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Watts Cooking: Using a Microwave to Prepare Bacterial Media for Inquiry-Based Experiments

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Abstract
Microbiology provides an excellent opportunity to capture student interest, encourage exploration, and to begin the development of research skills. With a low power microwave, similar to the type found in homes, and a short list of materials easily obtainable and/or found in many biology laboratories, you can begin to open this exciting world to your life science and biology classes. Microwaves are available at very reasonable prices, and can substitute for a much more expensive laboratory autoclave. Your students can choose and design inquiry investigations as well as learn basic laboratory techniques.

Bacterial Culture
Students across all grade ranges and ability levels are naturally fascinated by the growth of microorganisms, especially those growing in their environment. However, in order to observe and study bacteria, growth media, or food that the bacteria need to thrive, must be prepared and sterilized. In a research or clinical laboratory this is done by heating the media to 121 ºC and 15 psi pressure, a process known as autoclaving. This kills both vegetative and spore forms of microbes and is the most effective method of sterilization. However, an autoclave (figure 1) can be very costly and is not always available in schools with increasingly tight science budgets. Purchasing prepared media plates can be costly. Using a low power microwave provides an inexpensive alternative for preparing sterile media to be used in investigations of bacterial growth most often performed in schools. The depth of these investigations will depend on the knowledge base students bring to this experience.

Preparation and Sterilization of Luria-Bertani Agar for Bacterial Growth
Bacteria media plates will need to be prepared at least one day in advance of carrying out any experiment. The following procedure should be used to prepare sterile Luria-Bertani (LB) agar media, in a microwave, for use in experiments with bacteria. Other kinds of bacterial media may be prepared in a similar manner. Luria-Bertani broth is made with the same formulation, leaving out the agar; the agar (similar to gelatin in Jello-O) serves to make the media semi-solid.

Materials
- sterile swabs (Q-tips, though not sterile, are clean and work fine)
- balance
- microwave
- hot pads
- 1 ml pipetting device and 1 ml pipettes
- four sterile plastic petri dishes per student group
- one 250 ml flask or bottle per group (make sure it isn't too tall for the microwave)
- wax marking pencil or permanent marker
- tryptone
- yeast extract
- sodium chloride
- agar
- solution of 0.1 N NaOH
- distilled water
- beaker or tray containing 10% chlorine bleach for disposal of used swabs and Petri dishes

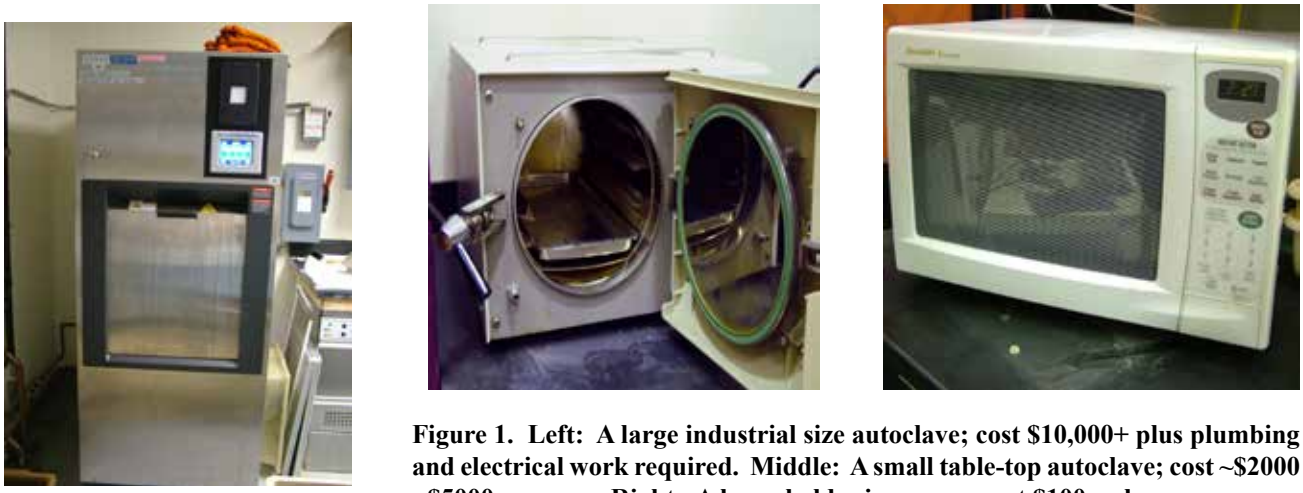

Figure 1. Left: A large industrial size autoclave; cost $10,000+ plus plumbing and electrical work required. Middle: A small table-top autoclave; cost ~$2000 - $5000 or more. Right: A household microwave; cost $100 or less.

Optional materials for Sabouraud's agar
- peptone
- dextrose

Procedure
1) Obtain four sterile Petri dishes and label the bottom with your name, date, and "LB," then turn the plates right side up. This identifies who made the plates, how old they are, and what media is contained in the Petri dish. The Petri dishes are already sterile, so do not take the lids off the plates until the media is poured into the bottom.
2) Weigh out the following ingredients for "LB" and place all of them into a 250ml flask or bottle:
1 gram of tryptone
0.5 gram of yeast extract
1 gram of NaC1
1.5 grams of agar

- Use a container that holds two to three times the volume of media you wish to make so that the media can boil vigorously without boiling over. Make sure your contained will fit in the microwave.

3) Add 100 ml of distilled water and 1 ml of 0.1 N NaOH. Do not put a lid on the container.

Students should be assisted with the following steps
4) Microwave the media for 60-90 seconds to bring the solution to boiling and to dissolve the ingredients. The media should boil vigorously for about 5 seconds, but microwaving for too long will boil the solution over. Once done, the solution should appear clear with no media granules visible. If the media is not clear, then continue microwaving in short 10-15 second bursts until all components are dissolved (figure 2).
- This procedure was worked out for a 1000 or 1100 watt microwave; microwave ovens of lesser power will require slightly longer times.
5) Once all of the ingredients have dissolved, microwave the solution for 15-20 seconds to bring it to boiling. The solution should boil vigorously again, but not boil over.
6) Bring the media to boiling two more times by repeating step 5 twice.
- Be very careful because the bottle is very hot. Media may superheat. This means that it may boil up when agitated, even after the initial rolling boil has stopped. Allow the bottle to stay in the microwave for a few minutes before handling to reduce the danger associated with superheating. Use hot pads when handling the media from the microwave.
7) Place the bottle of LB agar media in a water bath, adjusted to 56 ºC, and allow the media to cool. Loosely cover the bottle with its lid or a piece of foil. This temperature will prevent the media from solidifying, but allow it to be handled more safely and easily. Petri dishes may be poured immediately after microwaving, with close and careful supervision, but it is best to

Figure 2. Left: Flask with poorly dissolved agar; note the cloudiness of the liquid and the powder granules at the bottom. Right: Flask with agar that has been properly heated in a microwave to thoroughly dissolve the agar. Note that the liquid is clear.

cool the media to avoid student burns and melting plates.

8) Pour the liquid LB agar media into the bottom of the four petri dishes. Pour slowly and stop when the media has covered the bottom of the plate (about 20-25 ml). Be sure to work in an aseptic manner. This means do not leave the Petri dishes open on the lab table and replace the covers as quickly as possible.

9) Allow the plates to sit at room temperature until the agar has solidified. Do not move or tilt the plates until the media has hardened. Plates may be stored at room temperature for several days before use. Plates should be stored inverted, with the solidified agar media in the "top" so that condensation (a potential source of contamination) can't fall onto the agar (figure 3). For longer storage, place the inverted agar plates in a plastic bag to prevent dehydration of the media and store them stacked in the refrigerator. Plates may be stored in the refrigerator for one month or longer.

Bacterial Incubation

Once the media plates have been inoculated with bacteria, the Petri dishes are incubated upside down. The dish will rest on its cover and the bottom, containing the media, will be on top. This prevents any moisture, which may have condensed in the lid when the warm media was poured, from falling into the bacteria and contaminating them or disrupting colony formation. Most bacteria like to grow in warm places. Usually 37 ºC, which is also body temperature, is perfect. Obtaining this temperature, however, requires an incubator that many classrooms may not have. A very viable alternative is to grow the bacteria at room temperature, usually about 25 ºC. The bacteria will still grow, just more slowly than they would at 37 ºC. Try to pick one of the warmer locations in the room, such as away from the windows in the winter. Avoid placing the Petri dishes directly onto a radiator or heating vent, however, as the metal may get hot enough when in direct contact with the plastic Petri dish to melt it and the agar. Growth is usually assessed after 24 hours, but plates may need to incubate for 48 hours or longer if incubation temperatures are less than 37 ºC.

An Inexpensive Incubator

A heating pad and Styrofoam container can be used to make an inexpensive incubator. Place a heating pad in the bottom of a plastic or Styrofoam bin, then put four jars in each corner and put another bin on top of the jars. Put the plates in the upper bin, and cover your incubator with a lid.

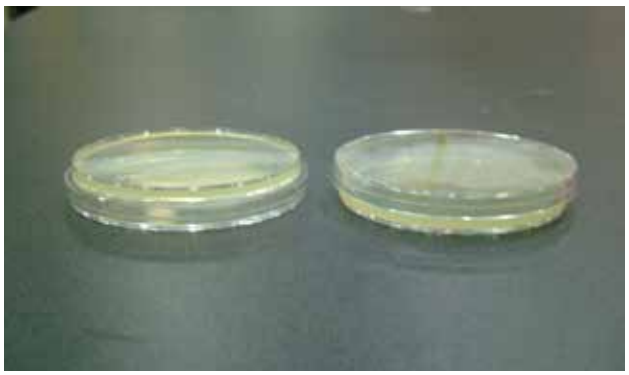

Figure 3. Left: A Petri dish turned upside down. Right: An agar-filled Petri dish with the agar in the bottom; note the condensation on the lid of the dish. Petri dishes with agar should be stored upside down, and dishes should be incubated upside down to prevent condensation from falling onto the inoculated agar.

Dry Heat for Sterilization

Your household oven can be used to dry sterilize items such as glassware and forceps. Make sure that the items you wish to sterilize will not melt or burst into flames. Forceps can be wrapped in aluminum foil and beakers and flasks can be covered with aluminum foil. Make sure that any screw-on caps are loosened. Place the items in an oven heated to 170 °C (340 °F) for one hour. Allow the items to cool to room temperature before using.

Classroom Inquiry and Data Analysis

As early as the elementary grades, students can use prepared agar plates to investigate such phenomena as the antibacterial effectiveness of different brands of liquid soaps or hand sanitizers. Students can touch their fingers to the media before and after using the product to study the reduction of bacteria on washed or treated hands. Plates should be taped shut immediately after inoculation and not opened by the students when they examine their results.

The more sophisticated experimenter may wish to vary the amount of soap or sanitizer used or the length of time spent washing hands. For a different environmental study, students may wish to investigate the quality and quantity of bacteria found in different locations such as on telephone mouth or ear pieces, buttons on a candy machine, drinking fountains, lockers, water faucets (no toilets; or swabbing other students), and so forth, in their school. Using swabs, such as Q-tips, they may swab different identified areas to pick up the bacteria then gently rub the swab across the media plate, being careful not to drag the swab on the agar and tear the media.

Data analysis for classroom bacterial growth experiments can be both quantitative and qualitative. Students can count the number of bacteria obtained from a given location and compare quantities at different sites. Besides observing the numbers of colonies, students can also identify types of organisms by colony morphology. Discussion of these colonies could include the way in which the organisms spread out on the media as well as a comparison of their shape, color, and texture. Incubation at room temperature might produce the growth of mold colonies as well as bacteria. Molds tend to prefer temperatures lower than 37 ºC. The more advanced learners could be encouraged to classify the microbes and relate them to the locations where they were found.

Science Fair Investigations

Bacteriology experiments provide a wealth of investigations that students can easily extend into science fair investigations. Students can experiment with changes in the physical and chemical conditions of the media to determine the effect on bacterial growth. For example, changes in temperature and pH will alter bacterial growth, as will changing the salt concentration (osmotic pressure). Many household products claim to be germicidal, killing microbes, or bacteriostatic, stopping or slowing their continued growth. Students may test whether these agents actually perform as claimed by using a modification of the Kirby-Bauer disc assay (Bauer et al, 1966). This assay traditionally uses discs which have been impregnated with antibiotics to test whether a particular bacterial isolate is treatable with an antibiotic. Students may test their own compounds by using sterile filter discs, approximately 1 cm in diameter (Scheppler et al, 2003).

Inoculation of Agar With Bacteria

1) Begin with an overnight liquid culture of bacteria, or scrape some bacteria off of an agar plate and place it into liquid media making a bacterial suspension. Good strains to use include *Escherichia coli* (MM294 or K-12), *Staphylococcus epidermidis*, and *Bacillus cereus*. These are common organisms found in and on humans and in the environment, but are considered safe to use in the classroom.

2) Dip a sterile swab or Q-tip into the bacterial culture and inoculate an LB agar plate by gently wiping the bacteria-laden swab all over the surface of the agar.

Preparation of Test Agents

3) Prepare a solution of the desired test agent. For convenience, solutions may be poured into

Figure 4. Filter paper discs, impregnated or soaked in a solution being tested for anti-bacterial properties, is gently placed onto Luria-Bertani agar that has been inoculated with B. *cereus.*

sterile empty petri dishes. It may be desirable to test different concentrations of the agent by diluting the product according to the manufacturer's directions for use. Many products are intended to be used at full strength.

- Potential test solutions include various drinks (coffee, tea, soda); different spices (salt, pepper, cumin, ginger, hot peppers); different mouth wash brands; cleaning products, and so forth. Your students can choose, conduct background research, and provide rationales for their choices (Scheppler et al, 2003).

Application of Agent-Impregnated Discs to Bacterially Inoculated Media

4) Using a sterile forceps, dip a sterile filter disc in the solution and place the disc onto the surface of the agar. Students will need to set up appropriate controls (figure 4).

Bacterial growth

5) Incubate the plate, inoculated with the bacteria and containing the disc at 37 ºC for 24 hours; incubate longer at room temperature

6) Observe the plate for bacterial growth. If the inoculated bacteria are sensitive to the antibiotic, an area free of bacteria growth will be observed around the outside of the disc.

Analysis

Students can measure the diameter of the area free of bacterial growth. Replicates can be averaged, and standard deviation determined. Students can perform simple statistical analysis comparing one test agent on different bacteria, or comparing different test agents on the same bacteria (figures 5 and 6.)

Bacterial Transformation

Using plasmid DNA to change bacteria is one of the laboratory exercises included in the Advanced Placement (AP) Biology curriculum (College Board, 2012). A very simple procedure for performing this lab, adapted from Hanahan et al (1983), is included in the AP lab manual, and in Scheppler et al (2000). Many teachers purchase prepared kits. Purchasing the individual components to perform this laboratory may cost more initially, but there will be plenty of reagents which can be used for multiple classes as well as science fair investigations. The reagents, if stored according to the manufacturer's instructions, will last for several years or longer.

Microbe Isolation

Students may wish to experiment with different media to select and isolate different types of organisms. LB agar, which will grow bacteria preferentially, and Sabouraud's agar, which will grow yeast (fungi) may be used. The lower pH of Sabouraud's agar inhibits many bacteria, but favors growth of fungi. The recipe for Sabouraud's agar is 1 g peptone, 4 g dextrose, and 1.5 g agar per 100 ml distilled water. The mixture is microwaved for sterilization in a manner similar to the LB agar media and plates are poured using sterile petri dishes as discussed previously.

Microbes in Soil

There are approximately 10^8 bacteria in a gram of soil. The numbers and types of microorganisms isolated from soil will vary depending on the time of year and the location and quality of the soil sample. Students might compare different soil types such as sand, garden soil, dusty soil from a baseball diamond, and so on. This experi-

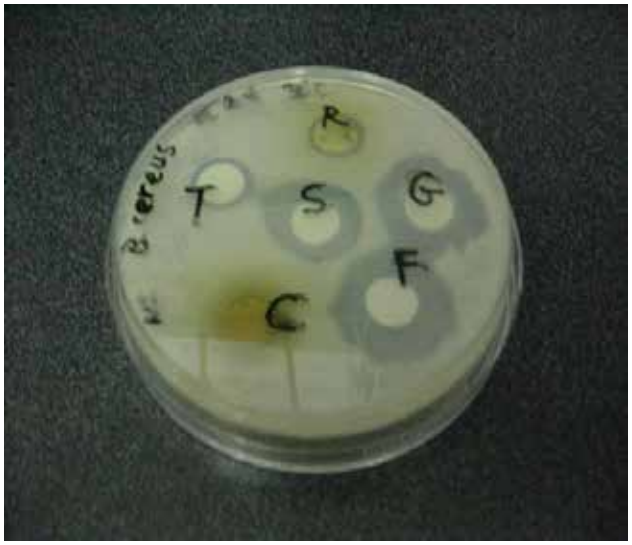

Figure 5. A Kirby Bauer disc assay. Filter paper discs were inoculated with various spice solutions and placed onto Luria Bertani agar inoculated with *B. cereus*. The plate was incubated overnight at 37°C to allow the bacteria to grow. Note that the discs labelled F and G have large zones of clearing around them, where the bactera did not grow. The discs labelled T and R have small small zones of clearing. The disc labelled S has a medium zone of clearing, and the disc labelled C shows no clearing.

ment could potentially develop into a year-long class project where students collect a soil sample each month, characterize the microbes present in the sample, and then correlate their data with environmental conditions such as temperature, rainfall, pH of the soil, and other factors.

To isolates bacteria from soil, students place 1 g of soil into 100 ml of sterile water. After vigorous mixing, 1 ml amounts of the microbe and water mixture are spread onto both LB and Sabouraud's agar plates using a sterile swab or spreader. Place the used swab in a beaker or tray of 10% bleach for disinfection. The plates are then incubated for one to seven days at room temperature and growth is observed. Fungi may require longer incubation periods for growth than bacteria. Students may need to spread smaller amounts of the soil inoculum onto the plates to obtain countable numbers of bacteria and fungi.

Safety Considerations

Students must use safe practices, which include good laboratory techniques during an experiment, proper clean-up after the experiment is finished, and adequate clean-up if a spill or other accident occurs. *E. coli* (MM294 or K-12) is an appropriate bacterium to use for bacterial transformation, altering growth conditions, and examining growth inhibition since it was designed for laboratory use and is not a wild-type strain. It is very important that any household items that you adapt for use in the classroom be used only for classroom experiments, and not revert to use for food or other non-laboratory activities.

When isolating bacteria from the environment it is possible that students may find bacteria that have the potential for causing illness. Although they are exposed to these organisms all the time, one must take appropriate precautions. Tape the dishes closed before students make and record their observations and do not allow them to open the dishes. When they are swabbing areas of the environment, do not allow them to swab other students, especially the mouth, nose, throat, ears, and so forth.

Note that some science fairs, expositions, and competitions may restrict where students and schools obtain their microbial cultures. It is possible that they will not permit investigations to be entered into a science competition that have used microbes grown from a primary culture obtained from humans or other warm-blooded organisms because of the risk of culturing a human pathogen. (A primary culture is one obtained directly from the organism.) Educators should review

Figure 6. A ruler can be used to measure the zone of inhibition around a compound impregnated disc and student can collect quantitative data and perfomr statistical analysis.

these guidelines and consider their own level of expertise when conducting experiments using microbes. There are many reputable biological supply companies that sell known, pure, and safe microbial cultures that can be used in these types of experiments.

At the elementary school level it is recommended that media plates be prepared for students, and that teachers encourage them to take part in the experimental design. Beginning in the middle school years, students should be able to mix ingredients together to make and pour their own media plates. Making bacterial media allows them to practice their laboratory skills of measuring both solid and liquid components. Students can then use the plates they made to conduct their own experiments.

Once the experiments are completed, you must disinfect the materials before discarding them. While autoclaving is the gold standard for sterilization, both in preparation of media for an experiment and for disposing of materials, other methods are satisfactory. An inexpensive alternative for disposal is household bleach. Prepare a 10% bleach solution and soak any swabs, equipment, and bacteria-containing petri dishes in it for at least an hour. This is sufficient to kill the bacteria and disinfect the surfaces. Follow your institution's guidelines for disposal.

Discussion

Preparing bacterial media in the microwave is an inexpensive and effective way to provide opportunities for students to participate more in laboratory experiments and to actually design their own experiments. These are appropriate exercises for inquiry-based learning.

I have successfully prepared LB agar in the microwave with many students as well as educators in various workshops. The younger students, ages about 10 years old (middle school age) and older, are closely supervised and assisted when pouring their plates. The classroom is set-up with two weighing stations and one microwave, so other activities are planned concurrently. Faculty workshop participants unanimously found the activity of preparing bacterial media in the microwave easy and useful. Most had not prepared agar petri dishes before. Discussions centered around the many ways that the plates could be used to examine bacterial growth in the classroom. All agreed that making the LB agar plates from scratch was less expensive then purchasing the media already prepared or purchasing a pre-packaged kit experiment. Using this method, the bacterial transformation laboratory from the AP Biology curricula becomes accessible to all biology students and could be used as a tool for various science fair investigations.

Once you have mastered preparing your own agar media for the growth of microbes, many experiments become easy to perform. For more ideas, obtain a microbiology laboratory manual and try out other experiments. Most experiments can be modified to address the needs and interests of your students. The possibilities are unending and your students will experience new growth.

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