Finding a Bacterium's Family Tree

A lab experience focusing on growing, observing and classifying a bacteria sample

A bacterial based lab experience is one of the key components linking health issues with microbiology. This project is designed to reinforce prior knowledgeand combine biogenetics lab practices, microscopy, and computer based research into a valuable and rich learning experience. The goal of this project is to influence student perception of bacteria's place in the micro biome. When students begin to understand bacteria as members of a robust living, thriving community, they will be better equipped to understand the purposes underlying good health practices. The overshadowing objective of this lesson is to transform students' perception of bacterium from a "germ" into a living thriving organism.

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The last 25 years have witnessed an unprecedented development of molecular evolution, phylogenetic and population genetic methods. On one hand, the advent of PCR technologies has allowed for the generation and rapid accumulation of nucleotide sequence data from many organisms including several eukaryotic species, bacteria, viruses and eventually the full human genome. On the other hand, the increase in computational speed of computer clusters, as well as desktop and laptop computers, has allowed for the implementation of sophisticated algorithms that would not have been computationally feasible just two decades ago.

The discovery of fast-evolving viruses, such as HIV and HCV, poses special challenges to evolutionary theory. The understanding of both inter- and intra-host evolution of these viruses is crucial and has broad applications ranging from molecular epidemiology to drug resistance, pathogenesis and forensics. Molecular evolution of pathogenic viruses includes experimental work to isolate and sequence viral strains from different hosts or from the same host over time, DNA and RNA sequencing techniques, as well as the development and application of phylogenetic and population genetic methods to gain insights on the interplay between viral evolutionary patterns, origin and spread of epidemic outbreaks and pathogenesis.

More recently, our lab has also been investigating the molecular evolution and phylogeography of pathogenic bacteria such as MRSA and V. Cholera. Phylogenomics and phylogeography of bacteria is a new exciting field of research, based on the analysis of genome-wide SNPs, using state-of-the art phylogenetic methods and the Bayesian coalescent framework. Full genome bacterial sequences are obtained with the Illumina technology and analyzed with in-house pipelines implemented in the Galaxy software platform.

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AUTHOR'S NOTE

With the advent of high school level high stakes testing and End of Course exams, Biology curriculum has been somewhat adjusted to accommodate the success of the students within the context of test performance. Unfortunately, in the general Biology course setting this necessitates limited coverage of certain areas of study. The current mindset categorizes the need to know as the need to know for testing purposes. As a result of this trend of thinking some areas of Biology have been minimalized in the high school Biology classroom. Studies in the area of bacteria are easily passed over in exchange for more time in the more critical areas of the Biology needs of the moment.

Students who have the good fortune of being included in IB or AP or even Biology Honors courses are given a far better biogenetics experience than their middle tracked peers. They often are afforded the opportunity to participate in fundamental laboratory lessons that promote the skills necessary in culturing and processing bacteria cultures. Often the downside of their experiences is associated with the absence of expensive analytical lab equipment necessary for genetic sequencing and analysis. High schools in the proximity of research facilities are often afforded opportunities to take advantage of programs designed to contribute to educational endevours. In many cases the inaccessibility of equipment can be overcome by substituting virtual assets. A meaningful learning experience in the area of bacteriology can establish student attitudes about the role of bacteria as a living thing as it interacts with humans.

Regardless the course level, a meaningful biological study experience should include a bacteriological component because of its relevance to personal health issues. Information concerning bacteria can be meaningful in an environmental setting characterized by salmonella, meningitis, tuberculosis, MRSA and other bacteria related problems. It is important to see bacteria as a community of living entities that share our biology in an unavoidable fashion. Knowing more about bacterial characteristics, beneficial and detrimental, can serve to guide decisions that will impact the lives of our learners.

An effort to introduce students to the world of bacteriology should include several components designed to produce a successful learning experience. Students need to be equipped with a basic accademic knowledge about the physiology and function of bacteria. It is on this foundation of existing knowledge that new knowledge can be built. Because of the miniscule size of bacteria advanced microscopy skills need to be acquired. All meaningful science is connected to data collection and resulting research. Done in a meaningful way, high school students can come away from bacterial studies with a richer more meaningful understanding of bacteria and how they relate to the world that all living things exist in.

INTRODUCTION – WHAT STUDENTS NEED TO KNOW ABOUT BACTERIA

Prokaryotes are the most abundant organisms on earth. Just a handful of humus rich soil contains a larger number of prokaryotes than the number of humans that have ever lived. Bacteria, a prokaryote subjected to various environmental challenges, has responded resulting in great diversity.

The surface structure of bacteria plays an important role in determining the nature of its coexistence with eukaryotic organisms. The surface structure of bacteria consists of a cell wall similar to that found in eukaryotic plant cells. The basic material used in the bacterial cell wall is peptidoglycan. Peptidoglycan is composed of a sugar polymer cross-linked by short polypeptides.

Because of surface structure differences, bacteria can be classified into two general groups, gram-positive and gram-negative. Gram-positive bacteria have a simpler wall structure constructed of large amounts of peptidoglycan. Gram-positive bacteria can pose negative health effects on a human host (ex: staphylococcus) but generally gram-negative bacteria are thought to pose greater health risks. The gram-negative bacteria's wall are more complex and lipopolysaccharides (carbohydrates bonded to lipids). The lipid component of the gram-negative cell wall are toxic to the human hosts causing fever and shock. The gram-negative cell walls tend to protect the bacteria from the human body defenses and acts to resist antibiotic corruption. The gram-staining portion of this project helps to emphasize important differences in bacterial communities.

The motility factor in bacteria contributes to bacteria function. It is often generalized that bacteria move through a whipping process of a single flagella. It is interesting to note that though this may be the case in some examples some bacteria have multiple flagella, sometimes at both ends, sometimes all around the cell. Some species of bacteria are capable of achieving speeds of 190 miles per hour. Most importantly, bacterial movement is usually characterized by taxis, movement away from or toward stimulus.

Bacterial DNA is similar to eukaryotic DNA with a few exceptions. Whereas eukaryotic DNA is linear, bacterial DNA is made up of a circular chromosome. Bacterial DNA has fewer proteins and has no discrete membrane bound location. The area where the chromosome ring resides is called the nucleoid. Bacteria also have some much smaller DNA rings called plasmids. Plasmids usually contain only a few genes and carry out their replication process independent of the main bacterial DNA.

The bacterial ribosomes differ from that of eukaryotic cells in the way of protein and RNA make up. These differences offer an effective strategy for overcoming bacterial infestations. Because of the composition differences antibiotics can bind to bacterial ribosomes blocking protein synthesis while having no effect on the eukaryotic cells.

The nature of bacterial reproduction suggests factors influencing successful survivability and adaptation to the environment. One noteworthy factor is bacteria's ability to reproduce quickly in a favorable environment. Some strains of bacteria are capable of reproducing once every 20 minutes. In a perfect setting a single bacterium cell could become a colony outweighing the earth in just 2 days. The reason that this doesn't happen is because of limiting factors such as limits in nutrients, toxicity in metabolic waste, competition, and predication. A short reproduction cycle is accompanied with a fast rate of mutation. The success of bacteria as a species is connected to their ability to withstand harsh environmental conditions.

One of the survival strategies of bacteria is its ability to form an endospore structure. An endospore is a packaging unit that is used during a time of threatening environmental factors. The endospore packages DNA removing water content with the purpose of reanimating the DNA at a more favorable time. Research has shown that an endospore has the capacity of storing DNA for hundreds of years.

TIPS ABOUT THIS CURRICULUM

Lesson Plan Format: All lessons in this curriculum unit are formatted in the same manner. In each lesson you will find the following components:

KEY QUESTION(S): Identifies key questions the lesson will explore.
OVERALL TIME ESTIMATE: Indicates total amount of time needed for the lesson, including advanced preparation.
LEARNING STYLES: Visual, auditory, and/or kinesthetic.
VOCABULARY: Lists key vocabulary terms used and defined in the lesson. Also collected in master vocabulary list.
LESSON SUMMARY: Provides a 1-2 sentence summary of what the lesson will cover and how this content will be covered. Also collected in one list.
STUDENT LEARNING OBJECTIVES: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.
STANDARDS: Specific state benchmarks addressed in the lesson. Also collected in one list.
MATERIALS: Items needed to complete the lesson. Number required for different types of grouping formats (Per class, Per group of 3-4 students, Per pair, Per student) is also indicated.
BACKGROUND INFORMATION: Provides accurate, up-to-date information from reliable sources about the lesson topic.
ADVANCE PREPARATION: This section explains what needs to be done to get ready for the lesson.
PROCEDURE WITH TIME ESTIMATES: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.
ASSESSMENT SUGGESTIONS: Formative assessment suggestions have been given. Additionally, there is a brief summative assessment (pre/post-test) that can be given. Teachers should feel free to create additional formative and summative assessment pieces.
EXTENSIONS: (ACTIVITIES/LITERATURE) There are many activities and reading sources available to augment and enhance the curriculum. They have been included. If you find additional ones that should be added, please let us know.
RESOURCES/REFERENCES: This curriculum is based heavily on primary sources. As resources and references have been used in a lesson, their complete citation is included as well as a web link if available. All references and resources are also collected in one list.
STUDENT PAGES: Worksheets and handouts to be copied and distributed to the students.
TEACHER MASTERS: Versions of the student pages with answers or the activity materials for preparation.

Collaborative Learning: The lessons in this curriculum have been developed to include many collaborative learning opportunities. Rather than presenting information in lecture format and teacher driven, the activities involve the students in a more engaged manner. It can be difficult to communicate instructions, particularly for students who are visual learners. For these students, use of visual clues such as flowcharts and graphics can help them understand how they are to move to different groups.

Groups: Most of the lessons are carried out in groups. While it isn't necessary for students to remain in the same groups the entire unit, if they work well together, it may foster students to think deeper as they are comfortable with their teammates and willing to ask questions of each other.

Inquiry-based: The lessons in the curriculum invite students to be engaged and ask questions. They work through background information in a guided fashion, but are challenged to think beyond what they have read or done. The teacher serves as the facilitator in these activities, not the deliverer of information.

Technology: Lessons have been written to be mindful of varying availability of technology in schools and homes. Some of the lessons would be very well suited to online environments and if your students are able, you might wish to engage in some of the technology modifications.

Content: Often we teach in a manner that is very content heavy. This unit provides an opportunity to apply concepts learned and put them in the context of coexistence of species. The lessons aren't designed to turn students into microbiologists, but rather equip them with a working knowledge of bacteria and how they impact human life.

Implementation notes: This curriculum should be modified and adapted to suit the needs of the teacher and students. To help make implementation easier notes have been included in lessons as needed.

Extensions: For those teaching the AP Biology curriculum, bacterial transformation is a required laboratory. While bacterial transformation can be very useful in the research lab and certainly it helps students understand how bacteria gain new traits, it is also somewhat abstract from their daily lives. Other than the concept of antibiotic resistance (which is extremely important for students to understand), there isn't a great deal of application for them. Students can grow a bacterial colony, conduct a gram stain lab and use a supplied genome sequence to identify the bacterium and its related family.

Science Subject: Biology

Grade and ability level: 9-12 students in advanced biology

Science concepts: DNA, mutations, replication, transcription, translation, genetics, and cell structure

LESSON SUMMARIES

LESSON ONE: Starting a Bacteria Culture
This project will be introduced to the students as a lab based learning experience composed of four procedures. Student activities will begin with a pretest activity that will be used to guide instructions and activities. Students will then watch a short video clip about bacteria and disease (You tube - https://www.youtube.com/watch?v=KM1DcGOY-tA) The first procedure will be composed of a jigsaw activity focusing on prior knowledge in the area of prokaryotic cells. This will be followed by a short lab activity involving bacteria culturing.

LESSON TWO: Preparing the Colony for Observation and Making Observations
This lesson begins with a microscope lab activity involving students following the gram staining protocol. In addition to this students will be given a water sample and instructed to investigate and identify a visible single cell organism. Students will then observe a sample from their stained bacteria cultures. Pictures will be taken by students using their cellular devices (phones, Ipads, etc). This will be concluded by completion of an exit card requiring that they summarize their work with comparison/ contrast statements based on their observations.

LESSON THREE: Research Component
As a whole class activity students will watch a NCBI tutorial. You tube has a number of video clips. The teacher should investigate these and decide which would be most appropriate for their classes. Emphasis needs to be placed on access to related websites from NCBI, the BLASTING , and the resulting data and conclusion from BLASTING.
As a second part of lesson three, students will BLAST a supplied genetic code given the assumption that the code is relevant to their bacterial colonies (each lab group will have a unique code). This process will supply them with information that they will use to identify bacteria and related families.

LESSON FOUR: Reporting out
In lesson four students will be asked to create mini posters reflective of their work. It is anticipated that the posters will include vocabulary terms, illustrations of their microscope observations, and information relevant to the code that they have blasted.

LESSON FIVE: What We Have Learned-Final Assessment
This lesson will begin with test designed to reflect summative learning for this activity. Lesson five will conclude with the presentation of a video featuring top 10 diseases caused by bacteria (You Tube https://www.youtube.com/watch?v=t9zwcOCSJ2w). This project will conclude with exit card composed of 3 questions teacher designed reflecting the impact this project has had on student life habits.

LESSON SEQUENCING GUIDE

Since the classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. Below is a suggested pacing guide that can be used when planning to use this curriculum.

45 minute periods

	Day 1	Day 2	Day 3	Day 4	Day 5
Day 1	Lesson 1 Pretest	Lesson 2 Introduction of the activity &	Lesson 3 Bacteria (Agar plate) culturing	Lesson 4 Jigsaw activity	Lesson 5

Lesson1	(10 minutes)	video clip (10 minutes)	(15 minutes)	(25 minutes)	
Day 2 **Lesson2**	Microscopy lab (Gram staining, observation of ameba, observe stained bacteria (50 minutes)	Exit card 3 things I learned today (8 minutes)	Out of class assignment Vocabulary in graphic organizer format		
Day 3 **Lesson3**	NCBI tutorial (30 minutes)	Blasting code & analysis of BLAST (28 minutes)	As an out of class activity students will use NCBI to research info relative to bacteria BLAST		
Day 4 **Lesson4**	Each group will create mini poster (30 minutes)	Each group will report out (present mini posters to class) (28 minutes)	Out of class assignment Bacteria worksheet		
Day 5 **Lesson 5**	Post test (30 minutes)	Video presentation (10 minutes)	Exit cards (8 minutes)		

VOCABULARY

Endospores: A thick coated, resistant cell produced by some bacterial cells when they are exposed to harsh conditions.

Eukaryotic cell: A type of cell with a membrane enclosed nucleus and membrane enclosed organelles.

Flagellum: A long cellular appendage specialized for locomotion.

Gram stain negative: Describing a group of bacteria that have a cell wall that is structurally more complex and contains less peptidoglycan than Gram stain positive.

Gram stain positive: Describing a group of bacteria that have a cell wall that is structurally less complex and contains more peptidoglycan than Gram stain negative.

Nucleoid: A non-membrane bound region of a prokaryotic cell where the DNA is concentrated.

Peptidoglycan: A type of polymer in bacterial cell walls consisting of modified sugars cross linked short polypeptides.

Pili: In bacteria, a structure that links one cell to another at the start of a conjugation.

Plasmid: A small circular, double stranded DNA molecule that carries accessory genes separate of those of a bacterial chromosome.

Prokaryotic cells: a type of cell lacking a membrane enclosed nucleus and membrane enclosed Organelles (bacteria and archea).

Taxis: An oriented movement toward or away from a stimulus.

NEXT GENERATION SUNSHINE STATE STANDARDS – SCIENCE

Benchmark	Lesson						
	1	2	3	4	5		
SC.912.L.14.4: Compare and contrast structure and function of various types of microscopes.		X					
SC.912.L.14.6: Explain the significance of genetic factors, environmental factors, and pathogenic agents to health from the perspectives of both individual and public health.	X	X	X	X			
SC.912.L.16.9 Explain how and why the genetic code is universal and is common to almost all organisms.	X	X	X	X	X		
SC.912.L.16.10 Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.	X		X	X	X		
SC.912.L.18.1 Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules.	X				X		
SC.912.N.1.1 Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following: 1. pose questions about the natural world, 2. conduct systematic observations, 3. examine books and other sources of information to see what is already known, 4. review what is known in light of empirical evidence, 5. plan investigations, 6. use tools to gather, analyze, and interpret data, 7. pose answers, explanations, or descriptions of events, 8. generate explanations that explicate or describe natural phenomena (inferences), 9. use appropriate evidence and reasoning to justify these explanations to others, 10. communicate results of scientific investigations, and 11. evaluate the merits of the explanations produced by others.	X	X	X	X	X		
SC.912.N.1.2 Describe and explain what characterizes science and its methods.	X	X	X	X	X		
SC.912.N.1.3 Recognize that the strength or usefulness of a scientific claim is evaluated through scientific argumentation, which depends on critical and logical thinking, and the active consideration of alternative scientific explanations to explain the data presented.	X			X	X		
SC.912.N.1.4 Identify sources of information and assess their reliability according to the strict standards of scientific investigation.	X	X	X	X			
SC.912.N.1.6 Describe how scientific inferences are drawn from scientific observations and provide examples from the content being studied.	X			X	X		
SC.912.N.1.7 Recognize the role of creativity in constructing scientific questions, methods and explanations.	X	X		X			

SC.912.N.2.4 Explain that scientific knowledge is both durable and robust and open to change. Scientific knowledge can change because it is often examined and re-examined by new investigations and scientific argumentation. Because of these frequent examinations, scientific knowledge becomes stronger, leading to its durability.	**X**	**X**	**X**	**X**			
SC.912.N.2.5 Describe instances in which scientists' varied backgrounds, talents, interests, and goals influence the inferences and thus the explanations that they make about observations of natural phenomena and describe that competing interpretations (explanations) of scientists are a strength of science as they are a source of new, testable ideas that have the potential to add new evidence to support one or another of the explanations.	**X**	**X**	**X**				
SC.912.N.3.5 Describe the function of models in science, and identify the wide range of models used in science.	**X**			**X**	**X**		
SC.912.N.4.1 Explain how scientific knowledge and reasoning provide an empirically-based perspective to inform society's decision making.				**X**			
SC.912.N.4.2 Weigh the merits of alternative strategies for solving a specific societal problem by comparing a number of different costs and benefits, such as human, economic, and environmental.				**X**			

BACKGROUND INFORMATION

General background information is given here. Information is shared that can be used to help adjust the direction of activities to better meet the needs of specific groups of students.

Bacteria are single prokaryotic cells. As a prokaryotic cell bacteria has no well-defined nucleus or membrane bound organelles. The operations center of the bacteria containing genetic information resides in a single loop of DNA. Some bacteria also have smaller genetic rings called plasmids. Plasmids contain small amounts of genes that serve as a defense over other bacteria and antibiotics. Bacteria have a short life span that is characterized by eating and reproducing. In the presence of adequate food resources and a favorable environment, bacteria populations can expand dramatically. Bacterial response to preditation serves to fuel genetic mutations as an act of survival. Preditation can be from other bacteria, other life forms, chemical, or antibiotic. Bacteria's quick reproduction rate, as little as 20 minutes, precludes a much advanced rate of genetic change in contrast to other species.

Bacteria are classified into 5 groups according to their shapes: spherical (cocci), rod (bacilli), spiral (spirilla), comma (vibrios) or corkscrew (spirochaetes). They can exist as single cells, in pairs, chains or clusters.

Bacteria can be found in soil, rocks, the ocean, and even in the artic. Some bacteria live in or on plants and animals. It is estimated that there are 10 times as many bacterial cells as human cells on humans. In humans, a large portion of bacteria are found in the digestive system. In nature, bacteria break down dead biological matter resulting in a recycling of nutrients. Some bacteria are responsible for crop damage and food spoilage, but as a whole, relatively few bacteria are associated pathogenic activity.

Bacteria reproduce by binary fission. In the binary fission process a single celled bacterium divides into two identical daughter cells. This process produces two DNA molecules. Next the bacterium splits in half producing two bacterium cells. The two resulting bacterium are clones of the parent cell. Bacteria reproduction is promoted by adequate nutrients and favorable environmental factors.

Growing bacteria is an easy to achieve lab project. Bacteria are everywhere and grow rapidly and easily with the proper preparation. Typically, bacteria growing projects use petri dishes, agar, and either sterile swabs or inoculating needles. Agar is s gelatinous medium designed to provide nutrition and a comfortable growing environment for bacteria. Even though agar is the medium of choice, that is not to say that a similar substance could not be used. The goal is to produce a growth site that is sterile and not already inhabited by bacteria. Bacteria samples can be acquired by swabbing mouth, skin, soil, or household surfaces. Environmental bacteria are not typically harmful to healthy individuals however it is always a good practice to wear disposable gloves when working with any type of bacteria in the lab.

One of the keys to the successful growing of bacteria is proper preparation of the equipment and materials being used. The preparation process basically involves sterilizing all materials before beginning. This practice will help to eliminate corruption due to unwanted bacteria. One popular means of doing so is the water bath method. The water bath temperature should be from 170 to 190 degrees F. The agar should be allowed to cool to 120 F before proceeding to the next step.

After all materials are sterile and ready for use, pour agar into the petri dishes and allow to stand for one hour. When the agar has solidified it is time to apply the bacteria sample to this medium. The bacteria sample should be carefully applied to the agar in a zig zag fashion. For best culturing, samples should be zig zagged in one direction and then again at a 90 degree angle to the first application. This gives the new colony good access to the agar surface and a good opportunity to grow and thrive. Colonies started in this fashion should be ready for use having been allowed to grow overnight. An excellent illustrated instructional resource for growing bacteria can be found at http://www.hometrainingtools.com/a/bacteria-experiment-guide ,

As a general rule, bacteria is one of the smaller single cell organisms. Because of this the microscopes found in most high school labs are not capable of showing bacteria as more than a spot. Best case scenario, students would be able to see a moving spot that they had grown. There is no possibility to see organelles in their samples. There are numbers of

internet sites that provide pictures of bacteria. In order to enhance the lab experience students need to conduct some type of activity that emphasizes the scientific process. This can be achieved through Gram staining.

Generally speaking, bacteria can be catagorized into two groups : gram positive and gram negative. Most gram negative bacteria are though to be invasive and harmful while gram positive are less so. This is not altogether true. You can find beneficial and harmful in both groups. Gram staining is a simple staining process that allows classifying of the bacteria.

Gram staining involves four steps:

- Apply crystal violet stain to a smear of bacteria culture
- Add iodine (binds crystal violet and traps it in the cell)
- Wash with alcohol
- Counterstain witrh safranin

The gram stain website http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/Gram2.htm gives step by step details for this process.

Once the bacterial sample has been gram stained, the sample can be observed on a microscope and classified by color. After this process, students should be able to predict some of the characteristics of their colonies. Gram staining is going to allow students to predict the composition of the cell walls of the bacteria. This should also indicate the nature of the cultures they have grown.

LESSON ONE: STARTING A BACTERIA CULTURE

KEY QUESTION(S): What properties make bacteria a living species?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes (set up agar plates and related materials)
- Student Procedure: 45-60 minutes

LEARNING STYLES: Visual, auditory, and kinesthetic

VOCABULARY:

Endospores: A thick coated, resistant cell produced by some bacterial cells when they are exposed to harsh conditions.

Eukaryotic cell: A type of cell with a membrane enclosed nucleus and membrane enclosed organelles.

Flagellum: A long cellular appendage specialized for locomotion.

Nucleoid: A non-membrane bound region of a prokaryotic cell where the DNA is concentrated.

Peptidoglycan: A type of polymer in bacterial cell walls consisting of modified sugars cross linked short polypeptides.

Pili: In bacteria, a structure that links one cell to another at the start of a conjugation.

Plasmid: A small circular, double stranded DNA molecule that carries accessory genes separate of those of a bacterial chromosome.

Prokaryotic cells: a type of cell lacking a membrane enclosed nucleus and membrane enclosed Organelles (bacteria and archea).

Taxis: An oriented movement toward or away from a stimulus.

LESSON SUMMARY: This project is presented to the students as an activity to reinforce concepts that they have obtained in previous units. This activity will begin with a pretest designed to start the thinking process. Students will be shown a 2 minute video indicating a link between bacteria and illness. Next, students will engage in a jigsaw activity designed to help students to recall previous knowledge about eukaryotic cell structure and function. The instructor will demonstrate the simple technique of using an agar plate to establish a bacteria colony. The students will then duplicate the process. Plates will be set aside for lesson 2 activities.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Relate to bacteria as a prokaryotic cell.
2. Compare and contrast bacterial DNA from eukaryotic DNA.
3. Define bacteria's mode of movement.
4. Explain the composition of bacteria's cell wall.
5. Describe bacteria's survival response to harsh environmental pressures.
6. Suggest factors that promote robust evolution in bacteria.

STANDARDS:

SC.912.L.14.6
SC.912.L.16.7
SC.912.L.16.9
SC.912.L.16.10

MATERIALS:

- 1 copy of *Teacher Pages: Jigsaw – Bacteria structure and function*
- 1 copy of *Teacher Pages: Worksheet Master*
- Copies of *Student Bacteria Fact Sheet*
- Copies of *Student Worksheet: Starting a Bacteria Culture*
- Pretest
- Agar
- Agar plates
- Sterile swabs

BACKGROUND INFORMATION: Teachers are encouraged to be familiar with the Student Worksheet prior to the activity. This activity specifically focuses on the fundamentals of bacterial structure and function to help students understand what they are so they might participate in the activities of this project and better understand the protocols that will be used.

If for the sake of time the teacher decides to prepare the culture medium in advance, the time line and scenario will be as follows, however if the teacher chooses for the sterilization and agar preparation to be part of the lab experience this lesson will most likely take two days.

The suggested method of conducting the jigsaw activity can be as follows. Distribute the *Student Bacteria Fact Sheet* to student groups. Please note that the fact sheet is divided into sections. Assign each group a different section. After enough time for students to read their parts, have each group report out the information that they learned from their section.

ADVANCE PREPARATION:

1. Make copies of the pretest.
2. Make copies of the Student Worksheets and Fact Sheets.
3. Make necessary preparation for the video clip (https://www.youtube.com/watch?v=KM1DcGOY-tA).
4. If teacher chooses to prepare agar, plates and accessories in advance of the lab, this process may take up to one hour.
5. Layout lab stations with agar plates and necessary accessories.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (10 minutes)Administer pretest explaining it as a necessary part of the project.

2. (10 minutes) Introduce the project by revealing and describing the four parts of the activity: starting a bacterial culture, observing the culture using a microscope, learning how to use the NCBI website, and BLASTing the sequenced genome data. Introduce and discuss the essential question. Show short video clip relating bacteria with health issues https://www.youtube.com/watch?v=KM1DcGOY-tA.

3. (15 minutes) Have students assemble in groups of 4. Have groups move to lab stations. Demonstrate the procedure used in starting a bacteria culture using agar plates.

4. (25 minutes) Have students set aside the bacterial cultures. Distribute Student Fact Sheets in preparation for a jigsaw activity.

5. Outline the jigsaw activity for students.

6. Assign sections for each group.

7. Have each group report out a summary of what they have learned from their reading.

8. Depending on time, have students to do work sheets or let that be an out of class assignment.

ASSESSMENT SUGGESTIONS:

- Student worksheet can be checked for completion.

EXTENSIONS:
ACTIVITIES: A take home vocabulary assignment.

- Students will place vocabulary words for this section in a self-constructed graphic organizer. The idea is to promote an understanding of bacterial structure and function.

RESOURCES/REFERENCES:

- Campbell Biology, AP Edition, Ninth Edition, 2011
- Background information: http://www.microbiologyonline.org.uk
- Background information: http://www.medicalnewstoday.com
- Background information: http://www.hometrainingtools.com/a/bacteria-experiment-guide

Student Bacteria Fact Sheet

General Facts

Bacteria are single prokaryotic cells. As a prokaryotic cell bacteria has no well-defined nucleus or membrane bound organelles. The operations center of the bacteria containing genetic information resides in a single loop of DNA. Some bacteria also have smaller genetic rings called plasmids. Plasmids contain small amounts of genes that serve as a defense over other bacteria and antibiotics. Bacteria have a short life span that is characterized by eating and reproducing. In the presence of adequate food resources and a favorable environment, bacteria populations can expand dramatically. Bacterial response to preditation serves to fuel genetic mutations as an act of survival. Preditation can be from other bacteria, other life forms, chemical, or antibiotic. Bacteria's quick reproduction rate, as little as 20 minutes, precludes a much advanced rate of genetic change in contrast to other species.

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Growing Bacteria

Bacteria reproduce by binary fission. In the binary fission process a single celled bacterium divides into two identical daughter cells. This process produces two DNA molecules. Next the bacterium splits in half producing two bacterium cells. The two resulting bacterium are clones of the parent cell. Bacteria reproduction is promoted by adequate nutrients and favorable environmental factors.

Bacteria are everywhere and grow rapidly and easily. Bacteria are usually grown in petri dishes using agar as a nutrient solution. Agar is s gelatinous medium designed to provide nutrition and a comfortable growing environment for bacteria. Even though agar is the medium of choice, similar substances could be used. The goal is to produce a growth site that is sterile and not already inhabited by bacteria. Bacteria samples can be acquired by swabbing mouth, skin, soil, or household surfaces. Environmental bacteria are not typically harmful to healthy individuals however it is always a good practice to wear disposable gloves when working with any type of bacteria.

One of the keys to the successful growing of bacteria is proper preparation of the equipment and materials being used. The preparation process basically involves sterilizing all materials before beginning. This practice will help to eliminate corruption due to unwanted bacteria. After all materials are sterile and ready for use, pour agar into the petri dishes and allow to stand for one hour. When the agar has solidified it is time to apply the bacteria sample to this medium.

Gram Staining the Bacteria

As a general rule, bacteria is one of the smaller single cell organisms. Because of this the microscopes found in most high school labs are not capable of showing bacteria as more than a spot. Best case scenario, students would be able to see a moving spot that they had grown. There is no possibility to see organelles in their samples. In order to enhance the lab experience students need to conduct some type of activity that emphasizes the scientific process. This can be achieved through Gram staining.

Generally speaking, bacteria can be catagorized into two groups : gram positive and gram negative. Most gram negative bacteria are though to be invasive and harmful while gram positive are less so. This is not altogether true. You can find beneficial and harmful in both groups. Gram staining is a simple staining process that allows classifying of the bacteria.

Gram staining involves four steps:

- Apply crystal violet stain to a smear of bacteria culture
- Rinse with water (1 minute)
- Add iodine (binds crystal violet and traps it in the cell)
- Wash with alcohol (10 seconds)
- Counterstain witrh safranin
- Rinse with water (1 minute)

Once the bacterial sample has been gram stained, the sample can be observed on a microscope and classified by color. After this process, students should be able to predict some of the characteristics of their colonies. Gram staining is going to allow students to predict the composition of the cell walls of the bacteria. This should also indicate the nature of the cultures they have grown.

STUDENT WORKSHEET: LESSON 1

Name: _____ Date: _____

Home group members: _____

A. Guiding Questions

Directions: Use the Student Bacteria Fact Sheet to answer the questions below.

1. Discuss the general description of a bacterium's organelles.

2. What are plasmids and what do they do?

3. What is bacteria's survival response to antibiotics?

4. What are the necessary conditions for bacteria to grow and thrive?

5. Where are most bacteria located in humans?

6. What is one of the advantages of bacteria thriving in nature?

7. What is one negative affect of bacteria upon vegetables and crops?

8. Describe binary fission.

9. How do the offspring of bacteria compare to the parent cell?

10. Why is sterility of equipment so important in growing bacteria?

TEACHER WORKSHEET: LESSON 1

Name: _____ Date: _____

Home group members: _____

Directions: Use the Student Bacteria Fact Sheet to answer the questions below.

1. Discuss the general description of a bacterium's organelles.
   Not bound in membranes; less well organized than eukaryotic organelles

2. What are plasmids and what do they do?
   Small genetic rings containing a small number of genes focusing on combating attackers

3. What is bacteria's survival response to antibiotics?
   Mutation

4. What are the necessary conditions for bacteria to grow and thrive?
   Nutrients and favorable environmental conditions
5. Where are most bacteria located in humans?
   In the digestive system
6. What is one of the advantages of bacteria thriving in nature?
   They serve to breakdown dead materials and return them to the environment
7. What is one negative affect of bacteria upon vegetables and crops?
   Crop disease and spoilage

8. Describe binary fission.
   It is the method of bacterial reproduction. It involves a division process similar to mitosis
9. How do the offspring of bacteria compare to the parent cell?

   Clones

10. Why is sterility of equipment so important in growing bacteria?
    To prevent introduction of other (unwanted) bacteria

Bacteria Pretest Key

Short Answer

1. What process do bacteria use to reproduce?

   Binary fission

2. Contrast prokaryotic cells and eukaryotic cells.

   Eukaryotic cells highly organized/ prokaryotic cells have no membrane bound organelles

3. What are plasmids?

   Genetic rings containing a few genes

4. What causes bacteria to mutate

   Survival pressures

5. Are all bacteria harmful?

   No

6. What is the difference in the genetic material in a bacterium and its offspring?

   No difference (clones)

7. What useful function does bacteria have in nature?

   Break down dead debre

Bacteria Pretest

Short Answer

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3. What are plasmids?

4. What causes bacteria to mutate

5. Are all bacteria harmful?

6. What is the difference in the genetic material in a bacterium and its offspring?

7. What useful function does bacteria have in nature?

LESSON TWO: MICROSCOPY LAB

OVERALL TIME ESTIMATE:

- Advanced Preparation: 30 minutes
- Student Procedure: 30 minutes

LEARNING STYLES: Visual, auditory, and kinesthetic.

VOCABULARY:

Gram stain negative: Describing a group of bacteria that have a cell wall that is structurally more complex and contains less peptidoglycan than Gram stain positive.

Gram stain positive: Describing a group of bacteria that have a cell wall that is structurally less complex and contains more peptidoglycan than Gram stain negative.

LESSON SUMMARY: Working in groups, students will extract bacteria samples from the cultures and create smears. These samplings will be subjected to the gram staining process. When the stained bacterial samples have dried, students will observe samples on the microscope.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Follow a sequential lab protocol
2. Develop new microscopy skills
3. Understand the significance of the two catagories of bacteria

STANDARDS:

SC.912.L.14.4

SC.912.N.1.1

SC.912.N.1.2

SC.912.N.1.4

MATERIALS:

1. *Agar plate samples*
2. *Sample of pond water*
3. *Microscopes*
4. *Gram Stain Chemicals : crystal violet stain, iodine, alcohol, and safranin*

BACKGROUND INFORMATION: Background information needed for this assignment is at the beginning of the guide.. Teachers should review the information before the lesson. Gram stain technique can be found at http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/Gram2.htm .

The purpose of the students observing a single celled organism is that they will understand the scope between the organism and bacteria – one is very visible while bacteria is more difficult to be viewed.

ADVANCE PREPARATION:

1. Prepare student lab stations with microscopes.

2. Have the staining chemicals at hand.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (5 minutes) Review the essential question (What have we seen about bacteria that indicates that they are a living functioning community).
2. (5 minutes) Demonstrate making a bacterial smear and the staining process.
3. (20 minutes) Have students duplicate the smear/gram stain process assisting as needed.
4. (10 minutes) Using an eye dropper, have students place a drop of the pond water on a slide in search of a single cell organism. When they find one they will take a picture.
5. (10 minutes) Have students view stained bacteria under the microscope taking pictures of what they see.
6. (8 minutes) Exit card : 3 things that I learned today.

ASSESSMENT SUGGESTIONS:

- Student work can be assessed based on their successful completion of the lab.

EXTENSIONS:
ACTIVITIES:

- Have students write a 2 paragraph reflective paper highlighting their microscope lab activity.

RESOURCES/REFERENCES:

- Gram stain reference: http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/Gram2.htm .

LESSON THREE: NCBI TUTORIAL

ESSENTIAL QUESTION(S): How can a online site contribute to understanding bacteria?
OVERALL TIME ESTIMATE:

- (30 minutes) Tutorial
- (28 minutes) BLAST Activity

LEARNING STYLES: Visual and auditory

LESSON SUMMARY: Lesson 3 focuses on the computer's role in analyzing sequenced genome data. At this point a bacteria culture has been grown, gram stained, and observed. The next logical step is for students to submit genomic sequence data for analysis. We take note that actual genomic sequencing cannot occur in a high school setting the inhibitive cost of sequencing equipment. For the sake of this project each lab group will be given a different short sample sequence. The teacher may allow students to assume that these are actual sequences of their colonies.

In this lesson, students will become familiar with specific parts of the NCBI website. Their focus will be directed toward the BLAST section. Before begin to explore the NCBI site, they will be directed to a NCBI tutorial site. This site contains many tutorial videos. There are 4 specific videos that instruct individuals on the use of BLAST.

In the classroom this could actually be handled in several ways. The BLAST tutorials could be assigned as out of the classroom work and planning on doing the actual BLAST the next day. If it seems more appropriate, the tutorials could be done in class one day and the BLAST the next. In the case of higher performing students, tutorial and BLAST could be done the same day. Each teacher knows their class and what would be the best learning situation for them. Within the confines of the teacher's design, students will take their assigned sample code, input the code into BLAST and use the output along with related features to identify the bacteria and phylogenetic tree in which it belongs.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Understand to some degree the results of the genomic sequencing process.
2. Be familiar with the basic aspect of gene analysis
3. Be able to relate a given bacterial culture to genetically related bacteria

STANDARDS:
SC912.L.14.4
SC.912.L.14.6
SC.912.L.16.9

MATERIALS:

- Sample Sequence Codes for each lab group
- Student accessible computers with access to the web

BACKGROUND INFORMATION: If this lesson represents a teacher's initial experience with NCBI or Blast, it would be a wise decision to make a time investment in exploring the NCBI site and some of the tutorials. In order to be able to facilitate students in this activity a working knowledge of the process and software is important. The NCBI website seems complex when first seen, however, when used for the purposes of this project it will be found to be quite manageable. Note: The NCBI site will be an excellent resource to create sample code for the project.

ADVANCE PREPARATION AND IMPLEMENTATION NOTES:
Even though thus far student work on this project has been small group oriented, this lesson is best implemented using independent work. The instructor will need to decide how computer access will work best for their school setting. If

using a computer lab is an option, the lab should be reserved. If your school exercises a student based bring your own device policy, this could be done using student smart phones personal computer devices. If the only workable option was the use of teacher laptop and computer projector, that could be worked too. At any rate before this lesson is pursued it will be necessary to determine how the online curriculum will be accessed.

- Print out the sample genetic code.
- Be prepared to access the needed web sites:
- http://www.ncbi.nlm.nih.gov/ for the NCBI site
  http://www.ncbi.nlm.nih.gov/home/tutorials.shtml for the tutorial site

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. (30 minutes) Students will be given the address to the tutorial website where they will find the four BLAST tutorials and watch them while taking notes.

2. (15 minutes) Students will be given the sample genomic code and ask to go to the NCBI website and BLAST the code.

3. (15 minutes) Students will be asked to document the name of the bacteria and list any closely related specimen. Note: this can be used as an exit slip.

ASSESSMENT SUGGESTIONS:

- Participation grades for the exit slip and paper/poster presentation.

EXTENSION:
ACTIVITY:

- Students compose a reflective paper listing the results of lesson 3. This paper should include the name of the sample bacteria, name of related bacteria, and current scientific information concerning this strain of bacteria (can be found on the NCBI web site).

RESOURCES/REFERENCES:

- NCBI website: http://www.ncbi.nlm.nih.gov/
- Tutorial website: http://www.ncbi.nlm.nih.gov/home/tutorials.shtml

LESSON FOUR: MINI POSTER: FROM COLONY TO GENOMIC SEQUENCE

KEY QUESTION(S): How did our choice of bacteria for our cultures affect sequencing results?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 15 minutes
- Student Procedure: 58 minutes

LEARNING STYLES: Visual and kinesthetic.

LESSON SUMMARY: At this point in the project students have:

- Established a bacterial colony
- Gram stained a sample of the bacteria
- Observed the stained sample
- BLAST a sequence sample
- Completed a writing assignment concerning the identity of the bacteria, related bacteria, and written a short summary of findings concerning the bacteria

This lesson will involve students doing a mental reflection on what they have learned during this project and construct a mini poster illustrating what they have learned. Before the process begins the teacher will briefly review the higher points of the past activities. Students will be encouraged to use all handouts, vocabulary, and the internet if needed. The resulting poster should be composed of detailed illustrations. The finished product should serve as a representation of their learning experience.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Demonstrate their overall knowledge of bacteria through graphic representation
2. Show their understanding of bacteria as an individualized species responding to environmental pressures
3. Illustrate the results of researching professional articles concerning the specific BLASTed bacteria

STANDARDS:

SC.912.L.14.6
SC.912.L.16.7
SC.912.L.16.9
SC.912.L.16.10

MATERIALS:

Poster paper

BACKGROUND INFORMATION:

One of the important objectives of this project is to help students to visualize bacteria as a living species. As a living species, bacteria colonies are influenced similar to other species. The vitality of a bacterial colony is affected by the availability of nutrition, favorable environmental factors, and the presence of predators.

Through gram straining students are helped to understand that there are distinct differences in the wall structures of bacteria. The designation of gram negative and gram positive suggests the characteristics of the different strains. The expectation of the gram staining lab would be that all of the bacteria specimens would be the same (negative or positive). It is possible that because of inadvertent contamination there may be samples of both positive and negative).

The learning strategy behind the mini poster project involves students creating an illustrated artifact that they have learned. The mini poster provides students an opportunity to collaboratively share what they have academically acquired. A value added benefit is the possibility of additional learning from the peer presentations.

IMPLEMENTATION NOTE:
Students should be encouraged to utilize all of the materials that they have used during the previous parts of this project. It is noteworthy that they duplicate the visual components that they took pictures of during the microscope observations.

ADVANCE PREPARATION:

- Set up lab stations with poster paper and colored pencils

PROCEDURE:

- Have students work in their lab groups
- Have them structure their posters using vocabulary, students guides, and any other helpful resources.

ASSESSMENT SUGGESTIONS:

1. Rate poster creation and presentations.

LESSON FIVE: WRAPPING IT ALL UP

OVERALL TIME ESTIMATE:

- (30 min) Assessment
- (10 min) Video- bacteria and health
- (10 min) Summary of project

LEARNING STYLES: Visual

LESSON SUMMARY: At this point students should be prepared to demonstrate mastery through assessment. As a wrap up to this activity students will be shown a short video relating life style, hygiene, and disease.
At the conclusion of these activities the instructor will summarize what has been learned. This should include bacterial colonization, gram staining, microscopy, and BLASTing.

STUDENT LEARNING OBJECTIVES:

The student will be able to...

1. Demonstrate mastery of curriculum used during the project
2. Make connections between bacteria and disease

STANDARDS:

SC.912.L.14.6
SC.912.L.16.2
SC.912.L.16.3
SC.912.L.16.4
SC.912.L.16.5
SC.912.L.16.9
SC.912.L.18.1
SC.912.L.18.4
SC.912.L.18.11
SC.912.N.1.6
SC.912.N.3.5

PROCEDURE WITH TIME ESTIMATES:

1. (30 minutes) Students will take a test structured to demonstrate mastery of project learning
2. (10 minutes) Students will watch a video associating bacteria and disease
3. (10 minutes) Project summary

RESOURCES/REFERENCES:

- Bacteria/ disease video: https://www.youtube.com/watch?v=t9zwcOCSJ2w

Bacteria Test

Short Answer

1. What process do bacteria use to reproduce?
2. Contrast prokaryotic cells and eukaryotic cells.
3. What are plasmids?
4. What causes bacteria to mutate?
5. Are all bacteria harmful?
6. What is the difference in the genetic material in a bacterium and its offspring?
7. What useful function does bacteria have in nature?
8. How quickly can bacteria reproduce
9. What does Gram staining indicate?
10. Contrast gram negative bacteria from gram positive bacteria

Bacteria Test

Short Answer

1. What process do bacteria use to reproduce? *Binary fusion*
2. Contrast prokaryotic cells and eukaryotic cells. *Eukaryotic cells are well organized; prokaryotic organelles have no membranes*
3. What are plasmids? *Small circular genetic structures holding a few genes*
4. What causes bacteria to mutate? *Environmental and predatory pressures*
5. Are all bacteria harmful? *No*
6. What is the difference in the genetic material in a bacterium and its offspring?*No difference*
7. What useful function does bacteria have in nature? *Breaking down biological debree*
8. How quickly can bacteria reproduce? *20 minutes*
9. What does Gram staining indicate? *The nature of bacteria's cell wall*
10. Contrast gram negative bacteria from gram positive bacteria *Gram negative cell walls are structured differently*