Curriculum
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Isolation and Characterization of Bacterial Cellulase Producers for Biomass Deconstruction: A Microbiology Laboratory Course †

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The conversion of biomass to biofuels presents a solution to one of the largest global challenges of our era, climate change. A critical part of this pipeline is the process of breaking down cellulosic sugars from plant matter to be used by microbes containing biosynthetic pathways that produce biofuels or bioproducts. In this inquiry-based course, students complete a research project that isolates cellulase-producing bacteria from samples collected from the environment. After obtaining isolates, the students characterize the production of cellulases. Students then amplify and sequence the 16S rRNA genes of confirmed cellulase producers and use bioinformatic methods to identify the bacterial isolates. Throughout the course, students learn about the process of generating biofuels and bioproducts through the deconstruction of cellulosic biomass to form monosaccharides from the biopolymers in plant matter. The program relies heavily on active learning and enables students to connect microbiology with issues of sustainability. In addition, it provides exposure to basic microbiology, molecular biology, and biotechnology laboratory techniques and concepts. The described activity was initially developed for the Introductory College Level Experience in Microbiology (iCLEM) program, a research-based immersive laboratory course at the US Department of Energy Joint BioEnergy Institute. Originally designed as an accelerated program for high-potential, low-income, high school students (11th–12th grade), this curriculum could also be implemented for undergraduate coursework in a research-intensive laboratory course at a two- or four-year college or university.

INTRODUCTION

At the Joint BioEnergy Institute (JBEI), we have demonstrated that a complex, open-ended, inquiry-based course can be appropriate and highly beneficial for students at an early stage of their scientific training. Over the past ten years, we have administered a program that targets an underrepresented segment of the future STEM workforce as a means to increase its diversity. Students targeted in this

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program include those from low-income households and underrepresented backgrounds who show high potential and an interest in education. The students are typically the first generation in their families to attend college, frequently come from minority backgrounds, and have a range of preparation from their respective high school courses. With this student population, we have found that early exposure to microbiology, biochemistry, molecular biology, and bioinformatics can be a formative early experience.

The Joint BioEnergy Institute is a Department of Energy Bioenergy Research Center managed by Lawrence Berkeley National Laboratory that has academic, national laboratory, and industrial partners (1). The mission of JBEI is to develop advanced biofuels and bioproducts from carbon stored in plant biomass that can serve as a replacement for gasoline, diesel, jet fuels, and petrochemically derived commodity chemicals (Fig. 1) (2, 3). To harness plant biomass, cellulose, which is the most abundant plant polymer in plant cell walls, must be deconstructed to form sugars such as glucose (4–7). This biomass can then be used to grow microorganisms engineered with the ability to biosynthesize advanced biofuels from the released sugars (8). To realize this goal of transforming biomass to biofuels, however, the first step is to devise strategies to break down cellulose and liberate the sugars that will feed the metabolism of these fuel-producing microorganisms. To biologically transform cellulose to sugar, a key component is the identification of candidate cellulases, enzymes that hydrolyze cellulose (9). Enzymatic transformation of the biomass to sugar is one of the most expensive parts of this process, and thus cellulase discovery is extremely important. Cellulase-producing organisms are commonly found in samples collected from soil or compost (10–12). During the course of this laboratory exercise, students isolate bacteria from samples collected from different environments, which they assay to determine the presence of cellulase activity. They characterize the cellulase activity and use bioinformatic analysis to identify the organism responsible for cellulase secretion. Unlike other biofuel-related lab courses that focus on the biofuel production host, fermentation, and/or other downstream aspects, this course provides a unique experience in understanding the upstream process of generating sugars from biomass (13, 14). This lab complements other biofuel-related courses and provides an understanding of how enzymatic cellulase biotechnology fits within the entire biofuel production pipeline. This activity ties together a breadth of laboratory skills in addition to relaying the interplay between microbiology, environmental science, and sustainability. While other inquiry-based labs focus on teaching the overall general laboratory skills, here we aim to bring focus to environmental and sustainable bioresearch. With the increasing interest in developing sustainable technologies for the production of biofuels, bioproducts and other important chemicals, the presented inquiry-based lab course provides a unique focus intended to broaden scientific laboratory skills while supporting research in sustainable and environmental technologies.

Intended audience and prerequisite student knowledge

This course is currently being offered as an immersive, advanced eight-week summer laboratory program for high school students. The course is organized much like an introductory undergraduate course, with a level of rigor that would make it highly appropriate as a course for undergraduate students at two- or four-year colleges and universities with adequate laboratory facilities. Prerequisites for the iCLEM program, and thus the course, include high school biology and algebra I, with chemistry and algebra II highly recommended. A basic understanding of the central dogma of biology, microbiology, and the chemical elements is important to understand the laboratory experiments presented in the course. Algebra is useful for generating sample standards and graphs plotted in excel or other similar programs. As currently administered, instruction is provided to ensure that students have an adequate background in the scientific method, sterile technique, preparation of solutions, and use of basic laboratory equipment, such as micropipettes and microcentrifuges. Students should have a fundamental understanding of the skills and concepts outlined above. Because this program was originally developed to meet the needs of students with varying levels of preparation, it can be tailored to a broad range of students. One of the largest

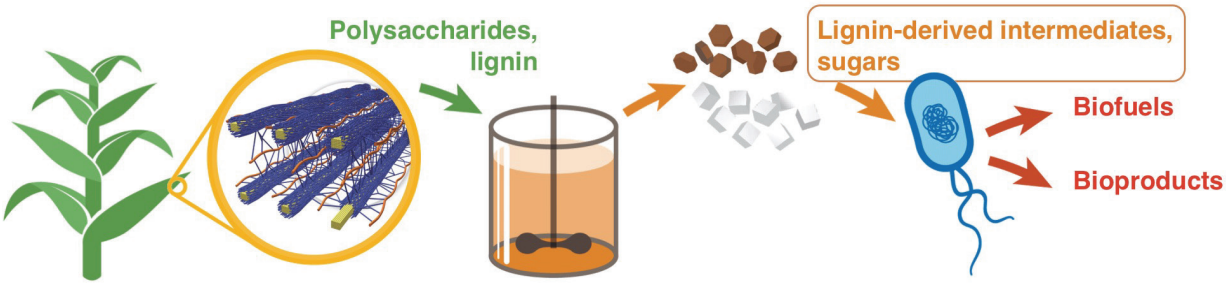

FIGURE 1. Biomass to biofuels and bioproducts pipeline. The activity described falls under deconstruction, the process of discovering enzymes that break down polysaccharides to form sugars and lignin-derived intermediates (indicated by orange text and box) that can be metabolized by bacteria to generate petrochemical replacements.

changes for adapting the program to a two- to four-year university course would be removing some of the basic information regarding laboratory techniques that might have been covered elsewhere.

Learning time and learning objectives

Although originally designed as an immersive program, this curriculum can be modified to be used over a typical 14-week academic semester. The major goal is to isolate environmental bacteria identified as having cellulase activity, assay the isolates' cellulase activity, and identify which species these isolates are by amplification and sequencing of 16S rRNA genes, which identifies the phylogenetic affiliation of the isolate. The learning objectives are set out in Table 1.

PROCEDURE

Materials and student instructions

A detailed list of all required materials for the course (including media, reagents, solutions, and protocols) is provided in the laboratory manual. An example Excel sheet for analysis of the dinitrosalicylic acid (DNS) assay is also included (Appendix 6). As a final project, this activity should require a summary of the results, either through a short oral presentation and/or an exam. We have included assessment questions, answer keys, and a rubric in Appendix 7. These can be used to draw inspiration for quizzes and other evaluative assignments.

Faculty instructions

Suggestions for faculty are included mainly in Appendix 2 and throughout the teaching manual. One major recommendation when time is an issue would be for instructors to monitor cultures independent of the lab class cycles to ensure they are growing at an appropriate rate for the activity in between scheduled lab courses.

Briefly, the requirements of the laboratory curriculum include biosafety level 2 (BSL2) recommended facilities, 30 and 35 degree (centigrade) shaking incubator space, equipment compatible with standard molecular biology manipulation (pipettors, thermocyclers, gel electrophoresis), multichannel pipettes, UV-visible absorbance readers, and standard microbiology equipment (e.g., inoculation loops, bunsen burners for sterile technique, centrifuges, petri dishes).

Suggestions for determining student learning

The manual includes questions that probe the students' conceptual understanding of the material outlined. They can be found in Appendices 7 and 8. These questions can be adapted for weekly laboratory reports or used in pre- or post-activity quizzes or tests. For a college course, these questions can be used in an evaluative way, whereas they were used in a non-evaluative way in the initial course due to its structure. We strongly recommend these questions be drawn upon for designing evaluative assessments such as tests and quizzes. Additionally, we believe this course would be highly appropriate for majors in microbiology or molecular biology.

Sample data

Sample data for the DNS assay are included in the Appendix 6.

Safety issues

It is strongly recommended that BSL2-certified facilities be used for this activity, including all standard BSL2 precautions, such as storage of isolates and waste requisitioning, because the students isolate and work with unknown organisms. Prior to this activity, the students should be familiarized with ASM (American Society of Microbiology) Biosafety Guidelines and the safe handling of samples and other laboratory resources (15). Furthermore, to ensure they have the skills and knowledge needed to work with their unknown samples, the students need to show that they are competent to work with BSL1 organisms (Escherichia coli K12). Appropriate institution-specific biosafety training should take place prior to starting the laboratory course. Because of the unknown nature of the isolates, biosafety

TABLE 1.
Module learning objectives and methods of assessment.

Learning Objectives At the end of this activity, students will be able to:	Assessment Method
1. Describe the role of biomass deconstruction in the biofuels pipeline.	Pre/Post assessment or exam
2. Describe important microbiology and molecular biology concepts.	Pre/Post assessment or exam
3. Apply the scientific method to organize, collect and analyze data.	Worksheets and/or exam
4. Communicate scientific concepts confidently and display confidence in their scientific abilities.	Pre/Post assessment or exam or final group presentation

should be strongly emphasized throughout the laboratory activities. There is no creation of an organism presented in this manual that presents risk for human health, and the risk of encountering a BSL2 level organism is low, but adequate precautions should be taken nonetheless.

The DNS assay reagent described in Section 2.1 of Appendix I contains phenol. Students should be made aware of chemical burn risk, and the reagent waste should be appropriately requisitioned.

DISCUSSION

Field testing

This activity has been field tested through 10 years of the iCLEM Program at the Joint BioEnergy Institute with 10 different cohorts of eight high school students. The high schools that the students came from (all in California) include: Oakland Technical High School (Oakland), Berkeley High School (Berkeley), Richmond High School (Richmond), Mount Eden High School (Hayward), Lowell High School (San Francisco), Oakland High School (Oakland), Skyline High School (Oakland), Lighthouse Community Charter School (Oakland), San Leandro High School (San Leandro), Irvington High School (Fremont), Pinole High School (Pinole), Middle College High School (San Pablo), Oakland Unity High School (Oakland), Salesian High School (Richmond), Marshall High School (San Francisco), Vista High School (Richmond), Bunche High School (Oakland), East Oakland School of the Arts (Oakland), El Cerrito High School (El Cerrito), Oakland Charter High School (Oakland), Emery Secondary School (Emeryville), American Indian Public High School (Oakland), Lionel Wilson College Prep (Oakland), Mission High School (San Francisco), Lincoln High School (San Francisco), Life Academy (Oakland), San Francisco International High School (San Francisco), Hercules High School (Hercules), Realm Charter School (Berkeley), Galileo Academy of Science and Technology (Galileo), Kennedy High School (Richmond), Castlemont High School (Oakland), Wallenberg High School (San Francisco), McClymonds High School (Oakland), Envision Academy of Arts and Sciences (Oakland), Making Waves Academy (Oakland), and Concord High School (Concord). Data for this publication were collected through standardized surveys in the 2017 and 2018 cohorts.

Among the 58 high school students trained in this program, 98% of the students are from low-income underrepresented minority households; 81% are first-generation college students. A full 98% of student participants to iCLEM have continued their education at two- or four-year colleges and universities, and 80% have majored in science or engineering. National data for students with similar backgrounds demonstrate that only 45% are likely to attend college.

Evidence of student learning

In terms of assessing student learning, students showed improvement in conceptual understanding of the learning objectives (Table 2). In assessing the first learning objective, describing the role of biomass deconstruction in the biofuels pipeline, students improved from 56% to 72% (a 16% increase). Student ability to explain important microbiology and molecular biology increased from a weighted average of 67% to 94% (a 27% increase). In assessing learning objective 3, students are required to keep an organized and updated notebook, where all data are recorded. Furthermore, students are required collect, analyze, and interpret their own data. This is evident in the DNS assay (Section 2.1), where students are required to collect data, generate standard curves, and analyze and interpret data. A more detailed rubric for learning assessment of objective 3 can be found in Appendix 7.

For learning objective 4, the students in the 2017 and 2018 cohort demonstrated an increase in confidence in their own laboratory skills from a weighted average of 3.2 to 4.2 (a 24% increase) on a scale of 1 to 10, with 1 being the least confident and 10 being the most confident (Fig. 2). Their confidence in explaining science concepts to family increased from a weighted average of 3.9 to 4.3 (an 8% increase), and their ability to explain the importance of their research to peers increased from a weighted average of 3.9 to 4.6 (a 15% increase).

Unexpected outcomes

Unexpected outcomes in various sections of the laboratory can arise, depending on the context of the course. It is important to be as clear as possible to obtain the desired outcome. For example, we asked students to bring "environmental" samples that potentially contain cellulase-producing organisms. Most students brought environmental samples from the kitchen (e.g., fruit, legumes, rice) and soil or leaves from near their home. We would encourage a brief lecture prior to sample collection on the diverse environments where cellulase-producing organism can be collected (e.g., soils, ponds, bark, etc.), as some of the kitchen samples (e.g., rice) were unlikely to yield substantial amounts of cellulase-containing bacteria.

Suggested modifications

Although this course was initially developed as an accelerated college-level course for high school students, it should be relatively easy to adapt the manual to suit the needs of larger lab sections and the level of students at two- to four-year colleges and universities, especially for an introductory microbiology course. The following are suggested modifications:

- Coordinating and consulting with academic experimental instructors/coordinators to check project

TABLE 2.
Student Learning Assessment[a].

Assessment	Correct answers (pre-assessment)	Correct answers (post-assessment)
1. Describe the role of biomass deconstruction in the biofuels pipeline.	56.25%	71.88%
2. Describe important microbiology and molecular biology concepts.	66.96%	93.75%

[a] See Appendix 8 for full survey questions

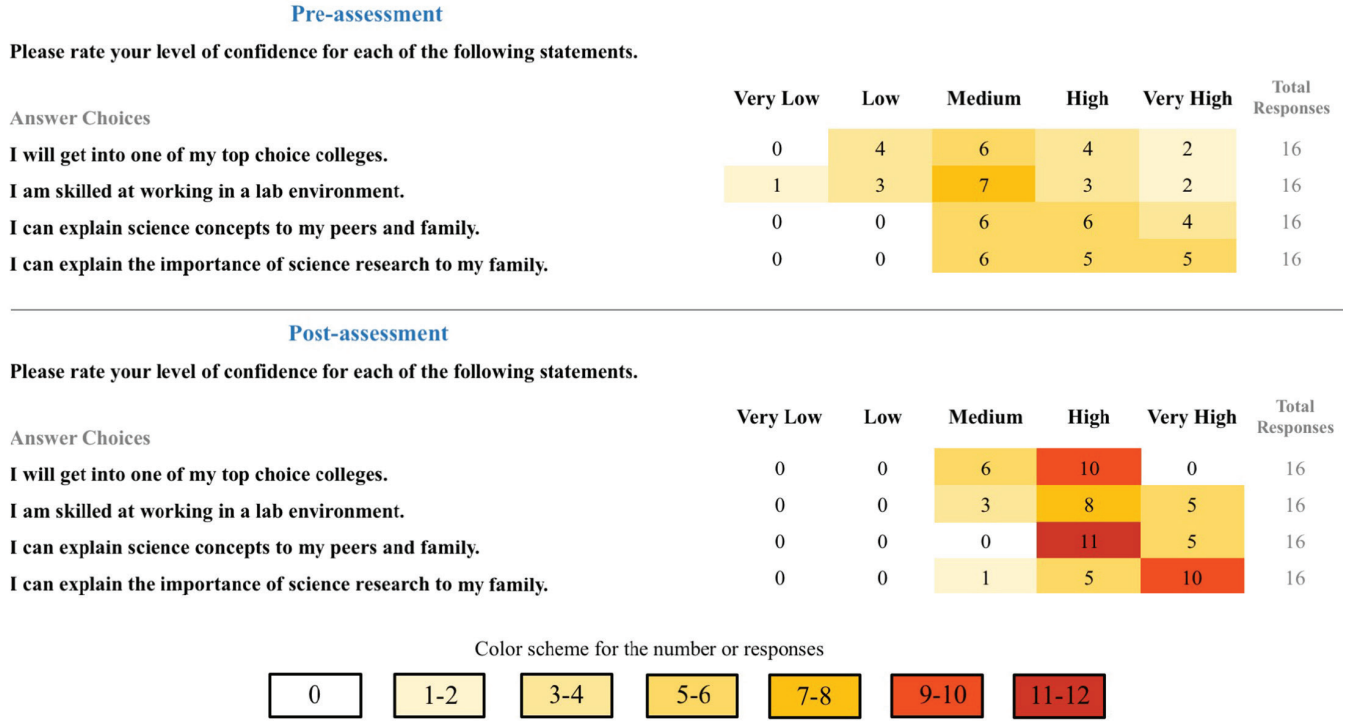

FIGURE 2. Student confidence assessment for learning objective 4 (see Table 1). Survey was on a scale of 1 to 10, with 1 being the least confident (Very Low) and 10 being the most confident (Very High).

feasibility and assess laboratory resources. In particular, ensuring that all BSL2 lab resources are current and in place.

- Have instructors check cultures and remove plates from the incubator on days when a lab class is not scheduled, so that successive days are not required.
- Some of the additional basic laboratory instruction is likely unnecessary in a more advanced course setting. We recommend consulting with academic experimental instructor/coordinators to inspect student support.

SUPPLEMENTAL MATERIALS

Appendix 1: List of reagents and instrumentation
Appendix 2: Faculty instructions
Appendix 3: Student handouts and protocols
Appendix 4: Recipes and detailed protocols
Appendix 5: Glossary and additional references
Appendix 6: Worksheets and templates
Appendix 7: Recommended exam questions and rubric
Appendix 8: Module pre-/post-assessment questions and rubric

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