

BACTERIA: THEY ARE EVERYWHERE

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Type of Entry:

- Project

Type of Activity:

- This is a hands-on activity that allows students to investigate common surfaces for the presence of bacteria and learn a simple procedure for culturing bacteria in a laboratory setting and assemble DNA by the method of PCR.

Target Audience:

- This lab can be used in any life science, biology or advanced biology classroom.

Abstract:

- This activity allows students to understand that bacteria are indeed everywhere. Most bacteria are beneficial and help maintain the environment by degrading waste materials, man-made chemicals and pollutants. Only a few bacteria can poison or cause disease. In this activity, students will illustrate that any surface at school will have bacteria present by using sterile swabs on selected surfaces and transferring the bacteria to the agar filled petri dishes. The students will grow the colonies in a controlled environment and will identify shapes and sizes of the bacterial colonies present. The students will also test which over-the-counter cleaner, bleach, soap and water, or various anti-bacterial agents, kill the bacterial colonies best.

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Background Information:

Part I:

- **Teacher notes:**

Lab groups of four students are used for this activity. This gives all students an opportunity to do a part of the lab and learn to share responsibility of helping others in a group lab.

The teacher will need to pour or plate the agar into the petri dishes a few days before needed and store in a refrigerator. This allows the agar to be firm so the students cannot tear it as easily. The petri dishes should be stored upside down to avoid condensation dripping on the agar.

The bacteria grown in the petri dish, seen with the eyes only, is called colony morphology. The bacteria seen on a microscope slide is cellular morphology. Therefore, to actually see the colony shapes you may need to gram stain the bacteria. However, for general biology students to actually see bacteria grown from places they come in contact with everyday is extremely important and not necessary to gram stain for students to grasp the idea.

- ***Class time needed:**

The class time needed for this lab can be arranged at the discretion of the teacher depending on the school's schedule and period time. It is recommended that several entire classes be devoted to the exercise in the beginning. The length of time recommended for the plates in the lab to incubate successfully is 72 hours or three days. Therefore, four to five classes maximum should be ample time to complete the project.

- **Preparation time:**

One half hour to one hour. All materials can be prepared several days in advance.

- **Required of students:**

Students will need to bring pencils, notebooks and text to class. They will need to work with their groups in a cooperative learning manner.

Materials Needed:

- *distilled water (aliquot into very small containers for each group)
- *petri dishes (approximately 60 x 15mm) or larger if you prefer (2 per group)
- *nutrient agar
- *sterile cotton swabs (4 per group)
- *incubator
- *Bacteria, area where students choose to swipe
- *paper
- *pencil
- *colors
- *five over-the-counter cleaners, (bleach, soap and water, anti-bacterial cleaners)
- *paper towels

Procedure:

- Discuss the way bacteria reproduce, develop, and grow. The students should be introduced to the idea that bacteria exist in many forms. Many bacteria can be recognized by their shape as seen under the microscope. Since individual bacteria cannot be seen with the naked eye, the students will grow colonies of bacteria containing several million individual bacteria that are visible to the naked eye. Introduce the shapes of the colonies and their names to the students. Rod shaped bacteria are called Bacillus. Berry or circle shaped bacteria are called Coccus. Bacteria, shaped like chains of berries, are called Streptococcus. Bacteria, shaped as a bunch of berries, are called Staphylococcus. Comma shaped bacteria are called Vibrio. The students should be able to draw these different shapes for further notes in this project.
- Divide the class into groups of four students per group. At this time, each group may choose a location they wish to swab with permission from the teacher. Each group must also choose one over-the-counter clear.
- Each group will receive equipment needed for the lab.
- The students in the group will divide each petri dish containing the agar into two halves, labeling one half, I, and the other half, II with a permanent marker on the back of the plate, agar side. One half will be used as a control.

- Each group will then go to the area chosen and carefully remove the sterile swab from the wrapper, dip in distilled water and swab the area chosen for their project, being careful not to touch any other surface including their fingers.
- The students will then remove the top of the petri dish and lightly wipe the contaminated swab on the agar in a two half pattern, side-to-side, up-and-down, being very careful not to tear the agar, then place the top back on the petri dish as quickly as possible upon completion.
- The students will carefully place the contaminated swab back in the wrapper and dispose in the proper garbage.
- The students label the petri dish with their names and the beginning date.
- The students will then place their petri dishes in the incubator upside down to prevent condensation from forming on the agar/bacteria.
- The students will return to the area previously swabbed and clean the area with the chosen over-the counter cleaner. The students will then repeat the swabbing technique on the clean area, returning the petri dish marked mark with appropriate data to the incubator.
- They will learn that bacteria favor an environment that is fairly high in moisture and a temperature between 20 degrees and 38 degrees Celsius. Those conditions are best replicated in an incubator where they can be controlled for proper growth culture. However, colonies can be seen at room temperature of 22 degrees Celsius within 24-48 hours.
- Over the next three classes, the students will observe increasing growth in bacteria as it becomes visible. Each colony represents an individual bacterial cell that has multiplied enough times to be seen. Students should determine whether there are different kinds of colonies growing in shape, color and size, representing different kinds of bacteria present on the surface of the agar in the petri dish. After determining the bacteria present in their culture, the students will compare and contrast that with other groups in the classroom.
- For the more advanced students: Students may examine the bacteria in the colonies by taking a small sample from the colony, mixing it with a drop of diluted crystal violet on a microscope slide, placing a coverslip on the drop and examining it under a high power microscope. Or the students may carry out a gram stain using a purchased kit.

- Each student in the group will keep a record of the data collected each day. The student will need to draw a picture of bacteria viewed in the cultures to be included in the data.

The last step, as a class will be to determine if the over-the-counter antibacterial agents, such as sprays, gels, and soaps or bleach will have any affect on their bacteria. Each group will have chosen one over the counter agent and used it to clean the surface of the bacteria on their groups chosen area. Each group will report the results to the class.

- You may also allow the students to design their own experiment to test various ways of cleaning their surfaces.
- As a follow up of the lab, the groups will be responsible for cleaning the location from which they chose to swipe at the onset of the lab, preferably with a bleach-based agent.

The students will have learned that not all bacteria will cause a disease from class discussion. There are good and bad bacteria, (from the human perspective, not the bacteria's). They also will have learned how easily bacteria can be transferred from one object or person to another by the transfer from area swabbed to petri dish; how the environment where bacteria prefer to live and produce can be controlled and even altered to prevent or increase bacterial growth by cleaning or not cleaning the area swabbed. The students also will have learned the best over-the-counter cleaner for hand washing is always soap and water and the best for surface cleaning is bleach.

Method of Evaluation:

- The students will be evaluated in the laboratory each day. They will be required to write an individual laboratory report containing all the data collected and a drawing of each phase of growth of the bacteria. The students will present as a group their conclusions to the class.
- The students will be evaluated by each other in the group, as well as by the teacher, for their cooperation and work ethics.
- Each student will be assessed individually and not as a group for the individual laboratory report.

Fun Fact:

- More students wash their hands after this lab activity than ever before!

Part II:

Purpose:

The purpose of Part II is to show the students how bacteria can be cloned and travel from one location to another location. The most likely culprit in school is the unwashed hands of the students. In this activity, four to five students will inoculate their unwashed fingers in the agar in the petri dishes, wash their hands and repeat the procedure, showing how much dirt and pathogens are carried on the unwashed hands.

The Polymerase Chain Reaction (PCR) will show how scientist can take a little DNA and make enough DNA to show who spread the bacteria or what bacteria was spread in the school.

Background Information:

- **Teachers notes:**

The second part of this activity involves the Polymerase Chain Reaction or PCR. The students will work on the project as a class. The students can do an at-home project on PCR to go along with the in class instruction.

- **Class time needed:**

The activity can easily be done in one to two class periods. The activity contains three sections: lesson/instruction, piecing together the PCR, and a hand sanitation exercise.

- **Preparation time:**

The PCR is made of Funnoodles, a styrofoam floating toy found at discount stores. The teacher will need to choose two different colors of Funnoodles and cut the Funnoodles in pieces of twelve-inch sections and then divide each section in half-length wise. You will need approximately sixteen twelve-inch section of each color, which can be done from three whole Funnoodles of both colors. The teacher will then need to take half of the pieces of each color and put them aside. These twelve-inch sections are to be the denatured half of the DNA fragment. The other half pieces will need to be cut again in nine and three inch sections. The three-inch sections will become the primers and the nine-inch sections will become the extensions of DNA nucleotides. To help with primer recognition, you may want to add several more twelve-inch sections of different colors. This should take no more than an hour to prepare.

The teacher will need to prepare the agar in the plates, as done in Part I. This can be done with Part I of the lab to save time. You will need two plates for each student you wish to test in hand sanitation. Four to five students per class should be enough to show how bacteria travels on your hands and how washing the hands helps to stop the transfer of bacteria.

- **Required of students:**

The students will need to bring notebooks and pencils to class; they will need to be able to work with each other as a class. The students can be responsible for doing an at-home project in a smaller scale of the PCR done in class.

Materials needed:

- eight Funnoodles (3 each of two colors and 2 of different colors)
- agar filled petri dishes (2 for each student up to five students)
- toothpicks
- hand soap (antibacterial)
- paper towels

Procedure:

- Discuss the principles of PCR with the students. PCR works by amplifying DNA fragment sequences. Even an extremely small amount of a certain DNA molecule to begin with, so little it can scarcely be detected, can generate literally billions of copies by PCR. As a result there are many, many uses for PCR.
PCR can be used in clinical diagnosis, reducing the valuable time before a diagnosis is made in a shorter frame. Viruses such as meningitis, hepatitis, and even the flu can be diagnosed by using PCR. Thus knowing what is wrong with the patient, a doctor can prescribe the correct medicine to the patient faster hopefully insuring a quicker recovery.
PCR can be used in genetic analysis for paternity cases, crime scenes, and to determine the heredity factor of a gene. PCR can be used, as in this activity, for genetic analysis to determine the source of a bacterial infection.
PCR is used in many other ways such as genetic engineering and for forensic analysis. There are countless possibilities for this wonderful invention called Polymerase Chain Reaction or PCR.

- The fundamentals of PCR begin with a segment of DNA that is to be amplified in order to generate many copies. For this to occur we need to follow three steps:
 - 1) Denaturation: First, an excess of primer, (three-inch Funnoodles---I used green and purple Funnoodles), which are typically a synthetic sequence of 20-30 nucleotides, is mixed with the DNA fragment (two different colors of twelve-inch Funnoodles held together with toothpicks—I used half green and half purple to form one double helix of DNA and half pink and half orange to form the second DNA double helix held together with toothpicks) to be amplified. This mixture of primer and fragment is heated (pretend) to approximately 90 degrees Celsius. At this high temperature, the double stranded DNA fragment (two different colors of twelve-inch Funnoodles) dissociates into single strands (Take apart the different colors of Funnoodles)
 - 2) Annealing of Primers: Next, the solution is allowed to cool to approximately 55-60 degrees Celsius (pretend). As it cools, the single fragment strands of DNA (pink and orange twelve-inch Funnoodles) reassociate into double strands. However, because of the large number of primer (three-inch green and purple Funnoodles), each of the fragment DNA (twelve inch green and purple Funnoodles) will pair up with the complementary primer (one three-inch green primer pairs up with one twelve-inch purple DNA fragment and one three-inch purple primer pairs up with one twelve-inch green DNA fragment held together with toothpicks, remembering to observe one at 5' and of DNA and the other at the 3' end of DNA) leaving the rest of the fragment single stranded (approximately nine-inches).
 - 3) Primer Extension: Now a very heat-stable type of DNA polymerase, called Taq polymerase is added along with a supply of all four nucleotides, (nine-inch sections of green and purple Funnoodles). Using the primer (three-inch sections of the green and purple Funnoodles that are already attached to the twelve-inch opposite color of DNA fragment Funnoodles with toothpicks), the polymerase copies the rest of the fragment as if it were replicating DNA (attach the nine-inch green or purple that matches the three inch primer to the opposite color fragment of twelve-inch DNA fragment). You now have two copies of the DNA fragment. Repeat this procedure twice with all new copies of the DNA fragments. If one fragment of DNA reproduces in one cycle to form two copies of the DNA fragment, then two DNA fragments will reproduce in the next cycles to make four copies and four DNA fragments will reproduce to form eight copies of the DNA fragment. This can continue to occur for forty to sixty cycles without losing integrity, forming millions of copies of the original one fragment of DNA.

- You may allow your students to form their own PCR experiment at home using such things as plastic colored straws, colored Popsicle sticks and colored sponges.
- The other way to show how much bacteria and other pathogens are carried on the students' hands is to have four to five volunteer students to gently press the end of their fingers to the agar plate, wash their hands and repeat the procedure on a different plate of agar. Remember to label the plates with the name of the student and before or after washing their hands. This has to be incubated at a temperature of 20 to 38 degrees Celsius for approximately 24-48 hours to see the outcome of the procedure. You can incubate the plates at room temperature of approximately 22 degrees Celsius and get good results. Students are amazed at the difference between before and after washing their hands.

Methods of Evaluation:

- Each student will be evaluated as to class participation during the PCR procedure.
- Each student will be evaluated on the take home project on PCR. It will be graded according to correctness and turned in on time.

STUDENT LABORATORY GUIDE:

Bacteria Culture Lab

I. Define the Problem

II. Hypothesis

III. Equipment Needed for Lab:

- distilled water
- petri dishes with agar (2)
- sterile cotton swabs (4)
- incubator
- paper
- pencil
- colors
- over-the-counter cleaner
- paper towels
- bacteria from _____

IV. Procedure:

- Decide on your group members (list names)
- Decide on location with permission from teacher
- Decide on one over-the-counter cleaner
- Receive equipment
- Divide each petri dish into two halves, labeling each half I or II on the back of the agar side of the petri dish
- Students will go to location approved by teacher
- Carefully remove the sterile swab from packet, being careful not to touch anything or anyone
- Dip the cotton swab in the distilled water
- Rub the swab, on all sides, on the area chosen by the group
- Carefully open the petri dish
- Lightly rub the swab on the agar in the two halves, one half should go side-to-side and the other half should go up-and-down.
- Quickly replace the top on the petri dish
- Carefully replace the swab back in the packet and dispose in the proper garbage
- Take the petri dish containing the bacteria back to the classroom
- Label the petri dish with the area swabbed, the date and time

- Place the petri dish in the incubator set at proper temperature
- Return to approved location and clean the area with the chosen over-the-counter cleaner
- Repeat the swabbing technique on the clean area placing results in the second petri dish
- Take the petri dish containing the bacteria from the cleaned area back to the classroom
- Label the petri dish with the clean area swabbed, the date and time
- Begin writing laboratory report
- Wait approximately 24-48 hours before removing from incubator
- Take each petri dish out of the incubator
- Examine your cultures for growth
- Collect data: Was there growth on both dishes? How much growth? If there was growth, what does it look like? Color a picture of what growth was seen in the culture. Was there an odor?(do not put the dishes directly to your nose-you may inhale spores from the bacteria)
- Return the petri dishes back to the incubator upon completion of data
- Wait 24-48 hours before removing from incubator
- Examine you culture for growth
- Collect data
- Complete laboratory report

V. Data collected

VI. Conclusion

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