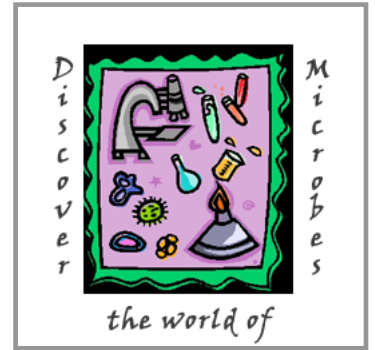


NABT 2006

Microbial Discovery Workshop

Friday, October 13 – Ballroom C
9:30am – 10:45am



Oceans of Microbes: Recovering Microorganisms from Your Local Environment

Facilitator: Peter J. Polsgrove, Northern Arizona University Flagstaff

Workshop Outline

- I. Introduction to ASM's Video Podcast and MicrobeWorld.org website.**
- II. *Intimate Strangers* - Episodes 5 & 6**
This video illustrates the microorganism ubiquity, isolation and growth.
- III. Microbial Discovery Activity - Kitchen Microbiology (It's Easier Than You Think)**
The American Biology Teacher, Volume 61, No.1, January 1999
- IV. Presentation of the Microbial Discovery on-line resources.**
Participants in this workshop will receive additional information on teaching resources available at the American Society for Microbiology website.

Did you use this activity in your classroom?
Please send questions/comments to:

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Kitchen Microbiology

(It's Easier Than You Think!)

Catherine Wilcoxson Stacey M. Shand Richard F. Shand

We are reminded continually of the presence of microorganisms in our surroundings. News reports of tainted food due to microbial contamination of chicken, beef or fruit are prevalent. Strep throat, acne and various other bacterial infections are common occurrences that are familiar to our students. As consumers, we are enticed to buy facial scrubs, mouthwashes, soaps and cleaners that inhibit bacterial growth. How effective are these products in controlling bacteria? Where do bacteria normally live? What conditions do bacteria require to live and reproduce?

These questions can be readily explored in middle and secondary classrooms using the following procedure to prepare culture media that can be used for the isolation and propagation of microorganisms. This procedure was devised and tested with the following goals:

1. To use supplies available locally in a supermarket or health food store
2. To reduce the process to its essential components
3. To produce a medium that would support bacterial growth equal to that of a professionally prepared medium.

The technique described herein is a simple and effective means to prepare the culture media needed to conduct microbiological investigations.

Materials

All the needed materials are shown in Table 1 and can be found in a

Catherine Wilcoxson and **Richard F. Shand** are Associate Professors in the Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640; e-mail: catherine.wilcoxson@nau.edu. **Stacey M. Shand** is at Flagstaff Junior Academy, Flagstaff, AZ 86004.

Table 1. Materials needed for *Kitchen Microbiology*.

- cotton swabs
- paper towels
- agar, 1 ounce, purchased at a local health food store
- measuring spoons—teaspoon and ½ teaspoon
- canned chicken broth—fat free, low in sodium, 14.5 ounces (410 ml)
- 40 sterile, plastic, disposable petri dishes, 15 × 100 mm
- 4 canning jars able to hold 8 fluid ounces
- manual can opener
- commercial disinfectant such as Lysol®
- big pot with lid—8 quart or larger
- aluminum foil
- 2 cups (500 ml) filtered or distilled water
- 1 cup (250 ml) glass measuring cup
- metal teaspoon or soup spoon
- permanent marker
- oven mitt
- tongs

kitchen or purchased locally, with the exception of the petri dishes. The sterile, plastic, disposable petri dishes are not expensive and can be purchased from most scientific supply companies for about \$4 or \$5 per 20 plates. Although alternative containers have been used (Blair & Bowen 1996), there is no substitute for microbiological petri dishes, and students might feel more like they are doing “real” science when they are using common scientific equipment.

Procedure

1. Clean the kitchen counter with disinfectant and a paper towel. Do not use sponges as they are usually heavily contaminated with bacteria.
2. Wash the canning jars and measuring cup in hot, soapy water. Thoroughly rinse out all the soapy water. Turn the jars and measuring cup upside down on a paper towel to drain.
3. Place the can opener and spoon in the large pot and cover with

water. Boil them for 10 minutes. Remove them from the boiling water with tongs and place them onto the paper towel next to the canning jars and measuring cup. The tongs will not be sterile, so they should be used at the end of the can opener and spoon, away from the end that will come into contact with the media. To determine the amount of water needed for boiling the canning jars, place the four canning jars into the pot and add water so that the level comes up about half way on the jars. Use care as the jars are empty and will tend to float. Remove the jars, replace the lid on the pot, and bring the water to a boil. While the water is being brought to a boil, prepare the agar.

4. Wash the top of the chicken broth can with hot, soapy water and rinse.
5. Add 200 ml (7/8 cup) of filtered or distilled water to each of two canning jars (use the measuring

cup to measure). Then open the chicken broth and fill the remaining two canning jars with 200 ml (7/8 cup) of chicken broth. One can of chicken broth is enough for two jars. There should be two jars with chicken broth and two with filtered water. Be sure to use jars large enough so that the agar fills the jar halfway or less.

6. Add 1.5 tsp (about 3 grams) of agar to the liquid in each jar. Mix the agar with the clean spoon, starting with the water agar first, then the chicken broth agar. This sequence prevents the transfer of protein from the chicken broth to the water agar.
7. Make an aluminum foil cover (use 2 to 3 layers for each jar). Label the aluminum foil covers with a permanent marking pen to indicate the contents of each jar (e.g. WA = water agar; KM = kitchen medium). Place each jar into the pot of boiling water. Make sure to wear the oven mitt to protect your hand and check that the water level is *at or above* the level of the liquid in the jars. Add more water if needed. Put the lid on the pot.
8. Boil the agar for 35 minutes.* Do not start timing until the water in the pot is actually boiling. Gently swirl the agar in the jars every 10 to 15 minutes. Wear the oven mitt to protect your hand. Check the water level every time you swirl and add more water as necessary.
9. While the agar is boiling, remove the sterile petri dishes from their protective plastic sleeves by cutting off the very top of the sleeve with a pair of scissors. Save the sleeve. Label 20 dishes as "WA" and 20 dishes as "KM" with the permanent marker.
10. When the boiling has finished, turn off the power source and carefully move the pot to a cool surface. Leave the canning jars in the pot while the agar is cooling.
11. When the agar has cooled (50 to 55° C or about 20 to 25 minutes), remove one jar at a time from the pot. Wipe off the water on the outside of the jar with a paper towel. Remove the aluminum foil lid and pour enough agar into the bottom of a petri dish to just cover the bottom (about 20 ml). Replace the lid and carefully slide the petri dish to the back of the counter out

of the way. Make sure not to slosh the media onto the underside of the lid when you slide the plates to the back of the counter. Work steadily as the agar in the jars remaining in the pot will begin to solidify at about 45° C. Continue in this fashion until all the agar has been dispensed.

The water agar was included to demonstrate that a nutrient source is necessary for microbial growth. Steps 5, 6, 7 and 9 can easily be amended so that only the kitchen medium is prepared.

*NOTE: This procedure was developed at an altitude of 7000 feet. The time for boiling was increased by 10 minutes at this altitude.

Inoculation, Incubation & Precautions

In addition to the chicken broth agar and the water agar that were made in the kitchen, agar that was professionally prepared (Difco Nutrient Agar®) was also used for a comparison study. To maximize viewing of colonies, all agar plates were labeled on their edges with type of agar, date and substance tested. On the back of petri dishes containing kitchen media, a line was drawn down the middle of the plate and the left side labeled "C" for control. Ten different sources were tested for microbes (see Table 2). Each cotton swab was carefully removed from the box and first streaked onto the control side of the kitchen medium to determine if any bacteria were present on the swab prior to inoculation. This process also "wets" the swab, which is advantageous as moist swabs will pick up more inoculum from dry surfaces than unmoistened swabs. Alternatively, swabs can be moistened using the water droplets that have condensed on the underside of the petri dish lids. The same cotton swab was then inoculated with the test substance, streaked onto the water agar, the other half of the kitchen medium, and the professional medium, in that order. After the plates were inoculated, they were taped shut (see below) and put back into the plastic sleeves. The opening at the top of the sleeve was folded over and closed with tape. If the plastic sleeve is unusable, a large plastic bag with a sealable closure will also work.

In this study, the plates were incubated at 98.6° F (37° C) for 24 hours in an incubator. However, incubation for 3 to 4 days at room temperature

works equally well, although growth of the bacteria is slower. Incubation times can be reduced by placing the plates in an area that is above ambient temperature, such as on top of a refrigerator, toward the back.

It is essential that students tape their petri dishes shut because it is possible that students will isolate pathogens and/or organisms that form spores. It is not safe for students and staff to be exposed to either of these.

Once the exercise has been completed, the cultures on the plates must be sterilized and made harmless. Unsterilized plates must not be disposed of in the garbage! The plates can be incinerated if the school district has an incinerator. Alternatively, arrangements can often be made with a local hospital, university, college or state health department to use its autoclave to sterilize the plates. If none of these alternatives is available, the plates can be sterilized in a pressure cooker for 20 minutes at 121° C and 15 pounds of pressure, and then disposed of in the garbage.

As a final word of caution, microwave ovens should never be used to sterilize the plates because:

1. Conditions for rendering the plates sterile must be arrived at empirically for each microwave.
2. Microwave ovens are almost always used to prepare food and are rarely dedicated solely to sterilization.

Results

Amount of colony growth and number of different types of colony morphologies can be easily observed and recorded. However, identifying bacterial or fungal organisms based solely on colony morphology is rarely definitive, although colonies with extensive hyphal growth (fuzzy in appearance) are usually fungal. Outdoor sources will probably produce more fungal growth than indoor sources. The vast majority of colonies recovered in this study were bacterial (Table 2).

The kitchen medium was as efficient as the professionally made medium in supporting colony growth. Very few colonies appeared on the control side of the kitchen media plates, demonstrating that either cotton swabs are relatively free of bacteria or that where colonies did appear, aseptic technique was comprised. Except for the fish tank water, almost nothing grew on the water agar plates; they contained no nutrients, demonstrating that agar is nonnutritive for most microbes. The

Table 2. Comparison of kitchen medium to professional medium in growth of microbial colonies from various sources.

Test Substance	Cotton Swab ^a	Kitchen Medium			Professional Medium	
		Water Agar (+/-) ^b	Growth (+/-)	Types of Colonies ^c	Growth (+/-)	Types of Colonies
Banana (surface)	0	-	+	4	+	6
Bottom of Shoe	0	+ (5 colonies)	+++	8	+++	10
Ear Wax	0	-	+	3	+	4
Fish Tank Water	2	++ (very small)	+++	7	+++	10
Kitchen Sponge	0	-	+++	3	++++	2
Money (dollar bill)	7	-	ND ^d	ND	+	3
Plant Leaf	1	-	+	2	+	2
Stove Top	2	-	+	2	+	2
Teeth	1	-	++	2	+	4
TV Dust	0	+ (3 colonies)	++	4	++	4

^aThis is a control where the cotton swab was rubbed onto the agar surface before it was rubbed onto the test substances (number of colonies reported).

^bGrowth: (-) no growth; (+ to +++) = different amounts of growth from several distinct and well separated colonies to confluent growth, sequentially.

^cIndicates number of different types of colony morphologies.

^dND: Not determined.

small colonies produced on the water agar from the fish tank inoculum were a result of nutrients in the fish tank water.

To determine if this procedure was accessible to a nonmicrobiologist, an undergraduate biology education major with little experience or knowledge of microbiology volunteered to repeat the experiment. With no help or advice from the authors, the student successfully replicated the above procedure. This success has encouraged him to incorporate *Kitchen Microbiology* into his student teaching experience.

Experimental Issues

We have found that there is simply no substitute for two of the materials used in our study—agar and petri dishes. Fortunately, agar is readily available at most health food stores (often located in the bulk foods section as “agar-agar”). Since agar is typically available in bulk, one can weigh out as little or as much as one requires. Its lack of accessibility as a nutrient to all but a few microorganisms, its superior gelling properties, and its local availability are all advantages that agar has over cornstarch and gelatin, which have been used as gelling agents (Blair & Bowen 1996). As men-

tioned earlier, sterile, plastic petri dishes can be obtained from many scientific supply houses or might be available as a donation from a local college, university, hospital or clinical laboratory. Alternatively, glass petri dishes can be purchased and reused, but are far less convenient when it comes to cleaning and sterilization.

We used low sodium chicken broth directly from the can. Canned chicken broth is sterile due to the processing procedure and is much more convenient than recovering the broth from a home cooked beef roast, as suggested by Blair and Bowen (1996). They prepared homemade beef broth because it lacked any monosodium glutamate (MSG), which they considered an inhibitor of microbial growth. However, MSG serves mainly as a “flavor enhancer” and only marginally as a food preservative (Atlas 1997). Blair and Bowen also suggest adding table sugar (sucrose); however, dextrose (D-glucose, also available at health food stores) would be utilized by a larger number of microbes.

Initially we had intended to use a pressure cooker in order to reach sterilizing conditions of 15 psi at 121°C for 15 minutes, but we really wanted to see what could be done without employing any sterilizing conditions.

Many items (e.g. paper towels, cotton swabs, aluminum foil) are essentially rendered sterile during production. Using previously unopened packages of paper towels and cotton swabs will help to minimize contamination. Since the chicken broth was already sterile, we only needed to wash the top of the can and boil the can opener and the spoon. The canning jars and measuring cups were washed in hot water and detergent. No other precautions were employed. The agar was weighed out from a common bottle into a plastic bag supplied by the store, and a large pot was used to boil the agar instead of using a pressure cooker.

Variations on a Theme

The protocol outlined above can be modified to use nutrient sources other than chicken broth. Examples of such alternatives and the composition of relevant nutrients in each are listed in Table 3. An excellent resource for preparing alternative microbiological media is the *Handbook of Microbiological Media* (Atlas 1993) in which recipes for Skim Milk Agar (p. 810), Tomato Juice Agar (pp. 916-17), and V-8™ Agar (p. 973) can be found. There are a few technical issues to note when preparing media from the nutrients

Table 3. Alternative nutrients for microbiological media.

Nutrient	Protein (g/200 ml)	Sugars (g/200 ml)
Skim Milk ^a	6.7	10.0
Chicken Broth	2.5	0.83
Beef Broth	1.7	0.83
Vegetable Broth	0	2.5
Carrot Juice	1.1	8.0
Tomato Juice	1.2	5.3
V-8™ Juice	1.2-1.7	6.5
Orange Juice	0.83	23.3

^aReconstituted to one cup (240 ml)

listed in Table 3. Skim milk powder contains spores (just as regular, pasteurized milk does) which are difficult to kill without autoclaving or using a pressure cooker. We have found that Pet™ and Carnation™ brands of skim milk powder have fewer spores than other brands. Spores in the skim milk that are not killed during preparation will germinate upon incubation and will form lenticular ("lens-shaped" or "football-shaped") colonies inside the agar. The skim milk powder should be heated gently (to avoid coagulation) and then brought to a boil so that it is fully dissolved. Note that skim milk agar can be prepared using just skim milk powder, agar and water (Atlas 1993); or, the skim milk powder can be added directly to the chicken broth agar (1.5 g powder/200 ml of water or chicken broth). If skim milk agar is successfully prepared, it will be white and opaque. It is a wonderful medium as many of the organisms in the environment secrete proteases (enzymes that break down protein) and zones of clearing will appear around these colonies as the milk protein is broken down.

Fruit juices generally contain insufficient protein (Table 3) to be used alone and should be mixed with chicken broth agar. However, fruit juices can be purchased as concentrates and are rich in sugars. We recommend mixing fruit juice concentrates with chicken broth in a ratio of 1:4 (e.g. 40 ml fruit juice concentrate to 160 ml chicken broth). This combination will make a rich and colorful agar medium. Similarly, tomato juice and carrot juice can be mixed with chicken broth in a ratio of 1:2 (e.g. 67 ml of tomato juice to 134 ml chicken broth). V-8™ agar can be made with just V-8™ juice and agar (Atlas 1993) if the variety of juice contains about 2 g of protein (alternatively, it can be mixed with chicken broth as described for tomato juice).

Finally, one should check the pH of the medium when it contains fruit or vegetable juice. These additives will often lower the pH between 4 and 6, which will prevent many organisms from growing. The pH can be adjusted to neutrality by adding a little base or, two sets of agar plates can be made, one with low pH and one with a pH near neutrality so that the effect of low pH can be observed.

Pedagogical Issues

An investigation of microorganisms is developmentally appropriate for middle level students as well as high school students. Students at this level should know that microbes are everywhere—on dust particles and wood, on human skin, and in fish tank water. The experience of discovering just where microbes are found is important for more complex understandings about nutrition and energy flow later on (National Research Council 1996).

Students should also know that microbes are very small; too small to see with your eyes. To see them, biologists put them onto media and allow them to grow and form colonies. These colonies are composed of millions of individual organisms.

This simple but effective procedure for preparing microbiological media is a beginning point for each investigation. Once the students are familiar with the technique, they are able to expand their investigations into topics that are relevant and of interest to them. Such topics might include: the impact of environmental factors (e.g. temperature, pH, metals, radiation, etc.) on microbes; the effectiveness of various disinfectants, antiseptics, antibiotics, soaps and mouthwashes; enumerating the number of microbes in various sources (e.g. as in hamburger or on vegetables); and elucidating

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growth requirements for various microbes. Inquiry into student-generated questions promotes student curiosity and helps them develop the ability to ask scientific questions, to design and conduct investigations to answer their questions, and to use their observations to arrive at a reasonable explanation for the question posed. Students can identify items used in the home that help prevent the spread of bacterial infection (e.g. disposable cups, disposable tissues, disinfectants, antibacterial soaps) and equally important, discuss the *beneficial* contributions of bacteria.

This procedure provides an opportunity to collaborate with the consumer science (formerly known as home economics) teacher to teach kitchen cleanliness, effectiveness of hand and machine dishwashing techniques, effectiveness of hand washing (see Brown & Williams 1990 for procedure), and other health related issues, especially if one takes samples from the kitchen area. An interdisciplinary approach can be further expanded to include mathematical analysis of data and the exploration of plagues and their economic effects on society.

Conclusions

Students are intrigued and interested in learning more about microorganisms because they affect their daily lives. Even though studies show that science classes do not receive adequate support for supplies and equipment (Suter 1996), microbiology is accessible to classroom teachers. Low cost materials and adequate substitutes for expensive equipment can be used to successfully conduct inquiry-based investigations. Teachers can perform this procedure even if they lack expertise in microbiology.

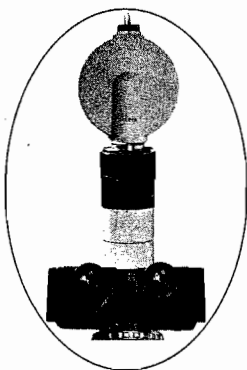
It is important that students engage in scientific inquiry. The inclusion of laboratories and hands-on activities in science classrooms is a goal of many science teachers. This goal is supported by the National Science Education Standards' emphasis on inquiry-based teaching and learning. Indeed, the motivation for developing this procedure came from a middle level student who was investigating a possible science fair project involving microbiology. We hope this procedure will enable you to engage students in posing microbiological questions and pursuing the answers to those questions.

Acknowledgment

We would like to thank one of the anonymous reviewers for suggesting the inclusion of skim milk agar, V-8™ agar, and fruit and vegetable juice agars.

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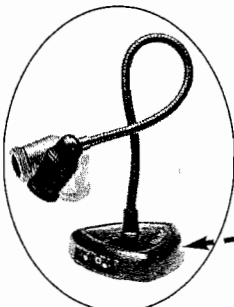
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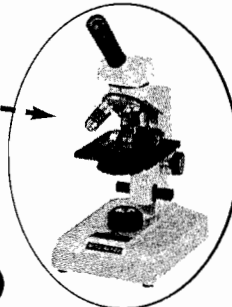


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Lesson Title:

Grade Level:

Course Title:

Teacher's Name:

Number of Students:

School District and Address:

Preparation

Yes

No

Notes:

Are the set-up procedures too complex for the purpose of the lesson?

Were the materials easy to acquire?

Did you have all applicable materials and equipment in your lab?

How much time did the lesson take to set up?

In class time?

Out of class time?

Lesson Design

Exceptional

Good

Poor

Unsatisfactory

Please rate the introductory activities.

Please rate the lesson/project

Please rate the experiment, in regards to student interest.

Handouts

Exceptional

Good

Poor

Unsatisfactory

Please rate the effectiveness of the teacher handout?

Please rate the effectiveness of the student handout?



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Assessment

Can this lesson can be accomplished in the amount of time indicated by the author?

Yes

No

If no, how much time did the lesson take?

Are you planning any follow up activities?

If so, what assessments or reviews will you be using? (tests, essay, discussion, etc.)

How were the national science standards addressed in this lesson?

General

Was this lesson effective in conveying a particular concept/activity?

Yes

No

Would you teach this lesson again in future years?

Would you recommend this lesson to a friend?

How would you change this lesson if you were to teach it again?

Podcast

Was the podcast appropriate for the intended audience?

Yes

No

Was the podcast clearly linked to the lesson objectives?

Was the lesson enhanced by the use of the podcast?

Notes: