be submitted under one of three categories: Biologic Research; Biology Education Research; or Demonstration of Techniques Applicable to the Teaching of Biological Sciences.

Abstracts should be submitted no later than April 15, 1976. Submit abstracts to Dr. Lawrence L. Lindauer, 3039 South Depew St., Denver, Colo. 80227.

Abstracts should not exceed 150 words in length. Notification of acceptance will be mailed before August 15, 1976.

Program Evaluation in Environmental Education

A bibliography of evaluation reports of environmental education curriculum and program materials has been prepared by John F. Disinger of ERIC/SMEAC. The bibliography also includes some references to environmental education classroom materials and is available from ERIC/SMEAC Information Reference Center, 1200 Chambers Rd., Suite 300, Ohio State University, Columbus 43212.