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CourseSource

Lesson

From Dirt to *Streptomyces* DNA

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Abstract

The purpose of this semester-long Lesson is to give students an authentic, course-based undergraduate research experience during which they learn basic and advanced microbiological and molecular biology techniques. This project begins with the isolation of a suspected *Streptomyces* bacterium from a soil sample and concludes with its identification. Students collect data, regarding colony and cell morphology, biochemical characteristics, the production of secondary metabolites, and employs the PCR using custom-designed primers to the *Streptomyces* 16s rRNA gene. The project culminates with the identification of their soil isolate using the National Center for Biotechnology Information (NCBI) web site to perform nucleotide blasts. The blastn program provides the final piece of evidence used to confirm, or not, the identification of their isolate as a *Streptomyces* from 16s rRNA gene sequence data, hence the title "From Dirt to *Streptomyces* DNA. In addition, the Lesson focuses on the *Streptomyces* bacteria to address several ASM aligned goals and objectives. These include prokaryotic growth phases and ways in which interactions of microorganisms among themselves and with their environment is determined by their metabolic abilities. In addition, this Lesson illustrates how microbial metabolism is important to a relevant societal issue, the need for new antibiotic discovery particularly given the rise of antibiotic resistance strains of clinically relevant bacteria. It also illustrates the microbial diversity of soil and the developmental/physiological strategies employed in such a competitive environment. This Lesson hopes to impart both the thrill and challenges associated with scientific discovery.

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Supporting Materials: Supporting Files S1. Dirt to DNA-Handout Introduction to the Actinobacteria (LAB 1); S2. Dirt to DNA-Handout Serial dilution and spread plate technique (LAB 2); S3. Dirt to DNA-Handout Count serial dilution plates and subculture to ISP medium 2 (LAB 3); S4. Dirt to DNA-Handout slide culture technique (LAB 5); S5. Dirt to DNA-Handout Check pure culture isolates for antibiotic action (LAB 6); S6. Dirt to DNA-Handout Grow suspected Streptomyces isolates in broth culture (LAB 7); S7. Dirt to DNA-Handout DNA extraction from broth culture of Streptomyces isolate (LAB 8); S8. Dirt to DNA-Handout Agarose gel electrophoresis of DNA extraction and UV scan (LAB 9); S9. Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10); S10. Dirt to DNA-Handout PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11); S11. Dirt to DNA-Handout *NCBI Nucleotide Blast (blastn) (LAB 12); S12. Dirt to DNA-Handout Laboratory Report Outline (LAB 13); S13. Dirt to DNA-Bacterial cultures, Media, Solution recipes, and Equipment; S14. Dirt to DNA-DNA extraction protocols instructor copy; and S15. Dirt to DNA-Student Supporting Files LAB 1-12.

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Learning Goals

- How does the survival and growth of any microorganism in a given environment depend on its metabolic characteristics? ASM Aligned Learning Goal - Metabolism (LAB 2).
- How are the interactions of microorganisms among themselves and with their environment determined by their metabolic abilities? ASM Aligned Learning Goal - Metabolism (LAB 2).
- Students will understand the unique metabolic capabilities *Streptomyces* use to support their growth and survival. Lesson specific goal (LAB 2).
- Students will understand and employ the basic principles of aseptic technique. Lesson specific goal (LAB 2 – LAB 7).
- How can humans utilize and harness microbes and their products? ASM Aligned Learning Goal - Impact of microorganisms (LAB 6).
- How do microorganisms interact with their environment and modify each other? ASM Aligned Learning Goal - Systems (LAB 6).
- Students will understand the significant role *Streptomyces* play in the production of antibiotics and our use of these secondary metabolites. Lesson Specific Goal (LAB 6).
- Students will understand the value of antibiotic production for the *Streptomyces* bacteria in a highly competitive soil environment. Lesson Specific Goal (LAB 6).

Learning Objectives

Students will:

- Master the technique of bacterial isolation in pure culture from an environmental source, in this case a soil sample. This isolation relies upon the basic microbiological techniques of serial dilution, spread plate, and the use of selective bacteriological media. Lesson Specific Objective (LAB 2 and LAB 3).
- Define the four phases of prokaryotic growth and describe what cells are doing during each phase. ASM Aligned Learning Objective - Metabolism (LAB 5).
- Give an example of and explain how microbial metabolism is important to a relevant societal issue (e.g., health and disease, bioremediation, agriculture, etc.). ASM Aligned Learning Objective - Metabolism (LAB 6).
- Be able to employ the agar plug method to demonstrate antibiotic production in pure cultures of *Streptomyces*. Lesson Specific Objective (LAB 6).
- Be able to employ and understand the need for two physical DNA extraction methods (the freeze/thaw cycles combined with grinding of the cells) and chemical extraction methods (lysozyme treatment of the cells) when using Gram + cells. Lesson Specific Objective (LAB 8).
- Understand both the principles of, and be able to interpret, an agarose gel electrophoresis of their DNA sample. Lesson Specific Objective (LAB 9).

Learning Objectives continued

Students will:

- Be able to explain the basic principles of the polymerase chain reaction and identify the individual responsible for its development. Lesson Specific Objective (LAB 10).
- Explain the function of each reagent in the PCR Master Mix. Lesson Specific Objective (LAB 10).
- Demonstrate competence in using the National Center for Biotechnology Information blastn program. Lesson Specific Objective (LAB 12).

INTRODUCTION

I developed this series of laboratory investigations because of my interest in soil bacteria (specifically the *Streptomyces*) and a desire to expose students to a research experience as part of their formal course work. Many students complete their undergraduate science education without an authentic research experience, as some course-based laboratory work is formulaic with predetermined outcomes. As a result, students describe the laboratory exercise as either it "worked" or it "didn't work" (1, 2). This view of laboratory work misses the point of hypothesis-driven scientific investigation. That is, an open-ended evidence-based investigation in which data is gathered and conclusions drawn from the data. The literature supports both the immediate and long-term value of course-based undergraduate research, including increased persistence in science (3), and "a positive impact on the development of students' conceptions and practice of scientific thinking" (4). Such course-based undergraduate research experiences, or CUREs, have five defining characteristics: 1) There is an element of discovery, so that students are working with novel data. 2) Iteration is built into the lab. 3) Students engage in a high level of collaboration. 4) Students learn scientific practices. 5) The topic is broadly relevant so that it could potentially be publishable and/or of interest to a group outside the class (5). One of the major goals of these CUREs is that they reflect a real research experience in order to give students a more accurate conception of how scientific research is done (5, 4). The Lesson described here, exposes students to several of these characteristics, while employing the tools of classical microbiology and modern molecular biology to investigate the characteristics and identity of their soil isolate. The Lesson begins with the working hypothesis that given the conditions of the initial isolation (using selective culture media) the student's soil isolate is a *Streptomyces* bacterium. The succeeding 12 weeks engage students in an in-depth study of their soil isolate reflecting several of the five characteristics listed above. In summary, the intent of the Lesson is two pronged. The Lesson engages students in an evidence-based research project focused on the *Streptomyces* and addresses several ASM aligned goals and objectives described below.

This Lesson requires a considerable investment of time and energy as students master basic and advanced microbiological techniques, from pure culture and serial dilution to DNA extraction, polymerase chain reaction (using custom primers to the *Streptomyces* 16s rRNA gene), followed by the use of bioinformatic-databases at the National Center for Biotechnology Information (NCBI). The PCR laboratory followed by the blastn at the NCBI web site, is the final piece of evidence to either confirm, or not, an initial identification of their isolate as a *Streptomyces*. Although the semester long series of laboratory exercises culminates with each student identifying their soil isolate as a *Streptomyces*, or not, the emphasis at each step in the process is the implementation of evidence-based decision-making. Each laboratory exercise requires the student to examine their results (their evidence) and then proceed to the next set of exercises. Each week students generate additional evidence as they move towards a final decision regarding the identification of their isolate. This investigation therefore mirrors the process of scientific investigation and illustrates the use of research as curriculum.

In addition to the two features of the laboratory described above, the structure of the laboratory setting promotes both learning and collaboration as students work in groups of 2-4 individuals for part of their investigation. The composition of student groups and grading of individual students is described below in the Assessment section. Eddy and Hogan (6) describe the elements of a course having moderate structure as including: 1. Graded preparatory assignments, 2. Student in-class engagement, and 3. Graded review assignments. They go on to state that, "The increased-structure format has students work in small groups, which may help students develop a more collaborative sense of the classroom. Collaborative learning in college has been shown to increase a sense of social support in the classroom as well as the sense that students like each other (6, 7). The goal of a collaborative classroom is further reinforced, as some students have already had research experience outside of class, as an independent research project. These students can therefore assist the learning of other members in their group. This Lesson employs the moderate structure characteristics as described above and as such enhances learning and inclusiveness.

Students begin this lesson by collecting soil samples from an environmental source of their choice. I tell them to choose a soil sample that has an earthy odor. This increases the chance of isolating a *Streptomyces*, as this genus of bacteria produces a volatile organic compound called geosmin (8, 9), responsible for the characteristic "earthy" odor of soil. Because this Lesson relies on isolates from the environment, and the *Streptomyces* are so diverse, outcomes will vary, and students may isolate an undescribed *Streptomyces* species.

The Lesson also provides an opportunity for the instructor to discuss the difference between primary and secondary metabolites, the role of natural selection on antibiotic production, and the origin of antibiotic resistance genes. This is particularly important given the rise of antibiotic resistant strains of clinically relevant bacteria. These include vancomycin resistant enterococci (https://www.cdc.gov › hai › organisms › vre › vr, accessed June 2019, Fan et al. (10), and carbapenem resistant Enterobacteriaceae (11).

The ASM-Aligned goals and objectives are described below and frame a pathway for the Lesson. Lesson-specific goals and objectives are described in the Lesson and as part of the supporting files and therefore are not discussed below. In summary, this set of laboratory exercises hopes to impart the excitement of scientific discovery coupled with the use of evidence-based decision making at each critical juncture in the Lesson.

Learning Goals

The numbering below is not always sequential; it refers only to the ASM aligned goals listed above. Lesson specific goals (3, 4, 7, and 8) are not discussed here.

1. How does the survival and growth of any microorganism in a given environment depend on its metabolic characteristics? ASM Aligned Learning Goal - Metabolism #1 (LAB 2).

The *Streptomyces* are soil-dwelling bacteria adapted to a highly diverse and competitive environment. Two metabolic capabilities enhance their survival. These bacteria produce antibiotics (as secondary metabolites) to interfere with the growth of other soil-dwelling bacteria. They also synthesize and secrete the enzyme chitinase. This enzyme degrades the cell wall of soil fungi, therefore reducing the competitive load of fungi, and releasing nutrients from the dead fungal cells. The presence of this enzyme is unusual in bacteria and renders the *Streptomyces* particularly well suited for the soil environment (12, 13).

2. How are the interactions of microorganisms among themselves and with their environment determined by their metabolic abilities? ASM Aligned Learning Goal #2 - Metabolism (LAB 2).

Streptomyces inhabit an environment rich in other microorganisms. Estimates of the density of bacterial species in soil are between 10^8 and 10^9 cells per gram and within 10-12 micrometers of each other (14). This density and proximity requires adaptations for survival. The *Streptomyces* have evolved several metabolic capabilities to enhance their survival, among them the production of antibiotics coupled with antibiotic resistance factors and chitinase production. This allows them to better compete for space and nutrients against other bacteria and mold.

The unique metabolic capacity of the *Streptomyces* to degrade chitin - they are one of the few bacteria capable of digesting chitin - is demonstrated by growth on chitin agar (12). This culture method uses chitin as the sole source of carbon, illustrating the selective nature of a growth medium containing a single carbon source while greatly enriching the isolation of *Streptomyces* directly from a soil sample. It also points out another metabolic strategy for surviving in the soil. *Streptomyces* take advantage of the abundance of chitin, particularly in the soil-dwelling insects and mold hyphae (8, 15) and, as a result, effectively compete with mold and other soil-dwelling bacteria for nutrients and space.

5. How can humans utilize and harness microbes and their products? ASM Aligned Learning Goal #5 - Impact of microorganisms (LAB 6).

Soiling dwelling *Streptomyces* are the primary producers of antibiotics. The vast majority of antibiotic discovery occurred between 1940 and 2000 with a peak during the 1960s (8). Of these, most were the products of *Streptomyces* metabolism. This Lesson illustrates both the promise and difficulty of isolating antibiotic-producing strains of *Streptomyces*.

6. How do microorganisms interact with their environment and modify each other? Systems - ASM Aligned Learning Goal #6 (LAB 6).

This Lesson can also demonstrate the production of antibiotics in both constitutive and inducible *Streptomyces* isolates. Not all *Streptomyces* isolates produce antibiotics when grown in pure culture. However, when grown in a competitive (stress-inducing) environment (growth of two isolates in close physical proximity to each other in culture) a conversion can occur. Some antibiotic non-producing isolates will produce an antibiotic in response to other organisms.

Learning Objectives

The numbering below is not always sequential as it refers only to the ASM aligned objectives listed above. Lesson specific objectives (1, 4-9) are not discussed here).

2. Name the four phases of prokaryotic growth and describe what the cells are doing during each phase. ASM Aligned Learning Objective #2 - Metabolism (LAB 5).

Streptomyces spp. provide an excellent tool to observe prokaryotic growth phases in an unconventional manner as these cells go through observable morphological (both cellular and colonial), and metabolic phases of growth. This morphological development is unusual in bacteria. Additionally, metabolic phases of growth are linked to the morphological growth phase. The initiation of *Streptomyces* cell growth, because of spore germination, may not be observable for up to 48 hours, a time that constitutes the lag phase. Initiation of the log phase of growth is apparent with the production of substrate vegetative hyphae, observable in cut sections of the agar or in a slide culture of the organism. As nutrient depletion occurs either in vitro or in the highly competitive microenvironment of the soil, *Streptomyces* enter what has been described as a second log phase of growth made visible by the formation of aerial hyphae and spore formation (16). These morphological changes, associated with growth phase, are observable with a dissecting and compound microscope (see Figures 1A, 1B, 6A, 6B, and 6C). The production of aerial hyphae, septation of the hyphae, and spore formation heralds the onset of secondary metabolite production, specifically antibiotics. After secondary metabolite production, *Streptomyces* enter the "stationary" growth phase. During this phase, spores remain viable for a considerable period, as long as 6-12 months.

3. Give an example of and explain how microbial metabolism is important to a relevant societal issue (e.g., health and disease, bioremediation, agriculture, etc.). ASM Aligned Learning Objective #3 - Metabolism (LAB 6).

Multi-drug antibiotic resistance is an alarming occurrence in many clinically relevant bacteria. The need for new antibiotics is at a critical juncture, as there are now extensively drug resistant *Mycobacterium tuberculosis* and carbapenem resistant Enterobacteriaceae (doi:10.1080/21505594.2016.1185577). I find it useful, when discussing this objective, to begin with a brief history of antibiotic discovery. This puts the subject into a historical context and demonstrates to students the dynamic nature of scientific discovery.

Antibiotic production was first observed by Alexander Fleming in 1928 but not used therapeutically until the 1940's during World War II and was effective primarily against pyogenic Gram-positive bacteria. Streptomycin was discovered in 1943

by Selman Waksman (17) and used as an effective cure for tuberculosis at that time. Emphasize to your students, that *Streptomyces* produce antibiotics as secondary metabolites during their growth, and that the variety of antibiotics produced by the *Streptomyces* is unequalled by other bacteria which has been put to great therapeutic effect since their discovery (8, 17).

Also emphasize that antibiotic production and resistance are phenomena that we view as recent, but in fact are ancient (18). *Streptomyces* evolved antibiotic production as a mechanism for survival in the competitive soil environment. These same antibiotic-producing cells also produce the resistance factors as a mechanism to avoid suicide (19). We are now faced with the alarming prospect of widespread antibiotic resistance. This resistance is due to the undisciplined use of antibiotics coupled with horizontal gene transfer of antibiotic resistance genes to pathogenic bacteria (20).

The initial isolation of *Streptomyces* from soil demonstrates the difficulty to isolate antibiotic-producing strains of bacteria and further highlights ASM aligned learning objective #3. My microbiology students, (both during the formal laboratory work and as an independent research project), isolated 14 *Streptomyces* of which two were constitutive antibiotic producers, two were inducible for antibiotic production, and the remaining ten did not demonstrate any antibiotic production. Having students isolate *Streptomyces* bacteria illustrates to them the difficulties inherent just in the isolation of a pure culture let alone the isolation of antibiotic producing cells. It also demonstrates the difficulty to isolate *Streptomyces* that produce antibiotics active against Gram-negative bacteria (due in part, but not exclusively, to the presence of their outer membrane); all of the student isolates, both constitutive and inducible, exhibited action against the Gram-positive bacterium Staphylococcus aureus. The entire exercise makes apparent the need for the discovery/production of new antibiotics as a way to address the profound global problem of single and multi-drug antibiotic resistance.

The Lesson described here, on the isolation of soil *Streptomyces*, contributes significantly to the already published undergraduate laboratory experiences on *Streptomyces*. This semester-long Lesson emphasizes both the microbiology and molecular biology of the organism. Its focus is not the isolation of an antibiotic producer (as is the case with all other published lessons) but a thorough examination of the cultural, morphological, metabolic, and molecular characteristics of the *Streptomyces*, and as such sets it apart from the other exercises. Students examine their isolate's morphology (both colonial and cellular) physiology (enzyme activity – chitinase activity - and secondary metabolite production), and its molecular/genomic characteristics (sequencing of its 16s rRNA gene by performing PCR) making use of the blastn program at the NCBI web site. In addition, the Lesson provides a detailed reference list on the *Streptomyces* for both the instructor and students. At the conclusion of the Lesson, students hand in a comprehensive laboratory report as a final assessment for the work, requiring the keeping of a detailed, weekly laboratory notebook. This illustrates for them the importance of careful record keeping as an essential feature of scientific investigation, an element not apparent in the other lessons described below.

There are several published lessons, outside of CourseSource, that are similar, in some respects, to the Lesson presented here. None, however, compare in scope, emphasis, and time commitment to this Lesson. The first lesson (Pinney and Kalil, Isolation of Antibiotic-Producing Actinomycetes from Soil) appeared in The American Biology Teacher in 1975 (21). This lesson is 2-4 weeks in duration and emphasizes only the isolation of an antibiotic producer. It employs basic microbiology techniques (aseptic technique and serial dilution for example) and only superficially discusses the unusual colonial, cellular and metabolic characteristics of the *Streptomyces*. It lists 3 references and as a result provides very little background on the *Streptomyces*. The second lesson appears in Benson's Microbiological Applications (22), Exercise 46 - Isolation of an Antibiotic Producer: The *Streptomyces*. This laboratory exercise, although longer (four weeks) and more detailed, also employs only basic microbiological techniques and has as its emphasis the isolation of an antibiotic producer from a soil sample. Unfortunately, there is no reference list to facilitate further investigation of the unique characteristics of the *Streptomyces*. As written, the Benson laboratory exercise is useful but of limited scope. A third lesson appears online in LibreTexts (23) and is also only a discussion of how to isolate an antibiotic producer from the soil. It describes basic microbiology techniques used during a 3-week investigation. Its description of the *Streptomyces* morphology and metabolism is short, and the exercise does not include any list of references. As such, it adds very little to the above referenced exercises. A fourth laboratory exercise appears online at the University of Wyoming virtual edge web page. This is a YouTube video, narrated by students, demonstrating serial dilution and plating of a soil sample. It describes the isolation of mold and *Streptomyces* from a soil sample. The web page shows images of mold on one set of plates and *Streptomyces* on another. The entire YouTube video and web page lack depth and are of very limited value. The fifth lesson (24) appeared in the November/December 2019 issue of The American Biology Teacher. This is a six-week study that emphasizes the isolation of an antibiotic producing strain of *Streptomyces* from a soil sample and employs basic microbiological techniques. It is the only exercise of those listed above (other than the laboratory exercise in the Benson Laboratory Manual) that provides a useful student-centered experience. The Lapaz lesson does provide a reference list. Although short, it includes published work by Chater and Hopwood, both excellent and necessary references for an understanding of the *Streptomyces*. The Lapaz paper is different from the Lesson presented here in its examination of antifungal metabolites of *Streptomyces* in addition to antibacterial. It also examines the metabolic capacity of the isolates to digest lipids, starch, and cellulose. This is an interesting part of the study; however, it is surprising that the authors did not look for the presence of the chitinase enzyme, a distinguishing feature of the *Streptomyces* and one that can be used to explain their adaptation to the soil and facilitate their isolation. In addition, it departs significantly from this Lesson by not addressing any molecular biology/genetics of the *Streptomyces*.

Finally, a M.Pharm thesis (25) by Rahman, published in 2008, although not meant as a laboratory exercise for undergraduate microbiology students, is a good reference for this Lesson. The Rahman study employs basic microbiological techniques used in the isolation of yet undescribed *Streptomyces* species from the soil, followed by testing of isolates for biologically active

compounds. Rahman's study employs selective media containing starch and casein as the sole source of carbon. Rahman reports that this method enriches for fungi and *Streptomyces*. It is surprising that none of the above-described laboratory exercises, including the Rahman thesis, use chitin agar as a selective medium for the enrichment of *Streptomyces* (as this Lesson does) although this method was first described by Hsu (12) in 1975. The Rahman dissertation also describes the identification of his isolates using the tools of molecular biology and finally sequencing 16s rRNA genes (using the NCBI blastn program) to conclusively identify his isolates. In some respects, the Lesson described here mirrors the format of the Rahman dissertation.

Intended Audience

The intended audience for this lesson is upper division (junior/senior level) biology and biochemistry majors taking a one semester 300 level course in microbiology. Students who take this course have often been involved in independent undergraduate research. As a result, they have had, in some cases, significant exposure and work with molecular biology techniques, such as SDS PAGE, DNA extraction, PCR, the Nanodrop spectrophotometer, and the use of agarose gels containing ethidium bromide. Other students have not had as much laboratory research experience and can benefit from group work with the more experienced students.

Required Learning Time

This is a semester-long series of laboratory investigations. Some of the 12-week laboratory sessions will take the entire three-hour laboratory period. In one or two cases, an additional hour of laboratory work will be necessary. Other lessons require 30-90 minutes of the laboratory session.

Prerequisite Student Knowledge

This is a junior and senior level microbiology course for biology majors. As such, students have had 4-6 semesters of both introductory and upper division biology and chemistry courses with laboratory. Therefore, students have background knowledge in cell structure and function, genetics, and general and organic chemistry. Students also possess the basic laboratory skills for record keeping and laboratory report writing. They also have an introductory knowledge of microscopy, pipetting, and electrophoresis. Nonetheless, each laboratory session is preceded with introductory comments and demonstrations that provide students with the necessary background to complete each week's work.

Prerequisite Teacher Knowledge

College or university instructors of microbiology have the background to teach this lesson. A basic understanding of *Streptomyces* structure and function is necessary (at the colonial, cell, and molecular level) and can be accessed from the reference list. Safe procedures in the microbiology laboratory are essential. Therefore, instructors should be familiar with and follow the ASM guidelines for handling BSL - 2 microorganisms. This lesson requires the instructor to guide students through basic microbiological laboratory techniques, such as aseptic technique, pure culture, and serial dilution. The teacher must also have knowledge in bacterial cell and molecular structure and bacterial growth patterns at both the colonial and cellular level. In addition, it requires teacher knowledge of molecular biology techniques in DNA extraction, agarose gel electrophoresis, the polymerase chain reaction, and use of nucleotide sequence databases at the National Center for Biotechnology Information. Detailed instructions for all techniques are in the supporting files.

SCIENTIFIC TEACHING THEMES

Active Learning

Active learning not only enhances student learning, but it can also contribute to an inclusive learning environment (26, 27, 28, and 6). In this Lesson, an active learning environment is fostered by several activities that focus on increasing course structure as a tool to promote active learning (6). These include: 1. Graded preparatory assignments, 2. Formulation, by students, of testable hypotheses with in class presentations, 3. Graded practice problems with in class presentation, 4. Small group work in which students think, pair, and share, 5. Independent data analysis followed by evidence-based-decision-making and, 6. Peer instruction.

Prior to several of the laboratory exercises (LAB # 2, 3, 6, and 8), students are asked (prompted) to read a research paper, refer to their text, class notes, or a supporting file and use the information in their reading to formulate (on their own) a short-written hypothesis or an explanation relevant to work in the next laboratory period. This provides additional structure to the laboratory by giving individual students time to think about the subject matter outside of class and then, either hand in their written work or upon arriving in class, share their thoughts with group members and the class (28). Specifically, in several of the lab sessions, student groups examine the results of their previous week's work and see whether the results support their hypothesis. After a few minutes of small group discussion (students are assigned by the instructor to a small group of 2-4 students) students in each group present their ideas to the rest of the class. Student presenters can take turns during the presentation or rotate during the semester as working groups are set for the semester. Either of these presentation methods allows evaluation of each student by the instructor.

In addition to formulating testable hypotheses and in class presentations related to the laboratory work, students are also given homework assignments related to the next week's laboratory. Homework assignments also increase course structure, promoting active learning (26, 6). Specifically, students are asked to come to class with serial dilution calculations relating to that day's work (LAB 3). Based on their own calculations and outside reading students are asked to predict which serial dilution of their soil sample will yield countable plates and the type of microbial growth that will occur on the different media. Their predictions are then compared against the actual plate count and type of growth. This work can also be presented orally in class (time permitting) or handed in if desired as one of several formative assessment assignments.

In addition to measuring microbial numbers and type in their soil sample, the students' main goal is to isolate and characterize a *Streptomyces* in pure culture. Examination of their plates, individually and with their group members, promotes evidence-based decision making. The group needs to arrive at a consensus, based on observable colonial features of each other's bacterial isolate. Students then decide collectively, which isolate (isolates) most closely resemble a *Streptomyces*. At this point, I ask the students to call me over to examine their isolates and help in their decision regarding which colony is

most likely a *Streptomyces*. In addition, students may decide to share their isolate with one of their classmates if they have been more successful in isolating a suspected *Streptomyces*. This also facilitates a sense of community and shared goal.

Finally, peer instruction is also an element of active learning by adding structure to the laboratory setting (28). The small group work that students do throughout the semester allows them to assist each other during their laboratory work. This actively engages all students in the project and increases their "stake" in the work. Peer instruction also promotes an inclusive environment as each student brings different skills to the group and therefore adds value to each person's contribution.

Assessment

This Lesson makes use of both formative and summative assessment tools to evaluate student learning. Within these two categories this Lesson employs, assessment of individual student performance, homework assignments, in-class oral presentation, multiple choice questions on the final exam, and a comprehensive laboratory report at the end of the project. At the beginning of the semester, I assign students to working groups based on my knowledge about them, their engagement with the major and previous research experience. As microbiology is a junior/senior level biology major course I may have known these students for 2-3 years. This allows me to form groups in which each member brings different strengths. I do not allow students to self-select their group, as this often results in groups that lack diversity. To ensure that all students participate in the group work, and can be individually assessed, (during the in-class presentations) each student in the group is required to present part of the group's work. Students are also told that each of them must participate equally in their group's presentation. If this is not possible, due to time constraints, the group can agree to rotate the "reporter" for each in-lab presentation.

In addition, as part of a formative assessment tool, students are asked to formulate testable hypotheses at several times during the semester-long project. During laboratory #2 and #3, students work in groups of two to four, depending on class size, to develop and present their hypotheses concerning type of microbial growth they anticipate on different culture media. This work requires that students demonstrate both a knowledge of relevant literature, as detailed in the lesson plan, and the proper interpretation of their results from the previous week's work. Classroom presentations also allow students to compare their work to other groups in the class and engage in self-reflection. During laboratory #6 (isolation of antibiotic producing strains of *Streptomyces*) students are again asked to work in their groups (for 10-15 minutes) to suggest reason(s) for the observed differences in antibiotic sensitivity between Gram-positive and Gram-negative bacteria. This allows the students to make connections between their observed laboratory results and lecture discussions on the structure of the Gram-positive and Gram-negative cell wall. Additionally, formative assessments also contribute to an inclusive learning environment. Each student in the group can contribute to the group's hypothesis and presentation of their results to the entire class.

The summative assessment tool is a detailed laboratory report on the semester-long investigation. To facilitate the writing of this report students are required to keep a detailed laboratory notebook throughout the semester. Students receive a comprehensive guide (Supporting file S12: Dirt to DNA - Laboratory Report Outline.) describing the requirements for their final report. This outline also serves as a rubric for the instructor and ensures uniform grading among the students. As described above, the final laboratory report on the *Streptomyces* comprises 1/3 of the student's final laboratory grade. In addition, a comprehensive written final laboratory exam contains multiple choice questions on the *Streptomyces* and interpretation of laboratory results. The method used to assign point values to each student (letter grade, point value, or percent) is up to the individual instructor and their overall grading system for the class. The laboratory portion of my class accounts for 30% of the overall microbiology course grade. In class presentations and written assignments account for 10% of the laboratory grade. The final laboratory report on the *Streptomyces* accounts for an additional 10% of the laboratory grade and the comprehensive multiple choice final laboratory exam counts for the remaining 10% of the laboratory grade.

Inclusive Teaching

Inclusive teaching requires that students feel comfortable in your classroom. "Being explicit about one's goal of cultivating an inclusive, equitable, and fair classroom learning environment reiterates that students and instructors are on the same side, not on somehow opposing sides, of the teaching and learning process" and "all students are explicitly welcomed into the intellectual discussion of biology" (28). Emphasize to your students that you, as the instructor, are committed to an inclusive classroom environment. To ensure inclusiveness tell your students that the course is structured in such a way to facilitate this outcome. Impress upon your students that scientific investigation is a collaborative effort that requires multiple inputs and talents. Tell them we each bring unique abilities and experiences to any situation, and that our success, as a class, on the *Streptomyces* project requires that each person contribute and that we as a class value each other's unique contribution and perspective. Remind your students that it is your hope, as their instructor, that all of them will become the next generation of scientific investigators committed to the scientific method and evidence-based decision making. Tell them that they will become the diverse face of the next generation of scientists (28).

In addition, as the instructor, it is important that you recognize the diversity in your classroom. Your class's diversity may include some of the following differences: race, class, poverty, gender, disability, and privilege or disenfranchisement (29). Before asking students to introduce themselves and identify diversity issues important to them you, as their instructor, can provide a comfortable classroom environment by introducing yourself to the class. Describe your background and diversity issues you deem important to create an inclusive classroom environment. You might also include a diversity statement in your syllabus.

Once you have initiated the discussion on diversity encourage your students to introduce themselves to the class at the beginning of the semester. Give them a chance to say who they are and what they hope to gain and give to the class. In addition, students should be encouraged to speak to you privately to assess their learning, progress, and concerns for the class. You should also ask them, during an office meeting, about their learning style, do they work best alone, or in a group. Do they excel at oral or written work? Let them know that you are available to meet with them in person, during office hours, or by email correspondence. Tell them that the class will provide an opportunity for them to participate and excel in different ways

and that you want them to tell you if they are uncomfortable, for any reason, during class and what things you as the instructor can do to facilitate their learning. In short, they matter to you as individuals.

Students work in groups of 2-4. This establishes a small community of learners in which each student can feel safe and included. I assign members to each group based on my prior knowledge of their prior research experience and observation of their engagement with their major. Some students have extensive out of class research experience, and these students should not group together. If students self-select the groups become homogenous and therefore do not reflect the diversity of the classroom.

As an example of an inclusive learning environment during LAB 3 and 4, students examine each other's work and then decide together which of their soil isolates most closely resembles a *Streptomyces* bacterium. These groups prepare a short in-lab presentation that gives each student a voice. It allows students who might be reluctant to speak to the entire class by themselves, to be part of a supportive group presentation that represents their work. This allows students with different learning styles (interactive group work as opposed to individual noninteractive work) to benefit from an interactive learning environment. This opportunity arises several times during the semester when students present their hypotheses regarding their group's laboratory work and their data. This Lesson also employs peer instruction which promotes an inclusive environment as each student brings different skills to the group and therefore adds value to each person's contribution.

Finally, as the instructor, you should take time to point out the diversity among *Streptomyces* investigators. Hopwood's *Streptomyces* in Nature and Medicine (8) provides an excellent resource documenting this point and the power of cooperative scientific investigation. I cannot emphasize enough how useful and important Hopwood's book is, as a resource, and to impress upon students the diversity inherent in the international *Streptomyces* project. The text highlights the international cooperation, among female and male scientists of different nationalities, brought together at the John Innes Centre in England. Individual investigators are described and given credit for their contributions. The book demonstrates the power of both diversity and cooperative investigation. I point out to students the names of individuals who contributed to the International *Streptomyces* project. For example, Hee-Jeon Hong from Korea working with Mark Buttner, from the John Innes Center in Norwich UK, discover one pathway for resistance to the antibiotic vancomycin (8, 30) and Janet Westpheling working with Xianyang Ni, from the University of Georgia in Athens, Georgia who together examine the promoter region of the *Streptomyces* chitinase gene (8, 15). These are not household names, but they are known in *Streptomyces* biology and demonstrate to students the diversity and collaborative nature of scientific investigation. There are many more examples and I strongly encourage you to obtain a copy of this book.

LESSON PLAN

Lesson Plan – Background

This lesson is a semester-long investigation of the soil-dwelling *Streptomyces* bacteria. Each laboratory runs for approximately 3 hours. The success students have isolating suspected *Streptomyces* in pure culture, determines the exact length of the project. Isolation of the organism in pure culture is essential to project success. During most weeks, additional standard microbiology laboratory work occurs along with each week's work on the *Streptomyces* investigation. The additional laboratory work is up to the instructor. In the past, I have been able to do considerable microbiology-specific laboratory work in addition to this series of exercises.

In a laboratory class of 16 students, perhaps three students isolate an antibiotic producer. That most students isolate a non-antibiotic producer is not a discouraging result. The purpose of this lesson is not to just isolate an antibiotic producer, it is to use evidence gathered over the course of the semester to say with confidence (evidence-based decision-making) that their isolate is or is not a *Streptomyces*. Additionally, my students have isolated both constitutive and non-constitutive antibiotic producers. These two types of *Streptomyces* isolates can be used to examine the phenomenon of antibiotic induction in non-constitutive isolates. One of my students performed this work as an independent research project.

Progressing Through the Lesson

The microbiology course that I teach consists of three fifty-minute lectures and one three-hour laboratory session per week. During the first fifty-minute lecture of the semester (prior to the first laboratory meeting), in addition to talking about the microbiology course policy, I describe the semester-long *Streptomyces* laboratory project. At this time, tell your students they should come to the first laboratory session of the semester with their soil sample. The location they use to collect their soil sample is up to them. I do however explain that the best chance of collecting *Streptomyces* is from a rich soil, not too compact, that has the characteristic soil smell. This earthy odor is due to the production of geosmin by the *Streptomyces* (9) and is an unmistakable odor once you have smelled it. I give them a sterile 50 ml Falcon tube to collect their soil sample and tell them not to open the tube until they are ready to collect their sample. When ready to collect their sample, they should fill the tube half-way to the 25 ml mark. Also tell your students to note the area they have sampled by marking it, if possible, or by taking a picture of the area with their phone. This will allow them to return to the area if they need additional samples. They should bring the tube with their soil sample to the first laboratory session.

During the first laboratory of the semester (LAB 1), students transfer their soil sample, all, or some of it, depending on the size of the sterile petri dish. The exact amount is not important. They want to achieve a single layer of dirt so it will dry thoroughly during the incubation period. Students incubate their soil sample at 30 degrees centigrade until the next laboratory session (5-7 days later). The drying process should reduce the microbial load of both bacteria and fungi and enhance the isolation of *Streptomyces* as they produce desiccation resistant spores (31). The Lesson then proceeds each week beginning with isolation in pure culture of their suspected *Streptomyces*, colonial, and cellular characterization of the isolate, followed by physiological and finally molecular characterization using *Streptomyces*-specific primers to its 16s rRNA gene. Students then conclude their study with a detailed laboratory report that presents all their data and the identity of their isolate. Additionally, I include student prompts at the beginning of

the student section (the handout for that LAB) in several of the supporting files. Some of the prompts are practical in nature and relate directly to that day's laboratory. For example, during the third laboratory period students look for typical *Streptomyces* colonies on their spread plates. The handout for LAB 3 asks students to look for images of *Streptomyces* colonies either in their text or appropriate web sites. If your students type into a Google search: "*Streptomyces* colony morphology" multiple sites and images of the *Streptomyces* colonies will be found. This will assist them greatly when they examine their own plates for typical *Streptomyces*.

Instructions for laboratory preparation by you and your laboratory prep room staff are included for each laboratory in the Lesson Timeline and supporting files. For laboratory 8 (DNA Extraction) and laboratory 10 (PCR) additional instructions are provided as part of Supporting file 13: Dirt to DNA- Bacterial cultures, Media, Solution recipes, and Equipment.

Lesson Plan

LAB 1

Supporting file S1: Dirt to DNA-Handout Introduction to the Actinobacteria (LAB 1).

The purpose of LAB 1 is to provide enough background information on the *Streptomyces* so students can begin their work on the isolation and characterization with a basic understanding of these bacteria. Students begin LAB 1 by transferring their soil sample to a petri dish and then drying it in an incubator at the beginning of the laboratory session. Considerable moisture may collect on the Petri dish lid during the incubation period. Should this occur, remove the lid, and replace it with a new sterile lid. Following the transfer of soil samples to the Petri dish the instructor will then proceed with the laboratory using Supporting file S1: Dirt to DNA- Handout Introduction to the Actinobacteria (LAB 1), as your lecture outline.

This supporting file begins with an introduction to the Phylum Actinobacteria, within which the *Streptomyces* reside. The *Streptomyces* are aerobic, filamentous, Gram-positive bacteria. The filamentous nature (hence the name *Streptomyces*, coiled filaments) makes them unusual, as most bacteria are unicellular. This handout describes/summarizes for the student the unusual cellular, colonial, and genomic nature of this group of bacteria. Excellent references for this introduction are Brock's Biology of Microorganisms (32) (page 499-504), a review of *Streptomyces* Morphogenetics by Flardh and Buttner (33), and Chater's reviews of the *Streptomyces* (34, 35). In addition, during this first laboratory meeting, it is important to place the *Streptomyces* in an evolutionary time scale. These bacteria are thought to have evolved 400 million years ago, coinciding with the colonization of the land by plants and the formation of soil (35). Their metabolic capacity to degrade chitin has figured largely in their ability to both compete in the complex soil environment and contribute to its formation.

LAB 2

Supporting file S2: Dirt to DNA-Handout Serial dilution and spread plate technique (LAB 2).

The purpose of LAB 2 is to introduce students to aseptic technique, the fundamentals of serial dilution, and the use of selective bacteriological media. Your students will also begin a formal laboratory notebook on the *Streptomyces* project. The students must be encouraged/required to keep detailed notes for each laboratory session. This will aid them greatly in the preparation of their final laboratory report and final laboratory exam.

Before beginning LAB 2 it is important to emphasize to your students that isolation of bacteria from environmental samples run the risk of isolating pathogenic bacteria. With that as a consideration, students must work under BSL - 2 conditions for this laboratory and laboratory 3, 4, and 6. Biosafety Level 2 conditions must be maintained until you are certain that each student isolate is a *Streptomyces*. Refer to these two links for a detailed discussion of BSL - 2 requirements. You may need to paste the URL into your browser:

- https://www.asmscience.org/content/journal/jmbe/10.1128/jmbe.v20i3.1975
- https://asm.org/getattachment/3c1eb38c-84d7-472f-aa9b-5d695985df21/2019-Biosafety-Guidelines.pdf

BSL - 2 requirements are discussed in more detail in the accompanying supporting file.

Your students will begin this laboratory session by retrieving their dried soil sample from the incubator. Each student will begin a serial dilution of their now dried soil sample, followed by the spread plate technique. You should demonstrate the technique, but you can also show your students the following YouTube video of the spread plate technique.

Prior to beginning the serial dilution of their soil sample, the instructor will describe and diagram the dilution scheme for a series of 10-fold serial dilutions to 1X 10-6. Explain to your students that the purpose of the serial dilution is to produce one or two sets of countable plates (between 30 - 300 colonies) within the dilution range. This is also the appropriate time to demonstrate how to calculate the number of organisms per ml/gm of their sample. I ask the students to come to laboratory the following week with an estimate of the dilution they think they will count on each of the three media, and why. In addition, I ask them to provide a sample calculation for that dilution to determine the number of organisms per ml/gm of their soil sample. Suggest to your students that they search for a published article on the number of microorganisms per gram of soil. They should then use the data in this article to defend their estimate.

At this time students should also meet in their groups (3-4 groups of 2-4 students, depending on your class size) and formulate hypotheses regarding the type of microbial growth (bacterial, fungal, or enriched for *Streptomyces*) they anticipate observing next week when they examine their spread plates. This will be the first-time students will meet in their group. Detail on the organization, dynamics, and grading of each group member was presented above in the section on Assessment and Inclusive teaching.

Your students will be using three types of agar media. T-soy agar an enriched medium on which all bacteria, mold, and *Streptomyces* will grow, Sabouraud's Dextrose agar a selective medium with pH of 5.5 (inhibits bacterial growth promotes fungal growth), and Chitin agar a highly selective medium due to its single carbon source (chitin). The *Streptomyces* are one of only a few bacteria that possess the chitinase enzyme. As a result, this medium greatly enriches for the *Streptomyces*, suppressing the growth of most bacteria and fungi (12,13).

Before beginning laboratory 3 next week, each student group should present their hypotheses regarding the type of microbial growth they expect to see on the three media and a justification for their hypothesis. Formulation of hypotheses by students serves as a prompt to encourage them to investigate the topic independently and be prepared for their laboratory exercise. This is in keeping with the theme of this Lesson, to facilitate student learning by adding structure to the Lesson. They should also present their sample calculations regarding dilutions and number of microorganisms-bacteria per gm/ml of soil sample. Their estimate should be evidence-based, not a guess. To inform their estimate you may want to prompt your students to access Raynaud and Nunan (14), Spatial Ecology of Bacteria at the Microscale in Soil. This article will provide published research data with which to make their estimate.

Also, inform your students that the spread - plate method produces a viable cell count (as opposed to a total cell count) under the given conditions for growth. It provides an estimate of the viable microbial content of the soil sample using T-soy agar, the mold content using Sabouraud's dextrose agar, and enriching for *Streptomyces* using chitin agar. The most important and accurate *Streptomyces* count will be on chitin agar. This medium will provide the best data for the number of *Streptomyces* per gram of soil.

LAB 3

Supporting file S3: Dirt to DNA-Handout Count serial dilution plates and subculture to ISP medium 2 (LAB 3).

The purpose of LAB 3 is to further refine your student's skills on aseptic technique, the streak plate, and to introduce them to counting serial dilution plates. They may also observe, for the first time, a *Streptomyces* colony exhibiting antibiotic activity. Your students begin this LAB by determining the number of microbial colonies per ml or gm of soil on each of the three-agar media used. Students should compare the actual values to their predictions from last week. You can take 15-20 minutes after students have counted their plates to present both the actual result and their prediction.

Identify suspected *Streptomyces* colonies on each of the three agar plates (the chitin agar plate will be the most productive) and aseptically pick these colonies for transfer to a fresh agar plate and streak for isolation of the organism. Students must pay particular attention to the plate of T-soy agar. This plate may show antibiotic production from a *Streptomyces* colony as a zone of inhibition surrounding that colony. If students observe such a colony on their T-soy agar plate they should of course try to isolate this organism in pure culture. This may take several rounds of streaking and re-streaking. See photomicrograph of *Streptomyces* colonies in this lab's supporting file for reference purposes.

LAB 4. (No Supporting file).

The purpose of this LAB is to continue the isolation of each student's *Streptomyces* isolate in pure culture. The success of the project depends on students having a pure culture of *Streptomyces*. This process may take several weeks, and therefore the instructor should devote 20 minutes during this laboratory session for students to re-streak isolated colonies from their previous week's agar culture.

You should, once again, stress the importance of aseptic technique as a prerequisite for isolation and identification of unknown bacteria from any environmental source. Without a pure culture of their isolate, no meaningful work is possible from this point forward. If students are unable to isolate *Streptomyces* in pure culture from their soil sample, assign them a collaborator in the laboratory who has successfully isolated a suspected *Streptomyces*. They should then streak their collaborator's isolate on a T-Soy or ISP-2 agar for their own use. Having students share their successful *Streptomyces* isolates has worked well in the past.

LAB 5

Supporting file S4: Dirt to DNA-Handout Slide culture technique (LAB 5).

The purpose of this LAB is to introduce students to the slide culture technique for the cellular characterization of their isolate, an essential step in the characterization of their suspected *Streptomyces* isolate. Begin LAB 5 by viewing the YouTube video on slide culture (fungal slide culture - YouTube). These videos (accessed by entering "fungal slide culture" into a Google search) describe the technique for slide culture of fungi. Use the same procedure for a slide culture of the *Streptomyces*. You will only need to watch the part on slide culture, not the staining section. There are many such videos on YouTube, and you can access any one of them prior to your lesson. Have the students watch the video prior to preparing their own slide culture. Next week your students will observe their slide culture directly without staining.

Explain to your students that a successful slide culture will allow them to observe the unique filamentous growth of the *Streptomyces* bacteria. By observing their agar plate cultures and their slide culture on a daily basis, they should be able to document, in their laboratory notebook, the temporal growth cycle from vegetative growth to aerial hyphae, and spore formation.

LAB 6

Supporting file S5: Dirt to DNA-Handout Check pure culture isolates for antibiotic action (LAB 6).

The purpose of this LAB is twofold. First, to introduce students to the agar diffusion method of antibiotic sensitivity testing (Kirby-Bauer Method) using the standard culture medium, Mueller-Hinton II agar. The second is to continue the physiological characterization of their isolate (they have already checked for chitinase production), by checking for antibiotic production. This will allow students to see the link between morphological development (appearance of aerial hyphae and sporulation) and the production of secondary metabolites, antibiotics. This laboratory, as with laboratory 2, 3, and 4, may involve bacteria that require BSL - 2 handling. Follow the requirements for BSL - 2 as described above with the link to the ASM site describing BSL - 2 procedure.

During laboratory six, students will check their pure culture isolates for antibiotic action. At this point in the semester I have covered in lecture the structure of the Gram-positive and Gram-negative bacterial cell wall as well as the structure and function of the Gram-negative outer membrane as a molecular sieve. Time permitting, ask your students to again form into their

small discussion groups (2-4 students) and suggest reasons for the differences in sensitivity to antibiotics between Gram-positive and Gram-negative bacteria. After a brief 10-minute discussion with their group members, you should ask one or two groups to present their reasons for the differences in antibiotic sensitivity between the two groups of bacteria.

Show your students the photographs, (Supporting file S5: Dirt to DNA-Handout Check pure culture isolates for antibiotic action (LAB 6), of the agar plug method (using sterile brass cork borer) for determination of antibiotic action. Also refer to, Supporting file S4: Dirt to DNA-Handout Slide culture technique (LAB 5), for the use of the sterile cork borer. Students should use the pure cultures of their *Streptomyces* isolates to check for antibiotic production against the following bacteria: Staphylococcus epidermidis, Staphylococcus aureus, and Escherichia coli. The first two bacteria are Gram-positive and the last one is Gram-negative. It is generally more difficult to isolate an antibiotic producer against the Gram-negative bacteria due to the presence of an outer membrane in these bacteria.

LAB 7

Supporting file S6: Dirt to DNA-Handout Grow suspected *Streptomyces* isolates in broth culture (LAB 7).

The purpose of LAB 7 is to begin the second half of the *Streptomyces* project, in preparation for the molecular characterization of student isolates. This requires growth of each student's isolate in pure culture with strict adherence to aseptic technique. Students begin this laboratory by inoculating their suspected *Streptomyces* isolates into broth culture, ISP medium 1. Students then incubate their broth culture in a shaking water bath at 30 degrees centigrade for 4 to 7 days. Incubation time, in days, will vary with the growth patterns of the isolate. In addition, the physical nature of the growth (turbid growth throughout the culture or clumps of cells at the bottom of the culture tube) will also vary with the growth characteristics of the isolate. At the end of the incubation period your students can also use this LAB to further confirm the filamentous nature of *Streptomyces* growth. They should be encouraged to prepare a wet mount of their culture for microscopic examination.

Both students and instructor perform this stage of the laboratory project in anticipation of DNA extraction and Polymerase Chain Reaction (PCR) using *Streptomyces*-specific primers to 16s rRNA genes. As the instructor, you will need to grow, in pure culture, both positive and negative control bacteria for DNA extraction and PCR. Ordering information for the bacterial cultures is in Supporting file 13: Dirt to DNA-Bacterial cultures, Media, Solution recipes, and Equipment. *Streptomyces* ordering information is also described below for your convenience.

Your positive PCR control will be *Streptomyces* griseus subsp. griseus (ATCC # 15395). This bacterium is available from the American Type Culture Collection (ATCC) in Manassas, Virginia (800 638 6597 or 703 365 2700, or www.atcc.org, or Email Tech@attc.org). The American Type Culture Collection sends an aliquot of the bacterium with detailed instructions on its handling and growth characteristics. These instructions are also available at the ATCC web site and can be downloaded and viewed prior to arrival of the culture.

LAB 8

Supporting file S7: Dirt to DNA-Handout DNA extraction from broth culture of *Streptomyces* isolate (LAB 8).

Protocol: DNA Purification from Gram-Positive Bacteria using the Gentra Puregene Yeast/Bact. Kit. This protocol is for purification of genomic DNA from *Streptomyces* fresh or frozen samples of 0.5 ml. Your students must familiarize themselves with this procedure prior to coming to the laboratory.

The purpose of this LAB is to introduce your students to the process of genomic DNA extraction from Gram-positive bacteria. They will also gain an understanding of the both the physical and chemical/enzymatic methods used to achieve this goal. In addition, they will be introduced to the use of the Nanodrop spectrophotometer and the importance of the 260/280 nm absorption ratio in determining the purity of their DNA extract. The following link from the University of Arizona provides an excellent summary for your students about interpreting Nanodrop results.

Hand out the procedure to your students the week before this laboratory or post it on the course's website. As this laboratory is quite time consuming, I spend little time during this laboratory period with introductory comments regarding the rationale behind the extraction method. See Supporting file S7: Dirt to DNA- Handout DNA extraction from broth culture of *Streptomyces* isolate (LAB 8), as significant work (2-3 hours) is required of the instructor prior to the beginning of the laboratory session.

Remind the students that this laboratory exercise (LAB 8) will take an additional hour and they will likely not finish their work until 6:00 PM, if they have a 2-5 PM laboratory. Tell them to be prepared to stay until the work is completed. I thank them in advance for their cooperation. This additional hour of student work assumes that the instructor has already prepared the cells as described in the accompanying supporting file.

Prompt your students to come to this laboratory session with a short, written explanation of the purpose behind both the physical (freeze/thaw grinding) and chemical (lysozyme treatment) isolation steps that precede the actual DNA extraction and isolation. You may also want to instruct them to include a brief explanation of peptidoglycan structure. Use this pre-laboratory assignment as a formative assessment tool. At the beginning of the laboratory session students should hand in this assignment for grading. Return the graded assignment during the next laboratory period.

LAB 9

Supporting file S8: Dirt to DNA-Handout Agarose gel electrophoresis of DNA extraction and UV scan (LAB 9).

The purpose of this LAB is to introduce your students to the technique of agarose gel electrophoresis. After your presentation of the agarose gel images of their DNA extracts from the previous LAB, they should understand the need for the DNA base-pair ladder (the information it provides) and be able to recognize the band on the gel that corresponds to genomic DNA. This supporting file is the procedure for preparation and DNA electrophoresis using an agarose gel. It also contains a scanned image of electrophoresed *Streptomyces* DNA. This file is primarily for your use, as you will perform the electrophoresis

of your student's DNA samples. However, I have also arranged the file so you can use it as a handout for your student's reference

At the beginning of the laboratory, you will present the general principles of agarose gel electrophoresis. Utilize the YouTube video (see below), as needed. Also, describe the appearance of genomic DNA on the gel and ask your students to predict the location of genomic DNA on the gel.

As the instructor, you will perform the agarose gel electrophoresis of your students' *Streptomyces* DNA (from LAB 8) prior to this laboratory meeting (LAB 9). I find this necessary to move the laboratory work along in a timely fashion. If you have the students perform this work, it will take an additional laboratory session to prepare the agarose gel, perform the electrophoresis, and visualize the DNA by UV gel scan. Once you have the students' DNA extracts electrophoresed, scanned, and photographed you can use the scanned image to discuss (during LAB 9) the principles and interpretation of agarose gel electrophoresis, the need for a DNA base pair (bp) ladder, and the function of ethidium bromide with particular emphasis on its safe handling and disposal. You can also take 10-15 minutes of the laboratory to show a YouTube video on agarose gel electrophoresis. There are many YouTube videos on agarose gel electrophoresis. Choose the video that best suits your class for time and content. The BioRad video is helpful. Distribute the scanned and copied gel image of your students' DNA in your course's shared file directory. Your students will need this for their final laboratory report.

LAB 10

Supporting file S9: Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10).

The purpose of this LAB is to introduce your students to the technique and theory behind the polymerase chain reaction (PCR). They should also understand the role of gene-specific primers and their interaction with Taq polymerase.

This supporting file is a template for the laboratory on the PCR using custom-designed primer sets. It includes information that you will need and a detailed procedure for students in your laboratory. Prior to photocopying the detailed procedure for your class, you will need to fill in the student assignments and hand out this procedure to the class the week before the PCR laboratory. This is an involved laboratory, and students must know their responsibility. However, students can complete the work within the scheduled laboratory time of approximately 3 hours. In the past, I have found that the students have finished their work a bit early.

Prior to laboratory, your students should have read the appropriate section in their text and laboratory manual explaining the basics of the polymerase chain reaction. You can then take about 15-20 minutes (no longer) to cover the basics of the PCR. Explain to them that they are now using the *Streptomyces* DNA that they extracted during last week's laboratory as the template from which they will amplify the *Streptomyces* 16s rRNA gene. At this time, you can present the photograph of the agarose gel you ran showing the isolation of genomic (high molecular weight) DNA. As part of your introduction, you will explain the role of the primers in directing Taq polymerase to the appropriate gene on their template DNA. You can also tell them that these primers have already been tested on DNA extracted from *Streptomyces* griseus (the positive control for the PCR) and student *Streptomyces* isolates, and should work on their DNA samples if they isolated a *Streptomyces*. Also, explain that the negative control DNA (Escherichia coli) should not result in any DNA amplification. As a result, they should not see a PCR product (band) in the gel lane associated with that reaction tube in the thermal cycler. I collect the PCR products at the end of the thermal cycler process and run the agarose gel on the PCR products for presentation during the next laboratory period.

In addition to explaining the technical part of this laboratory, I place great importance on the historical context surrounding both scientific procedures and the knowledge that enabled these procedures. This does two things for the students. It demonstrates that science is a dynamic process and the procedures and knowledge that we have are the result of others' work. In addition, it instills in them the need to honor the past. I make available to them the Perspective written by Thomas Brock describing his work in the discovery and isolation of Thermus aquaticus (44) and refer them to the patents placed by Gelfand (45) on Taq polymerase, and Mullis (46) on the PCR technique.

LAB 11

Supporting file S10: Dirt to DNA-Handout PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11).

The purpose of this LAB is to introduce your students to the power and usefulness of the PCR technique in providing data (nucleotide sequences) that can then be used to both identify and establish phylogenetic relationships among organisms. This LAB should also establish for your students the need for the appropriate positive and negative control organisms, *Streptomyces* griseus and Escherichia coli respectively. Finally, the students should understand why primers were designed to recognize the 16s rRNA gene and the historical significance of this gene.

Promptly after the PCR laboratory (LAB 10) you should send the PCR products to Genewiz (contact information below) for nucleotide sequencing. Do this immediately to ensure that the results come back from Genewiz prior to LAB 11. Supporting file S10: Dirt to DNA-PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11), explains the steps in PCR product submission and presentation. As part of your order to Genewiz, you will need to provide a copy of your agarose gel electrophoresis of the PCR products. When Genewiz has sequenced your PCR products you will now have access to the data (at the Genewiz website) in the seq and trace files. You can download this information from the Genewiz web site and make it available to your students. Present these files to your class during LAB 11. In addition, present the agarose gel electrophoresis UV scan-results to your class. They will need these data for their final report on the *Streptomyces* isolation. LAB 11 and LAB 12 represent the final pieces of evidence, in this case molecular data, necessary to confirm the identify of student isolates as a *Streptomyces*.

Up to this point the evidence gathered has been morphological (both colonial (LAB 3 and 4) and cellular, LAB 5), metabolic with the use of chitin agar as a single carbon source selective growth medium (LAB 2), and functional by the determination of antibiotic production (LAB 6). Your students will now make use of molecular data to finalize the identification of their isolate.

Again, I try to place the science in a historical context. The use of molecular data to establish phylogenetic relationships and identification of specific organisms dates back to the work of Carl Woese and George Fox (47). Their landmark 1977 paper in the Proceedings of the National Academy of Sciences, (Phylogenetic structure of the prokaryotic domain: The primary kingdoms) marks the beginning of the use of 16s rRNA gene sequencing as a powerful tool in establishing phylogeny and identity of living organisms. It is important to emphasize to students the power of this process and the insight Woese brought to bear by choosing ribosomal RNA genes, and therefore ribosomes, as a comparable structure, present in all living organisms, and therefore amenable to a comparative analysis.

Woese writes: "Phylogenetic relationships cannot be reliably established in terms of noncomparable properties. A comparative approach that can measure degree of difference in comparable structures is required… To determine relationships covering the entire spectrum of extant living systems, one optimally needs a molecule of appropriately broad distribution."

The sequence of nucleotides in 16s rRNA genes can now provide a barcode for identification of your students' isolate. The Woese paper also speaks convincingly against the use of noncomparable structures (the prokaryotic/eukaryotic dichotomy as stated by Stanier and Van Niel (48) as a means of establishing phylogenetic relationship. Woese writes, "eukaryote/prokaryote is not primarily a phylogenetic distinction, although it is generally treated so." I emphasize these important concepts as part of this laboratory sequence. Have your students read the Stanier and Van Niel paper (The Concept of a Bacterium) and the Woese and Fox paper as supporting references to this laboratory.

It is also important, again, to point out the historical context surrounding and informing the work students are doing. The use of DNA sequencing data from PCR is a recent innovation (44, 45, 46). Use this development to emphasize the dynamic nature of scientific investigation and the power of evidence-based decision-making. To further emphasize the dynamic nature of scientific investigation you can point out that investigations of the type they are doing use next generation DNA sequencing (NGS) of 16s rRNA genes to determine a comprehensive profile of microorganisms within an environmental sample. This is now accomplished without the need to culture individual microorganisms in the laboratory (49). This technique, NGS, is a powerful tool to quickly demonstrate, not only the diversity of the soil microbiome, but how environmental factors can change the makeup of the microbial community over time.

At the conclusion of LAB 11 and LAB 12, students should be able to present an evidence-based argument regarding the identity of their soil isolate.

Send PCR products to Genewiz. Detailed instructions are in Supporting file S10: Dirt to DNA-PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11). Genewiz is just one of several companies that perform PCR product sequencing. I list others in the supporting file.

LAB 12

Supporting file S11: Dirt to DNA-Handout NCBI Nucleotide Blast* (blastn) (LAB 12).

The purpose of this LAB is to introduce your students to the National Center for Biotechnology Information web site and the use of its BLAST programs. They will learn how to use the nucleotide sequence data provided by Genewiz (a nucleotide sequencing company) to establish a probable identify of their soil isolate.

LAB 12 represents the final piece of work and evidence in this semester-long project. At the conclusion of this laboratory, students will have all the data they need to begin writing their laboratory report. You will copy and hand out the accompanying supporting file to guide your students through the final analysis of their soil isolates making use of the nucleotide database at NCBI. Students should come to this laboratory with the nucleotide sequence of their soil isolate (previously accessed by them at the Genewiz web site or downloaded for them by you) ready to subject the sequence to a nucleotide BLAST at the NCBI web site. You will guide them through the following using Supporting file S11: Dirt to DNA-Handout *NCBI Nucleotide Blast (blastn) (LAB 12). This supporting file describes, in more detail, the steps listed below.

1. Copy their nucleotide sequence. Omit those bases at the beginning and end of their sequence indicated as "N."
2. Access the NCBI web site.
3. Click the tab for nucleotide BLAST.
4. Paste their nucleotide sequence into the accession box.
5. Use the default settings on this web page as indicated in the handout.
6. Click the tab at the bottom of the page for BLAST.
7. Once the BLAST is complete, students will have both a graphical and descriptive table of "sequences producing significant alignments."
8. Scroll down to the descriptive table and look for the best matches, usually the first several listed.
9. Click on the top match to access the query-subject nucleotide sequence pairs.
10. Examine the score, expectation value, identities, and gaps. Supporting file S11: Dirt to DNA - Handout *NCBI Nucleotide Blast (blastn), contains definitions of these terms.
11. Based on this final piece of data, your students are now ready to confirm their hypothesis regarding the identity of their soil isolate.

Students do not need to complete their analysis during the laboratory period. The purpose of this laboratory is to show them how to proceed with this final piece of data. They will work on their analysis outside of class. Before moving on to any additional laboratory work you have scheduled for this lab session, students should inoculate two ISP 2 agar slants of their isolate. They should choose a well-isolated colony from a previous agar plate to inoculate the slant and then incubate the slants at 30 degrees centigrade for 5-7 days. Slants should be stored at 4 degrees centigrade. Growth on the slant can remain viable for up to a year. If time permits you should freeze dry cells, from a broth culture, to produce a more stable sample of the isolates. Students interested in an independent research project can then use these cells.

LAB 13

Supporting file S12: Dirt to DNA-Handout Laboratory Report Outline (LAB 13).

The laboratory report outline provides a template for the student report. They should follow the outline as presented. It can also serve as a rubric for your grading of their papers. I post the Laboratory Report Outline on the course web site and/or handout a printed copy in laboratory. I spend 15 minutes explaining the laboratory report requirements and the due date. You can modify this file to reflect the points more accurately in the *Streptomyces* project that you emphasized.

TEACHING DISCUSSION

Effectiveness of the Lesson

I view the Lesson as effective on four levels. 1. Student reaction to the Lesson, 2. Demonstration of student understanding of the ASM aligned goals and objectives, 3. Fulfilling several of the five characteristics of a CURE as described by Auchincloss et al. (5), 4. Successful isolation and characterization of a soil *Streptomyces* by employing the techniques of microbiology and molecular biology.

1. Conclusions about the effectiveness of the Lesson come from my observations and interactions with students during the semester-long project and as demonstrated in their end of semester laboratory report. During the semester, the students were eager to pursue the project at each laboratory meeting. They enjoyed the work as evidenced by the positive attitude in the laboratory and their effective group collaboration throughout the semester. They were unwilling to not get it right. Some students came to my laboratory to redo parts of the work that did not turn out well for them during the scheduled laboratory period. They were determined and committed to the work. Overall, the class was enthusiastic about the project, and were willing to spend extra time at the end of several laboratory periods that required an additional 60-90 minutes to complete. Working after the end of a scheduled laboratory period often presents problems for the students and can result in a less than positive attitude. This did not occur. The students did not see the after-hours work as an inconvenience or a burden, but instead were committed to the project and its successful completion. This was also evident as group members were willing to share the burden of extra work outside of the prescribed laboratory hours. Group members would "stand in" for each other based on individual needs and commitments. They had formed a cohesive and collaborative working group.

Further evidence regarding the effectiveness of the Lesson stems from the desire of one of my students to pursue the class work, as an independent research project, for an additional two semesters. This is an example of what others, Hanauer et al., have described as "project ownership" (3). A brief description of this work seems appropriate as a desire to continue the work demonstrates both a sense of excitement and ownership in the project, both highly desired outcomes. During the independent research project, my student and I investigated the possibility of antibiotic induction in isolates that were non-antibiotic producers. We were able to induce two non-antibiotic producing isolates to antibiotic production by growing these cells in a competitive environment instead of in pure culture. This result was reproducible and started us thinking about the mechanism of induction. We hypothesized either plasmid transfer from the producer to the non-producer or the induction of an existing gene by soluble factor(s) produced by the antibiotic producer. Our preliminary results, by comparing (on an agarose gel) DNA extracted from the induced cells both before and after induction, did not support the plasmid transfer hypothesis. However, much additional work is needed to further explore this trait. Finally, as further evidence of the success of the lesson, students in my microbiology course concluded this work by presenting the results of their semester-long project during the university research day. These types of outcomes may then provide students with the confidence and interest to pursue research outside the formal classroom setting.

2. In addition, students demonstrated competence in their understanding of the ASM goals and objectives. Several of these are included as final exam questions and in the end-of-semester student laboratory report outline. Their written answers to these questions demonstrated a good level of understanding. However, the reports also laid bare deficiencies in their understanding of some ASM goals and objectives and highlighted the need for a more thorough discussion of these.

Some of the Lesson specific goals and objectives apply to technical competence in both microbiology and molecular biology. Students majoring in or taking biochemistry courses, and working on independent research outside of the formal class setting, commented on their greater confidence and understanding in their biochemistry course as a result of the work on the *Streptomyces* project.

3. Fulfilling several of the five characteristics of a CURE as described by Auchincloss et al. (5).

Auchincloss et al. define CUREs as:

a. Use of scientific knowledge by … "proposing hypotheses… using tools of science, gathering, and analyzing data and… communicating findings." This first characteristic is applied during the semester-long project. Students are asked, throughout the Lesson, to formulate hypotheses regarding their work. This activity is performed either alone as a prelab exercise, or in collaboration with their working group. Students then communicate their thoughts as part of a group presentation. Finally, students use the tools of science as appropriate to this discipline of study, in microbiology and molecular biology, to gather and analyze their data. This process is performed each week as additional data are acquired through different investigative methods.

b. Discovery "as students take ownership of their learning, they transition from seeing themselves as consumers of knowledge to seeing themselves as producers of knowledge" (5). As this research project holds out the potential for an original discovery, of an undescribed *Streptomyces* and its antibiotic, students start to see themselves as "producers of knowledge."

c. "Broadly relevant or important work." This relates to one of the ASM learning objectives: Give an example of and explain how microbial metabolism is important to a relevant societal issue. This Lesson demonstrates the difficulty in isolating antibiotic-producing strains of *Streptomyces* that demonstrate effectiveness against selected pathogenic bacteria. As such, it provides a "real life" experience in the ongoing need for antibiotic discovery.

d. "Collaboration … we propose that group work is not only a common practical necessity but also an important pedagogical element of CUREs because it exposes students to the benefits of

bringing together many minds and hands to tackle a problem" (5). Students engage in group work throughout this semester-long research project. This provides students with opportunity to contribute and value each other's thoughts and abilities.

e. "Iteration…Students learn by trying, failing, and trying again, and by critiquing one another's work, especially the extent to which claims can be supported by evidence." A central element of this laboratory work is the necessity that students reproduce their results. Or, if not being successful at their first attempt (isolation of a *Streptomyces* from the soil, or extraction of sufficient and useable quantities of bacterial DNA) repeating their work until they achieve a result that allows them to proceed to the next phase of the work. Students understood this requirement and worked hard to achieve it.

4. Successful isolation and characterization of a soil *Streptomyces* by employing the techniques of microbiology and molecular biology. During several years of engaging students in this Lesson they have isolated and characterized approximately 15-20 soil-dwelling *Streptomyces*. The characterization has included observations at the colonial and cellular level, metabolic characteristics (enzyme activity and antibiotic production) and finally the genetic identification of their isolates using custom primers to the *Streptomyces* 16s rRNA gene for their polymerase chain reaction. This work has been documented by their written end-of-semester laboratory report and a presentation of their work to the University community during its annual undergraduate research day.

Modifications and Adaptations

Assessment of student ownership

Although I did not measure student ownership, there is research demonstrating the effectiveness that course-based research has in promoting student ownership in their work. To assess student commitment to the work, you may choose to provide students with an anonymous evaluation of the *Streptomyces* project. This is a useful tool for the instructor to assess the success of the Lesson and gain insight into the strong and weak points of undergraduate research. You can use the survey provided in the paper by Hanauer and Dolan: The Project Ownership Survey: Measuring Differences in Scientific Inquiry Experiences (3). Students responding in the Hanauer and Dolan survey commented on the benefit of both undergraduate course-based research and small group collaborative work as promoting their learning.

This positive outcome is additionally supported by Hunter A-B: Becoming a scientist: the role of undergraduate research in students cognitive, personal, and professional development (51). In this study, "faculty note when students begin to work independently, they take "ownership" of the research project, become more willing to think creatively, or make decisions about next steps in the research." Hunter A-B also report that undergraduate research positively affects students' "confidence to do research and contribute to science, the significance of building professional relationships with faculty and peers, and (a) shift in their identity and sense of belonging that they express as "feeling like a scientist." It would be interesting to discover if your teaching of this Lesson also resulted in similar positive outcomes. The Project Ownership survey may provide useful information in this regard.

Assessment/Moderate increase in course structure

A moderate increase in course structure can be accomplished with this Lesson by requiring short written assignments for all or most of the Lesson specific goals and objectives as a formative assessment tool. The students would benefit by arriving at the laboratory session already having written a short answer (paragraph or two) addressing the goal(s) and objective(s) specific to that laboratory. For example: Lesson specific goal (LAB 2): Students will understand the unique metabolic capabilities *Streptomyces* use to support their growth and survival. This assignment should be given to your students the week before the laboratory to which it applies. These assignments will be handed in and given a point value like a quiz. You can incorporate several of these assignments throughout the semester and therefore provide the students with an opportunity to both arrive at the laboratory with an increased understanding of that day's work and also a way to positively benefit their grade. A brief discussion by you of the goal/objective (after their work has been handed in) will give them immediate feedback on their work. You should return the assignment the next laboratory period and meet with your students either individually or with their working group each week to go over the assignment. The benefit of a moderate increase in course structure is formally supported by Eddy and Hogan's 2014 study, (6) Getting Under the Hood:…for Whom Does Increasing Course Structure work? "Overall, the major pattern associated with student achievement was that exam points earned increased under moderate structure relative to low structure and increased disproportionately for black and first-generation students."

Class size

The laboratory class I have taught has 12-16 students. This number of students required significant out of class instructor preparation time. The size of the class can certainly be increased above that. However, an increase in class size would require a considerable increase in out of class instructor preparation time, more than the already considerable time for a class of 12-16 students. Additional resources both hardware (thermal cyclers for example) and supplies/reagents would also be necessary.

Laboratory work

a. Due to the multistep nature of the investigation, the instructor can choose to complete the entire 12-week project during a single semester or just the first six labs. The first six laboratory exercises, (emphasizing basic microbiological techniques), can stand-alone and yield meaningful results. For example, the first 6 investigations, Actinobacteria introduction (LAB 1), proceeding to the isolation of suspected *Streptomyces* in pure culture, slide culture of their isolate, and determination of antibiotic production (LAB 6) can work as an independent investigation. The next time/semester teaching the laboratory, the instructor can again teach labs 1-6 and then proceed to the next six investigations (LAB 7 - LAB 12). These latter exercises emphasize molecular biology techniques. They involve growing the isolate in broth culture followed by DNA extraction, agarose gel electrophoresis, and the use of polymerase chain reaction to amplify 16s rRNA genes from their suspected *Streptomyces* isolate. This stepwise approach will allow the instructor to develop a deeper understanding of the project.

b. Measurement of bacterial growth phases by wet weight measurement. In this Lesson, students identify *Streptomyces* growth phases by identifying changes in morphological development. An adaptation to this method could be a time

course study using wet weight measurements of *Streptomyces* growth in broth culture. Students would employ aseptic techniques and careful measurement of culture wet weights. If this is done during the 5th or 6th week of the project students should be able to perform this modification of the Lesson. This would, however, require a considerable investment of time and well executed collaborative work. Samples of the broth culture would need to be taken over a 24-72-hour time period with agreed upon time points and uniform measurements of wet weight. This technique is well described and illustrated in the paper by Horinouchi (16). Refer to Figure 2 in Frontiers in Bioscience 7, 2002.

c. Isolation of bld mutants from student cultures. The developmental sequence of *Streptomyces* also provides an excellent way to screen for mutants that do not produce antibiotics. These isolates are described as "bald" bld mutants (52), as they do not produce aerial hyphae, the necessary branch or decision point that precedes the production of secondary metabolites. These isolates are different from isolates that do go through the usual developmental sequence but still do not produce antibiotics in vitro. Students could isolate these bld mutants from their cultures and confirm the absence of antibiotic production in those isolates that do not advance in their morphological development.

d. Use of PCR products from previous classes as additional controls for the next class's/year PCR. Your students' Polymerase Chain Reaction products (using primers to *Streptomyces* 16s rRNA genes) should be stored at -20 degrees centigrade after each semester's work. These products can then be used as additional controls during the current year's PCR work. This adds a "generational" aspect to the project and demonstrates to the students that their work will be part of this ongoing study.

SUPPORTING MATERIALS

- S1. Dirt to DNA-Handout Introduction to the Actinobacteria (LAB 1).
- S2. Dirt to DNA-Handout Serial dilution and spread plate technique (LAB 2).
- S3. Dirt to DNA-Handout Count serial dilution plates and subculture to ISP medium 2 (LAB 3).
- No Supporting file for LAB 4.
- S4. Dirt to DNA-Handout slide culture technique (LAB 5).
- S5. Dirt to DNA-Handout Check pure culture isolates for antibiotic action (LAB 6).
- S6. Dirt to DNA-Handout Grow suspected *Streptomyces* isolates in broth culture (LAB 7)
- S7. Dirt to DNA-Handout DNA extraction from broth culture of *Streptomyces* isolate (LAB 8).
- S8. Dirt to DNA-Handout Agarose gel electrophoresis of DNA extraction and UV scan (LAB 9).
- S9. Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10).
- S10. Dirt to DNA-Handout PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11).
- S11. Dirt to DNA-Handout *NCBI Nucleotide Blast (blastn) (LAB 12).
- S12. Dirt to DNA-Handout Laboratory Report Outline (LAB 13).
- S13. Dirt to DNA-Bacterial cultures, Media, Solution recipes, and Equipment.
- S14. Dirt to DNA-DNA extraction protocols instructor copy.
- S15. Dirt to DNA-Student Supporting Files LAB 1-12.

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Table 1. From Dirt to *Streptomyces* DNA teaching timeline.

Activity	Description	Estimated Time	Notes
Lab 1 Preparation for laboratory – Introduction to the Actinobacteria.			
Prepare handout on the Actinobacteria, and possibly all other student lab protocols. Have sterile 50 ml Falcon tubes ready to hand out in lecture one week prior to first lab meeting.	Make one copy of the Actinobacteria handout for each student in the class. Handout Supporting file S1: Dirt to DNA-Handout Introduction to the Actinobacteria (LAB1). Alternatively, you may choose to duplicate all student protocols at once using S15: Dirt to DNA-Student Supporting files LAB 1-12.	About 15 minutes to prepare 10 sets	Place your order with the American Type Culture Collection (ATTC) for *Streptomyces griseus subsp. griseus*. See Supporting file S13: Dirt to DNA-Bacterial cultures, Media, Solution recipes, and Equipment for ordering information. You will need a culture of *S. griseus* as a positive control throughout the semester. If you choose to use S15: Dirt to DNA-Student Supporting files LAB 1-12 this will relieve you of the need to duplicate student handouts each week.
Lab 1 – Introduction to the Actinobacteria.			
Lecture on the Actinobacteria. Inform students that they must maintain a detailed laboratory notebook throughout the semester. They will need this for their end-of-semester final laboratory report.	Use Supporting file S1: Dirt to DNA-Handout Introduction to the Actinobacteria (LAB1) as your lecture outline. Adapt the outline to reflect your emphasis, regarding the *Streptomyces*. Transfer aseptically collected soil samples to sterile petri dishes and place in 30-degree centigrade bacteriological incubator. Dry for one week.	Give a 45–60-minute lecture on the Actinobacteria. Allow 15-20 minutes for student groups to formulate their hypothesis regarding which of the three media (used next week) will yield the greatest number of *Streptomyces* colonies and the type of growth predicted for each type of media.	Instructor must cover the following major points regarding the *Streptomyces*. Colonial and cell morphology, metabolic characteristics including chitinase and antibiotic production, and genome size, large and linear unlike most other bacterial genomes. Upon arrival of *S. griseus* from ATCC start the culture on ISP 2 medium or T-Soy agar. ATCC includes detailed instructions for culturing.
Lab 2 Preparation for laboratory – Serial dilution and spread plate method.			
Serial dilution and spread plate method of the dried soil sample. Prepare media for this week's laboratory.	Make one copy of the serial dilution handout for each student in the class and handout Supporting file S2: Dirt to DNA-Handout Serial dilution and spread plate technique (LAB 2) prior to the laboratory session. Have laboratory support staff prepare media. Prior to the laboratory meeting prepare a diagram of a 10-fold serial (to 10^{-6}) dilution of the now dry soil sample.	About 20-30 minutes to prepare handouts Media preparation will take several hours and can be done a week in advance of the laboratory	Check drying soil sample, remove excess moisture from the lid prior to laboratory meeting. Prepare 9ml sterile water dilution blanks, 7 blanks per student. Prepare sterile T-Soy, Sabouraud, and Chitin Agar petri plates, one set per student. Have available 1ml (6/student) and 5ml (2/student) sterile pipets and pipettors. Have available spinners for the spread plate procedure, 1 for every 2 students. Also, one glass spreader/student and 30ml 70% EtOH per students for flaming glass spreaders.
Lab 2 – Serial dilution and spread plate method.			
Serial dilution and spread plate method of the dried soil sample. Re-streak ATTC culture of S. griseus on agar medium in preparation for laboratory next week.	Diagram a 10- fold serial dilution (to 10^{-6}) of the now dry soil sample. Students perform, their soil sample dilutions. Student groups present their hypotheses regarding growth on different bacteriological media.	About 60 minutes About 25 minutes	Use Supporting file S2: Dirt to DNA-Handout Serial dilution and spread plate technique (LAB 2) for your introduction. Describe BSL- 2 requirements to your students. The ATCC *S. griseus* culture will serve as a positive control for students to compare to their soil isolates next week. It may take 7 days for your culture to grow and be useable for LAB 3 so it is important to start the culture now.

Activity	Description	Estimated Time	Notes
Lab 3 Preparation for laboratory – Count spread plates and subculture suspected Streptomyces colonies.			
Students will count the number of colonies on each of the three types of agar media. Support staff will prepare media for Lab 3.	Make one copy of Supporting file S3: Dirt to DNA-Handout Count serial dilution plates and subculture to ISP medium 2 (LAB 3) for each student in the class and handout prior to the laboratory session Prepare 2-3 plates of T-Soy agar and ISP 2 agar per student for subculturing of soil isolates.	About 1-2 hours.	The majority of the suspected *Streptomyces* isolates will come from last week's chitin agar plates.
Lab 3 – Count spread plates and subculture suspected Streptomyces colonies.			
Students will count the number of colonies on each of the three types of agar media. Students will subculture suspected *Streptomyces* colonies.	Students will decide which of the dilutions to count. They will look for plates with between 30-300 colonies. Students will subculture suspected *Streptomyces* colonies onto ISP 2 medium or T-soy medium.	About 45-60 minutes	Use Supporting file S3: Dirt to DNA-Handout Count serial dilution plates and subculture to ISP medium 2 (LAB 3) for your introduction. Maintain BSL-2 requirements during this laboratory. Students will compare their suspected *Streptomyces* colonies to the positive control bacterium. It is important to note that the *Streptomyces* show great variation in their colony morphology and the student isolates will likely not look identical to the *Streptomyces* organism from the ATCC.
Lab 4 Preparation for laboratory – Re-streak isolated colonies from lab 3 to confirm isolation.			
Support staff will prepare media for this week's laboratory. Students will examine isolated colonies from previous week's work. Students will subculture again suspected *Streptomyces* colonies.	Prepare 2-4 plates each of T-Soy and ISP 2 agar per student for subculturing of suspected *Streptomyces* isolates. Prepare 2-3 T-Soy or ISP 2 agar slants for stock culture inoculation of suspected *Streptomyces* isolates. Prior to the laboratory meeting instructor should check student plates for their success in isolating *Streptomyces*.	About 2-3 hours for media preparation.	There is no supporting file for this laboratory. Examine student isolates for *Streptomyces*. Note those that have a colony morphology consistent with the *Streptomyces*.
Lab 4 – Re-streak isolated colonies from lab 3 to confirm Streptomyces isolation.			
Students will identify suspected *Streptomyces* isolates from the previous week's streak plate.	Instructor will confirm the identity of the isolated colony as a suspected *Streptomyces* isolate. Students will subculture well isolated *Streptomyces* colonies.	About 15-20 minutes	Students should maintain a stock culture of their isolate. This can be on a slant or agar plate of either T-Soy of ISP 2 agar.
Lab 5 Preparation for laboratory – Slide culture technique.			
Have your support staff prepare 2 slide culture sets for each student in your laboratory.	Make one copy of Supporting file S4: Dirt to DNA-Handout Slide culture technique (LAB 5) for each student in the class and hand it out prior to the laboratory session. Show Supporting file S4: Dirt to DNA-Handout Slide culture technique (LAB 5). to laboratory support staff to illustrate the correct set up for the slide cultures.	Preparation of slide cultures will require 1-2 hours of support staff time.	The slide culture sets must be autoclaved prior to use by the students.
Lab 5 – Slide Culture Technique.			
Students will inoculate their *Streptomyces* isolate onto a slide culture. Instructor will maintain an agar culture of ATCC *S. griseus* to demonstrate the slide culture inoculation and its production of antibiotics next week.	Instructor will demonstrate the slide culture technique using the ATCC *S. griseus* culture as a source of inoculum. It may also be helpful to show the students the YouTube video demonstrating the technique. Incubate the slide culture at 30 degrees C and observe daily at a magnification of 100-400x. Students record the results of their observations.	About 60 minutes	Use Supporting file S4: Dirt to DNA-Handout Slide culture technique (LAB 5) for your laboratory introduction. Remind students to use aseptic technique when making their inoculations.

Activity	Description	Estimated Time	Notes
Lab 6 Preparation for laboratory – Agar plug method for determination of antibiotic production.			
Have your support staff assemble 3-4 sets of the cork borers (#4), 70% alcohol, and 24-hour cultures of bacteria for the agar plug method. Support staff will prepare 2-3 plates per student of Mueller Hinton (MH) agar for Kirby-Bauer testing of antibiotic production.	Make one copy of Supporting file S5: Dirt to DNA-Handout Check pure culture isolates for antibiotic action (LAB 6) and handout prior to the laboratory session Instructor will demonstrate the agar plug method for detection of antibiotic production by student *Streptomyces* isolates.	Agar plug set up and media preparation will require approximately 2-3 hours of support staff time.	You or your support staff will prepare, one to two days in advance, broth cultures of *E. coli, S. epidermidis*, and *S. aureus* for use in the agar plug method. Have one set of cultures (2 ml of each bacterium) for every two students in your laboratory. Have available sufficient sterile cotton tipped swabs for production of a lawn of bacteria on MH agar.
Lab 6 – Agar plug method for determination of antibiotic production.			
Agar plug method for determination of antibiotic production.	Instructor will demonstrate the agar plug method for detection of antibiotic production using the ATCC *S. griseus* culture. Students will check their suspected *Streptomyces* isolates for antibiotic production within 24-48 hours after inoculation.	This will require approximately 60 minutes.	Use Supporting file S5: Dirt to DNA-Handout Check pure culture isolates for antibiotic action (LAB 6) for your introduction. Tell your students that they must employ aseptic technique throughout the process.
Lab 7 Preparation for laboratory – Grow suspected Streptomyces isolates in broth culture.			
Instructor is prepared to grow ATCC *S. griseus* in ISP1 medium. Support staff prepare media for the laboratory.	Make one copy of Supporting file S6: Dirt to DNA-Handout Grow suspected *Streptomyces* isolates in broth culture (LAB 7) for each student in the class and hand it out prior to the laboratory session Support staff prepare sufficient quantities of ISP 1 medium. Each student will need 3-4 tubes of the sterile broth culture.	Approximately 1 hour.	ATCC *S. griseus* will serve as a positive control for mycelial growth characteristic and future work on DNA extraction and PCR.
Lab 7 – Grow suspected Streptomyces isolates in broth culture.			
Students inoculate suspected *Streptomyces* isolates in broth culture in ISP 1 medium. Instructor inoculates ATCC *S. griseus* into ISP 1 medium.	Each student will need 3-4 tubes of the sterile broth culture to inoculate their isolates.	30 minutes	Use Supporting file S6: Dirt to DNA-Handout Grow suspected *Streptomyces* isolates in broth culture (LAB 7) for your introduction. Emphasize the importance of aseptic technique. Incubate student isolates and ATCC *S. griseus* in a shaking water bath for 5-7 days at 30 degrees C.
Lab 8 Preparation for laboratory – DNA extraction from broth cultures.			
Extraction of DNA from broth cultures of *Streptomyces* isolates.	The instructor will copy and handout (one week prior to this laboratory) the protocol for DNA extraction from student isolates. Each student will follow the protocol as outlined in Supporting file S7: Dirt to DNA-Handout DNA extraction from broth culture of *Streptomyces* isolate (LAB 8).	30 minutes	Remind students that this laboratory will require that they stay an additional hour after laboratory to complete the DNA extraction. Reagent preparation for this laboratory is detailed and is included as part of Supporting file S13: Dirt to DNA-Bacterial cultures, Media, Solution recipes, and Equipment. For your convenience there is a separate supporting file, (Supporting file S14: Dirt to DNA-LAB 8 DNA extraction protocols instructor copy), that contains only the work required of the instructor.

Activity	Description	Estimated Time	Notes
Lab 8 – DNA extraction from broth cultures.			
Extraction of DNA from broth cultures of *Streptomyces* isolates and ATCC *S. griseus*.	The day of the laboratory, the instructor will begin the DNA extraction of three student groups' isolate by following the directions in Supporting file S7: Dirt to DNA-Handout DNA extraction from broth culture of *Streptomyces* isolate (LAB 8), "Things to do before starting." Students begin their work at *3 of the Qiagen procedure. Each student group will follow the protocol as outlined in their handout.	5-6 hours	Use Supporting file S7: Dirt to DNA-Handout DNA extraction from broth culture of *Streptomyces* isolate (LAB 8) for your introduction. Instructor should perform the DNA extraction of the ATCC *S. griseus* prior to the student laboratory. This can be done well in advance of this laboratory. Extracted DNA can be stored at -20 degrees Centigrade for a considerable period of time.
Lab 9 Preparation for laboratory – Agarose gel electrophoresis of DNA extracted from broth cultures.			
Agarose gel electrophoresis of DNA extracted from broth cultures of student isolates and ATCC *S. griseus*.	The instructor will perform the electrophoresis as described in Supporting file S8: Dirt to DNA-Handout Agarose gel electrophoresis of DNA extraction and UV scan (LAB 9) prior to the next laboratory session.	Requires 2 hours outside of class. Work performed by the instructor.	You may want to duplicate Supporting file S8: Dirt to DNA-Handout Agarose gel electrophoresis of DNA extraction and UV scan (LAB 9) for your students. This is your decision based on whether you have them perform the electrophoresis themselves. The images in this file may be useful for your students' final laboratory report.
Lab 9 – Presentation of agarose gel electrophoresis of DNA extracted from broth cultures.			
Presentation of agarose gel electrophoresis of DNA extracted from student broth cultures and ATCC *S. griseus*.	The instructor will present scanned images of agarose gel electrophoresis as described in Supporting file S8: Dirt to DNA-Handout Agarose gel electrophoresis of DNA extraction and UV scan (LAB 9).	30 minutes	Use Supporting file S8: Dirt to DNA-Handout Agarose gel electrophoresis of DNA extraction and UV scan (LAB 9) for your introduction.
Lab 10 Preparation for laboratory – PCR of student DNA.			
Polymerase chain reaction using student isolated DNA and DNA from ATCC S. griseus with established primer sets to *Streptomyces* 16s rRNA gene.	The instructor will submit (one week in advance) a request to Eurofins Genomics LLC for primer sets #4 and #5. Directions for the request are described in Supporting file S9: Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10).	Approximately 60 minutes	Supporting file S9: Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10) contains the instructions for both ordering the primer sets for the PCR and the PCR handout/instructions for the students. Prior to duplicating the student section of Supporting file S9: Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10) insert student names for their Groups and individual responsibility during the laboratory session and hand it out to the students the week before the laboratory. If you used S15: Dirt to DNA-Student Supporting files LAB 1-12, at the beginning of the semester, that handout has no student assignments listed and you will need to have a separate handout for this laboratory.
Lab 10 – PCR of student DNA.			
Polymerase chain reaction using student isolated DNA and ATCC *S. griseus* DNA (as your positive control) with established primer sets to *Streptomyces* 16s rRNA gene.	Students will work in groups of 3-4 using Supporting file S9: Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10) as modified by the instructor with student responsibilities. Use the student DNA samples having the best yield (ng/ul) and demonstrating antibiotic production (if possible).	The entire laboratory period	Use Supporting file S9: Dirt to DNA-Handout Polymerase Chain Reaction (LAB 10) for your introduction. Equipment and reagent setup for this laboratory is detailed and is included in Supporting file S13: Dirt to DNA- Bacterial cultures, Media, Solution recipes, and Equipment.

Activity	Description	Estimated Time	Notes
Lab 11 Preparation for laboratory – PCR Product Sequencing of 16s rRNA gene at Genewiz.com.			
PCR Product Sequencing of 16s rRNA gene at Genewiz.com.	The instructor will submit student PCR products to Genewiz.com for sequencing. Directions for the request are described in Supporting file S10: Dirt to DNA-Handout PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11).	Five to seven days.	This file contains the instructions for the instructor regarding submission of student PCR products to Genewiz for sequencing. This process may take a week. Therefore, quickly submit PCR products from last week's laboratory so they are ready for LAB 11 work. Student handout should be copied from Supporting file S10: Dirt to DNA-Handout PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11) to facilitate your discussion of their results.
Lab 11 – PCR Product Sequencing of 16s rRNA gene at Genewiz.com.			
Instructor presents the PCR Product Sequencing data of 16s rRNA gene from Genewiz.com.	Instructor uses sequence data and trace files from Genewiz to explain the class results.	60 minutes.	Use Supporting file S10: Dirt to DNA-Handout PCR Product Sequencing of 16s rRNA gene at Genewiz.com (LAB 11) for your introduction.
Lab 12 Preparation for laboratory – NCBI Nucleotide Blast (blastn).			
NCBI Nucleotide Blast (blastn)	The instructor prepares a handout of the Genewiz data and the screen shots from Supporting file S11: Dirt to DNA-Handout NCBI Nucleotide Blast* (blastn) (LAB 12) explaining the use of the NCBI web site for the nucleotide blast.	60 minutes.	This file contains the instructions for the nucleotide blast at the NCBI web site.
Lab 12 – NCBI Nucleotide Blast (blastn).			
NCBI Nucleotide Blast (blastn)	The instructor explains the Genewiz data and the screen shots from Supporting file S11: Dirt to DNA-Handout NCBI Nucleotide Blast* (blastn) (LAB 12) illustrating the use of the NCBI web site for the nucleotide blast.	60 minutes.	Use Supporting file S11: Dirt to DNA-Handout NCBI Nucleotide Blast* (blastn) (LAB 12) for your introduction.