MICROBIOLOGY RESEARCH MADE EASY

by Rod Sobieski

Research in microbiology is far less common than student undertakings in the other major areas of biology. This is partly due to the ease, because of size, with which the "macro" areas have systems to work with; thus projects are easily conceptualized and performed using familiar skills involving the visible world.

Generally one schedules a variety of regular classroom lab exercises in the "macro" disciplines which require no major pieces of equipment nor large quantities of different materials; then students often use these labs to generate ideas for research projects suited to their liking. Microbiology, by contrast, generally requires a variety of preparatory materials, many of which need to be sterile (culture media, bacteria or other microbial cultures, and equipment: autoclaves, incubators, oil immersion microscopes) in order to accomplish almost any teaching objective in this laboratory area. Therefore, students in many biological laboratory courses never have the opportunity to work with microbes because of these technical requirements. Thus, they do not gain the hands-on experience leading to the realization that bacteria, fungi, yeast, and blue-green algae possess the characteristics of life and are systems available to outside experiments and research.

Some teachers do no more in microbiology than have their students use nutrient agar plates to sample whatever whims the class members think of that day. Any exposure to this vast area of biology is better than none at all; yet this level of experience for the student does not allow an understanding of even the basic fundamentals of the science. In order to take the next step in the teaching process, problems must be solved as to how to supply sterile materials and equipment, most of which are not inexpensive. The goal of this Naturalist is to provide some insights into these problems so that the naive student can gain enough familiarity with the subject to do something as an extra classroom activity. Elementary and secondary students are considered in this discussion.

Beyond the First Step - Secondary level

Students need to have a working experience within the subject before a project can be reasonably constructed. That exposure requires at least a couple of classroom encounters dealing with the areas listed in Table 1, which are goals for the secondary school pupil. These basic experiences will provide the student with the realization that microbes possess the features of reproduction, metabolism, and irritability found in all forms of life and can serve as a basis for the formation of a research idea.

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TABLE 1. Classroom Experiences for an Overview of Microbiology.

<table>
<thead>
<tr>
<th>GOAL</th>
<th>MECHANISM TO ACCOMPLISH</th>
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<tbody>
<tr>
<td>Aseptic techniques</td>
<td>Must communicate philosophy of asepsis and working knowledge by using streak plates with mixed and pure cultures and subculturing from those plates.</td>
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<tr>
<td>Structure/function</td>
<td>Oil immersion microscopy of basic morphotypes of bacteria. Look also at spores, bluegreen algae, fungi, and unicellular protozoa.</td>
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<tr>
<td>Metabolism/physiology</td>
<td>Extracellular enzyme activity, such as starch plate hydrolysis, intracellular enzyme activity of fermentation, and even antibiosis, to consider ecological competition due to physiology. Heat treatment of sporeformer and nonsporeformer.</td>
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<tr>
<td>Ubiquity of microbes</td>
<td>Exposure plates to environmental sources (plants, animals, water, air, foods, etc.) use media for bacteria (nutrient agar), yeasts, and molds (Sabouraud dextrose agar.).</td>
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Beyond the First Step - Elementary level

To attempt accomplishing the goals in Table 1 with these students is unrealistic because (a) the inherent danger from flames used to insure asepsis and (b) cognitive limits of the students to grasp the significance of the goals and meaningfully integrate the knowledge. Nevertheless, it is very easy to convey a knowledge of the microbial world to enable even these students to take on projects exploring the usually unseen microbial world.

Special concerns here, however, are a must. These students need not, and in many cases should not, use any specialized equipment: flames, incubators, or pure cultures for that matter. In short, experiments should manipulate natural items using these items as sources of naturally occurring microbes. These same experiences should be short term and use set ups which are simple in design. Here, the supermarket can be source of bread or vegetable (slices) for culturing, pie tins, glass jars, and such as containers, and foil or clear plastic wrap for covers. Cotton swabs right from the package can be used for inoculating devices if one wanted to transfer the microbes from the substrate of isolation to some other medium. One can sample everyday foods and items onto slices of "medium," incubate at room temperature, then examine with a hand lens. A simple lens will enable viewing of colonial morphology of bacteria and fungi.
This approach will show the existence of microbes in various environmental sources and above all allow them to begin to ask questions about the unseen world. The suggestions for classroom activities in the lower grades found in Table 2 will also serve to acquaint the pupils with specific contributions in nature and in the lives of all of us that microbial activity provides.

**TABLE 2. Lower Grade Activities in Microbiology.**

<table>
<thead>
<tr>
<th>ACTIVITY</th>
<th>SPECIAL POINT</th>
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<tbody>
<tr>
<td>Easy Sources</td>
<td>Moldy fruit and cheese provide good examples of problems caused by microbes.</td>
</tr>
<tr>
<td>Milk</td>
<td>Use jars and alter the conditions, warm vs. cool, air vs. plastic wrap sealed, along with early vs. late dated samples.</td>
</tr>
<tr>
<td>Moisture effects</td>
<td>Dry cereal in jars vs. jar with cereal and a few milliliters of water (why raisins and other dried foods do not spoil).</td>
</tr>
<tr>
<td>Yeast balloons</td>
<td>Soda pop bottles with rubber balloons on top to trap gas. Use dried yeast, water, and some sugar (why bread rises).</td>
</tr>
<tr>
<td>Food and microbes</td>
<td>Examine food made by microbes, Swiss cheese, Blue cheese, Yogurt, compare sauerkraut with fresh shredded cabbage.</td>
</tr>
<tr>
<td>Plants</td>
<td>Activity to collect specimens showing evidence of disease—leaf spots, galls, and rusts.</td>
</tr>
<tr>
<td>Animal Waste</td>
<td>Very nice source of fungus—place one small piece of horse dung (biscuit) into chamber with moistened paper on bottom. In 2-3 days you’ll find numerous members of the fungus <em>Polobolus</em>, (Figure 1). Can also place small amount of bird dropping, etc., onto slice of potato, etc.</td>
</tr>
</tbody>
</table>

*Not due to a microbe but an insect, yet it is a disease due to parasitism.

Other lower grade activities that can serve as sources of generating ideas in the students for continuation of studies in microbiology are suggested below.

Are girls really "cleaner" than boys? Is that pretty, neat-looking, kissable blonde in class also a possible source of bacteria? Do boys have "dirtier" hair than girls? Is there such a thing as "filthy money?" The following are fun, even though they do not actually prove anything.

1. Snip several bits of hair from a number of boys onto the culture medium.
FIGURE 1. Diagrams of *Polobolus* fungus on horse dung.

Diagram courtesy of Dr. Richard P. Keeling.

Do the same for several girls on another sample of culture medium. Label each dish as to what it contains.

2. Snip some bits of finger nail from several boys onto the culture medium. Do the same for several of the girls.

3. Have several students slide their finger tips across the surface of the medium. Then have these students, using soap and water, wash their hands thoroughly, and do the same to another sample of growth medium.

4. Place a penny, dime or nickel, and quarter on a plate (or use separate growth systems if enough are available).

5. Put a well-worn dollar bill (teachers do not usually have larger denominations) inside the growth medium and leave for a few moments. Remove the bill and replace the cover.

6. Have several girls "kiss" (press their lips) to the growth medium then replace the lid. (The girls may want several boys to do the same on another plate.) Label each.

7. Keep one of your media sources untouched (sterile), to be used as a control.

8. Place all the inoculated and control containers with growth medium in an out-of-the-way place. If it is an area that is a bit warm (top of refrigerator, but not a heat register or radiator, for they are too hot) things will generally grow better. Examine the experiment during the next two or three days.
Observations and questions

a. How do you account for fewer bacterial and/or fungus colonies growing about the bits of hair from some people than about those from others? Do boys or girls harbor the greater number of bacteria and/or yeasts spores on their hair?

b. Can you see why a scratch is a potential source of infection, especially by a cat or other wild animal that never "cleans" its nails? Can you see a good reason why you should clean your nails regularly?

c. Does washing your hands carefully reduce the presence of bacteria? Do you now see why doctors, even after thoroughly scrubbing their hands before an operation, wear sterile surgical gloves?

d. Remember when your mother told you not to put coins in your mouth? Does what happens on the growth medium tell you why? Do as many bacteria occur on a penny as on a dime, nickel, or quarter? Can you think of a reason?

e. Could germs be passed on when kissing a person?

f. Do bacteria and fungi just "start" to grow (abiogenis), or do they have to come from somewhere (check your control plate). If bacterial colonies develop on your control plate, what does that tell you about the sterilization of your materials?

A word of caution: Be sure to inform your students that you are not looking for "germs" (disease causing bacteria)! Rather, you are demonstrating the fact that bacteria and fungus spores are everywhere, even on the "cleanest" of humans. Also be sure to point out that not only are most of these organisms harmless, but many are also extremely valuable to man.

The Most Difficult Part—The Experimental Question

The idea, questions, or observation triggering the research project is, in this author's opinion, the hardest aspect for students or teachers to originate in order to do research. Many times the interest may be there but the formulation of the experimental design is lacking. It is the design, the how to, that is the biggest hurdle to successful exploration of this area of biology. Projects proposed by students are often not simple in their design, hence are unmanageable in terms of the time or materials and supplies the researcher has available to do the project. For example, can the work be completed in the semester the student wants to do the work, or would the absence of a major item of equipment cripple the successful collection of observations or data? These kinds of concerns require the teacher to advise and counsel the young researcher. If you have any experience in microbiology (even a good laboratory associated course) do not hesitate to plunge into this new role, much of it is good old common sense. If you have no experience, then by all means have the student contact resource people that can help. Professors at colleges and
universities are often overlooked as resources in this field as are hospital laboratory personnel, dentists, and veterinarians. Industrial firms involved with food processing (canning, baking, etc.) and food preparation (regional offices), pharmaceutical houses (human and animal products), and microbiological supply houses making media and other specialty products are all staffed by people able to give the interested pupil insights into further directions or simply able to answer plaguing technical questions. Do not overlook research centers, if they are near you, for these same kinds of people and, importantly, state and county public health departments. Many segments of our society utilize microbiologists, who from their working experience can be valuable to the interested, eager, enthusiastic, and dedicated student wanting to explore this field of science.

Please note the last sentence of the previous paragraph, the student must have these listed qualities in order to be successful in his/her endeavor. Research requires these sorts of traits, for success does not come without hard work, intermixed with disappointments, repetitions, and evaluations until, finally, findings begin to answer the experimental question.

Thus, a very significant dimension to this most difficult part of research, the idea, is not an end in itself for the student because the student's attitude contributes just as much to a valuable and exciting experience. If a microbiologist acquaintance or teacher can be used as a resource by the student to help solve problems as they occur, this will give the researcher needed confidence in the pursuit at hand.

Other Sources to Overcome the Most Difficult Step

Often overlooked sources of ideas for students are laboratory manuals in microbiology. Some of the most extensive holdings here would be professors in colleges or universities. Some libraries refuse to order "manuals," so you may need to contact several institutions in order to find a library source, if you cannot find a cooperating faculty member at a local university. The reason for citing these sources is due to the emphasis different manuals present. Almost all of them do similar exercises in regard to exploring ubiquity, morphology and structure, metabolism, and aseptic technique. However, they differ dramatically in how they explore foods, antibiosis and antibiotics, controls of microbes and disinfection, flora, genetics, viruses, immunology, etc. The really good idea manuals will have a page or so of discussion over the exercise to provide an introduction to the topic. Four manuals cited below do not represent any type of ranking or selection process but are those meeting the goals listed above.

One source book should be mentioned separately for its excellent "how to" approach to laboratory techniques. "Sourcebook of Experiments for Teaching of Microbiology", edited by Primrose and Wardlaw, 1982, Academic Press. It provides nine different major areas of laboratory experiments in the field and has a total of 111 experiments. These are indexed by subject so that if the student is interested in viruses, he/she would find 11 experiments listed under viruses. The really nice feature that many lab manuals lack is a section within each experiment titled "Notes and Points to Watch." Here, the secret to success is given, either pointing out how one must do a particular step or how to save time. Likewise, the material section gives complete formulations and specific equipment needs. This is supposed to be a book for the undergraduate level, yet the secondary student involved with research will find it an invaluable source of ideas, with excellent "how to" references.

Yet another source worth mentioning is the national organization of microbiologists, the American Society for Microbiology* with has 8-9 different types of publications and audio visuals for teaching the subject. Although most of these are not oriented to the students we are concerned with, a few are mentioned. *Microbiology in your Future is, in single quantities, free for the asking, and details the myriad of career opportunities in the field. I recommend it for any of your students seeking directions in a College or University. They also have a lab manual entitled *Bringing Life to Microbiology*, which gives 26 exercises and is fewer than the number found in those four manuals cited above. Their audio visuals strike me as very expensive and geared to the college or university student. *Highlights in Microbiology* is a very fine yearly summary of happenings written for practicing microbiologists wishing to keep up with the subject tangent to their main interests.

The publication of the American Society for Microbiology titled EleSec has been a disappointment for me since it was supposed to be directed at teachers of elementary and secondary science. In the two years it has been published as a quarterly issue, there has been little to justify the cost of the 16 pages in a year's worth of this publication. They have reduced the subscription from $15 to $5.00 for 1983, and the topics sound as if they may finally be producing a worthy source of information for the intended audience. The topics for 1983 are Microbiology from the Kitchen, Simple Experiments with Baker's Yeast, Exploring with Protozoa and Algae and Microbial Life in Soil and Water. We will have to see if 1983 changes my opinion; however, I mention it to give you additional sources of information for the teaching of Microbiology and research.

*1915 I Street, N.W., Washington, D.C. 20006, self addressed stamped envelope needed for information, prices, etc.
What is the Last Step of a Project?

Like all worthy scientific undertakings, a major event in the project or research question is communicating the findings to others. Without the reporting of the results, the intent of the questions answered is lost. Granted, many research findings by young investigators may not carry the significance of the earth-shattering discoveries of established investigators, it is nevertheless important to have the student finish the process and understand that writing up the findings and presenting them to audience or science fair is part and parcel of good science. Research is, in fact, wasted research if the community interested in the work never hears or reads of the findings. Note that whatever the outcome of the student's queries, be they positive or negative, a presentation of the work is worthwhile in instilling in both the investigator and the audience that science is science. Although it may be harder to deal with completely negative findings, it is a finding, and the absence of an answer can be used by the investigator to suggest what new questions might be asked of the experimental system or how the same questions could be asked with an altered experimental design. Perhaps the same student could deal with the new direction as a junior if the original study was done as a sophomore. After all, science regularly progresses on the prior findings of others or earlier trails by the original investigator.

Mechanism for the Last Step

Most would probably respond by stating that the student should set up the data and analysis for a science fair, and that certainly is a very legitimate possibility. Since I am the Associate Director of the Kansas Junior Academy of Science, I want to speak to this vehicle as a mechanism of fulfilling this last, very needed, step in the scientific process of a student's project, no matter if the student is in the elementary or secondary system. The Kansas State Director is Dr. Ed C. Shearer, Chemistry Department, Fort Hays State University, Hays, KS 67601. He can supply a very informative handbook for student papers and what the Junior Academy requires for membership, etc.

Essentially, regions within the State's Junior Academy system hold meetings where students present their work in a formal fashion. A well written paper complete with Introduction, Materials and Methods, Results and Conclusions is given to the judges at a district or regional meeting. Those presentations and write-ups found to be superior are presented at statewide Junior Academy meeting, where students with similar subject material present their findings before another group of judges. For example, papers on physiology are all in one session while those on earth science in another. These meetings are organized in the fashion of the Senior State meetings in terms of having a session devoted to a particular subject area of science. An evening banquet for the Junior Academy participants and other attendees has a guest scientific speaker and ends with an announcing of student win-
ners. In the past, the top student scientist from the state was helped financially to attend a national meeting where elementary and secondary students from around the country presented their works. The national meeting is associated with American Association for the Advancement of Science and, in fact, over the last several years the Kansas winners have attended this national meeting of AAAS, the organization which also puts out the weekly publication \textit{Science}.

Let me emphasize one point, students do not need to present a paper to attend a regional or district meeting of the Junior Academy. I say this because if you are interested in stimulating interest in your students to explore extra classroom activities, take a group to spend a day at a Junior Academy meeting. The students will come away enthused over the fact that others their age and class standing do research that is exciting and interesting.

Another point to emphasize is that once the student has organized the results of the investigation, the opportunity for wider communication is always available. For example, a Junior Academy presentation can be used to enter other competition, such as the General Electric Talent Search. This yearly event at the national level can lead to substantial scholarships for furthering the student's education, if the work is of a superior rating. Hence a lot of "mileage," other outlets, can be used for this last step of the research process. Do not overlook your Junior Academy, along with science fairs and other opportunities for completing the scientific process.

Thus, research in Microbiology or any other field of science boils down to four major steps: 1) Identify a problem, 2) Solve the problem through experimentation, 3) Record the results or observations, and 4) Write up and report the findings, preferably in manuscript form for your State Junior Academy of Science.

More specifically for Microbiology, several major skills are required (aseptic technique, appreciation for microbes as ubiquitous, living creatures) along with sometimes difficult supplies and equipment. However, it is hoped that this issue of the \textit{KANSAS SCHOOL NATURALIST} has helped solve some of the hurdles in attempting to have students of several ages begin the process.

**Preparation of Materials**

Material preparations can be simplified to eliminate the high "tech" requirements for class experiences or research in microbiology. Several suggestions are offered below to overcome these requirements. Almost any University, hospital clinical laboratory, dental office, or veterinarian would be happy to fulfill the occasional request for a bacterial or fungal culture or to sterilize material for educational purposes.

Aluminum foil is an excellent wrapping for materials requiring and undergoing sterilization. Tubes of water, broth medium, and flasks of agar can have foil covers made from 2-3 layers of aluminum bent down over the mouth of the con-
tainer. All glassware can be easily sterilized in a home oven set at 350°F for 1.5 hours. Be sure the item being sterilized will not be damaged from the heat of the oven. Cotton, rubber, paper, and plastics will not do well in oven sterilization. Items not suitable for oven sterilization can be processed in a home pressure cooker in only 20 minutes once proper temperature and/or pressure (15 lbs. or 121°C) is reached. A small glass serves as a convenient tube holder. A pressure cooker for an identical time will be necessary if you plan to prepare your own agar medium in the form of plates or slants (see directions in next sections). Glass tubing can be crudely calibrated by weight (1 milliliter weighs 1 gram) then marked with a file, these make suitable 1 ml pipettes that can be repeatedly sterilized in aluminum wraps.

Speaking of pipettes, do not let mouth pipetting develop into any sort of habit, use a bulb. This is poor safety practice that could lead to trouble if caustic or hazardous materials were being mouth pipetted. In regard to safety, several other points are spelled out below:

1) Always wash hands before and after completing laboratory work.
2) Always disinfect the work area with disinfectant, use plenty and wipe off the excess with paper towels. (10% Chlorox works well for this and other disinfection uses also.)
3) Always use a bulb to pipette, never mouth pipette. The pipette may have the mouth end contaminated with microbes of hazardous and poisonous substances.
4) Never eat in lab even if you are not working on your experiment. You do not know what may have occurred on the work bench before you arrived!
5) No horse play with your friends, materials, equipment, or experiments at any time.
6) Report to your teacher any violations of these and other safety rules.
7) Any accidents should be immediately reported to your teacher or lab supervisor.

One can add or delete other safety and laboratory regulations as needed (regarding microscope handling, for example). All students must be made aware of basic fire equipment, location of eye wash stations, and showers, if available.

**Agar Medium**

In preparation of your own media, one can substitute tap water for distilled water without having the results of regular classroom activities compromised. For purposes of research projects, you should find a source; again the university or hospital clinical laboratory is a sure, free source. Nutrient agar plates can be purchased already poured from one or more preparation or science supply houses. The costs here are 5-6 times per plate for purchased media than in-house made agar
plates; however, for occasional use this may be a more practical source. To prepare your own media, the dry powder is weighed out in proper proportions, water added and then it is sterilized before pouring into sterile petri dishes. Flasks of medium should not be more than 1/2 to 2/3 full to prevent boiling over during the sterilization process.

If a hospital or other facility sterilizes your medium, do not be concerned about being present immediately following the process. After the molten medium solidifies in the flask, it can be stored for many days this way, then easily remelted in a boiling water bath.

For this, place the flask in water so that the medium is below the surface but do not have so much water that the flask floats and tips over which would allow water to enter the sterile medium and contaminate it. Once the medium completely dissolves, it should be tempered to about 42-45°C in a sink of water with occasional swirling of the flask. Another alternative is to cool the molten agar under flowing, cool, tap water with swirling until you can touch the flask to your cheek for a second or two without having it burn you (about 42-45°C). If you swirl too vigorously, bubbles will be created in the flask which are difficult to eliminate when plates are poured and they will have a cratered surface (flaming can remove bubbles from still-liquid media if need be). To overcome bubbles entirely, swirl the flask through a wide radius of about two feet or so; then reverse the direction. Swirling from the wrist generates many bubbles. The correct volume of agar to put into a petri dish (100 mm diameter) is between 17-20 mls. This can be gauged by noting when the surface of the dish is about 80% covered, then stop pouring and the delay will give the plate sufficient molten agar to fill the dish without over-filling it. It may require a gentle side-to-side movement to overcome surface tension to complete the covering. After pouring, the plates should be allowed to sit out (lids closed) on a bench top in order to dry excess moisture for one or two or more days depending on humidity and temperature in the building. Prepared, purchased plates require no drying.

Sterile wood sticks are inexpensive transfer devices to make subcultures and transfers. Only one or two sticks should be wrapped per package to prevent contamination once the foil is opened. Discarding of contaminated sticks can be done in 10% household chlorine bleach. Cotton swabs, sterilized in the pressure cooker or autoclave, are excellent to sample environment, animals, and plant surfaces and are discarded in the same manner. After soaking for one or two hours, the decontaminated materials can be disposed of. Petri dishes can be disposed of following autoclaving or thorough disinfection. Tubes of liquid media should be treated the same way. Tubes, slides, droppers, and other items used with microorganisms should be disinfected or sterilized prior to washing to remove the threat associated with working with any bacterium, fungus, or other microorganisms.
To make sterile vegetable slices, wash the item, cut it up on a piece of aluminum foil using a sterile knife. An easy way to sterilize a knife (or pair of forceps) is to dip it (not the handle) into a graduate cylinder or jar full of alcohol and, upon removal, pass it through a flame to ignite the utensil and allow the alcohol to burn away. Repeat this twice more and you have incinerated any contaminants.

Figure 2. Work area of an unorganized student.
One last point regarding safety concerns instilling in the young investigator the desire to organize his or her working area and to keep it neat and orderly. Mistakes and errors in procedure occur frequently if the young (or old) researcher is overwhelmed with a hodgepodge of dirty glassware, left over samples, old petri dishes, yesterday's returned homework, plans for the next issue of the school paper, etc. etc. In short, do not have your student mimic the organization of the lab pictured in Figure 2, instead use Figure 3 as your model.

![Figure 3. Same area organized for efficient research.](image)

Cover Photos: Courtesy of Dr. Hugh Gerlach, St. Francis Regional Medical Center, Wichita, Kansas.