Curriculum

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Hybrid Inquiry-Based Laboratory Curriculum Highlights Scientific Method Using Bacterial Conjugation as a Model

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Undergraduate microbiology students are exposed to the theory of the scientific method throughout their undergraduate coursework, but laboratory course curricula often focus on technical skills rather than fully integrating scientific thinking as a component of competencies addressed. Here, we have designed a six-session inquiry-based laboratory (IBL) curriculum for an upper-level microbiology laboratory course that fully involves students in the scientific process using bacterial conjugation as the model system, including both online discussions and in-person laboratory sessions. The student learning objectives focus on the scientific method, experimental design, data analysis, bacterial conjugation mechanisms, and scientific communication. We hypothesized students would meet these learning objectives after completing this IBL and tracked student learning and surveyed students to provide an assessment of the structure of the IBL using pre- and post-IBL quizzes and the Laboratory Course Assessment Survey. Overall, our results show this IBL results in positive student learning gains.

KEYWORDS conjugation, hybrid, IBL, inquiry-based laboratory, microbiology, scientific method, curriculum, laboratory, undergraduate

INTRODUCTION

Many undergraduate microbiology laboratory courses lack curricula that emphasize critical thinking skills and encourage students to participate in the scientific method. Traditionally, these courses implement "cookbook-style" laboratory exercises focused on content-driven curricula. While this approach is useful to generate reliable data, teach technical skills, and demonstrate important scientific principles, it lacks full integration of the scientific method and student participation in the experimental process. To face this challenge, laboratory courses are beginning to integrate inquiry-based labs (IBLs), which involve students in the experimental design process (1, 2). IBLs are designed to mirror authentic scientific research from developing the question and hypothesis, to evaluating data, to replicating experiments. This student-driven style of education is designed to increase student engagement and cognitive skills as a part of the educational outcomes (3).

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In addition to this curriculum challenge, the use of hybrid labs, where some or all content is presented using an online platform, is increasingly being used to combat limited resources, increased enrollment numbers, finite available classroom space, and limited funding (4, 5). The 2019 coronavirus disease (COVID-19) pandemic hastened the need for this type of innovation when in-person classes were rapidly transitioned to online platforms due to public health restrictions (6). Even with the availability of COVID-19 vaccines, continued restrictions resulting from variant outbreaks and lack of herd immunity continue to strain laboratory course resources. While this curriculum was developed initially with the COVID-19 restrictions in place, other emerging diseases, severe weather or climate conditions, and other unanticipated events could warrant similar hybrid laboratory class options.

To address these needs, we have designed a hybrid IBL curriculum for a pathogenic microbiology course using the *Enterococcus faecalis* peptide pheromone-induced conjugation system (7) to guide students through the scientific method from development of hypothesis, design of experimental procedure, analysis of results, and reproducibility of the experiment. By including both in-person and online teaching environments, we have relieved some of the pressures of lab space needed, as well as offering an online format that is more accessible and conducive to inclusive active discussions (8).

The *E. faecalis* conjugation model is an effective model for this IBL, as the technical simplicity allows students to explore external factors that may impact conjugation efficiency. The

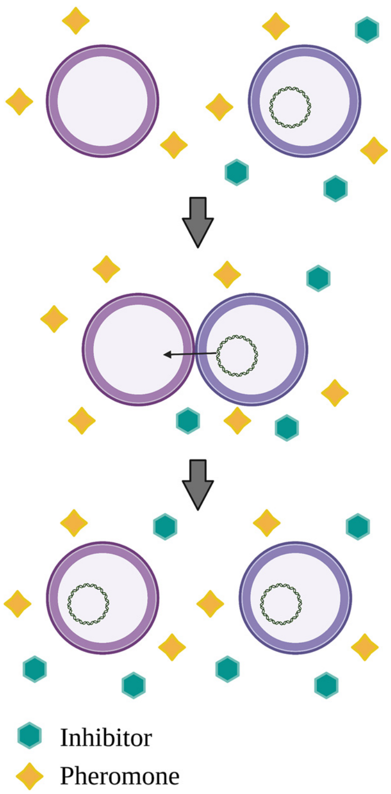

FIG 1. Plasmid-bearing donor *E. faecalis* strains (right) and plasmid-free recipient strains (left) both produced peptide pheromones (diamonds) that induced conjugation. Inhibitor peptides (hexagons) were produced by the donor strains. Conjugation can occur when the ratio of peptide pheromones exceeds inhibitor peptides and results in transfer of the plasmid. (Figure was adapted from reference 10 with permission of the publisher [Copyright 2005 National Academy of Sciences, USA] and created with BioRender.com)

procedure for mating is straightforward: students incubate donor and recipient strains and select for transconjugants based on antibiotic resistance genes found on the plasmids and chromosomes. Bacterial conjugation is also a critical concept for undergraduate microbiology education, as it is an important mechanism of horizontal gene transfer for virulence factors, such as antibiotic resistance genes (9). A summary illustration of this system, adapted from reference 10, is shown in Fig. 1. In this model system, the transfer of a conjugative plasmid (pCF10) bearing antibiotic resistance genes from a donor strain to a plasmid-free recipient strain is regulated by the ratio of conjugation pheromones and inhibitor peptides produced by the mating pairs. The chromosomally encoded sex pheromones are produced by both the donor and plasmid-free recipient cells and induce the expression of plasmid-encoded conjugative genes required for transfer of the pCF10 plasmid. This includes the expression of proteins on the cell surface of the donor and recipient cells that aid in the aggregation of cells required to form a mating pair and transfer of the plasmid. Donor cells, which contain pCF10, also produce an inhibitor peptide that prevents unnecessary expression of conjugation-specific genes when there are limited numbers of recipient cells present (11). In essence, conjugation between donors and recipients can only occur when the conjugative pheromones overcome the available inhibitor peptide. Previously, a Tips and Tools publication highlighted the usefulness of conjugation experiments (albeit with a different model system) in the teaching of the scientific method (12). However, that article described a smaller-scope curriculum taught solely in person and lacked assessment of student learning. Here, we present a fully developed IBL which utilizes a hybrid format of both in-person and online platforms. Further, we monitored student progress through the IBL to demonstrate successful outcomes in student learning.

Intended audience and prerequisite student knowledge

This IBL was designed for an upper-level pathogenic microbiology laboratory course with students who had previously taken Introduction to Microbiology lecture and laboratory courses.

Learning time and student learning objectives

The IBL timeline (Table 1) covered six 3-h sessions spanning 3 weeks and included time for students to observe results outside of class time. This schedule includes an optional medium preparation session (session 3), which could be eliminated if medium is prepared by the teaching team.

We designed our IBL with 6 key student learning objectives (SLOs), listed here. After performing the IBL, students should be able to:

1. Collaborate to develop a hypothesis statement.
2. Design and perform an experimental procedure to test a hypothesis statement.
3. Analyze conjugation data to determine experimental outcome.
4. Evaluate experimental design for alternative procedures.
5. Communicate scientific data in oral presentation format.
6. Peer-evaluate oral scientific presentations using a provided rubric.

PROCEDURE

Our first SLO focused on student collaboration to develop a testable hypothesis. Prior to the first online session, students were expected to review the provided reading materials, watch provided iBiology videos (https://www.ibiology.org/) and prerecorded lecture on the conjugation model and, finally, develop a specific question about bacterial conjugation efficiency (see Appendices S1 and S2 in the supplemental material). During the first online meeting, students were divided into breakout rooms according to the lab section they were enrolled in, and groups determined which student's question to pursue. They then collaborated to create a

TABLE 1
Timeline of IBL

Session no.	Location	Purpose	Prior to class, students will:	During class, the moderator will guide students to:
1	Online	Zoom breakout groups will discuss the scientific method, develop a question about the conjugation model, design a hypothesis, and then present the hypothesis to the lab group for discussion. Teaching team facilitates discussions.	• Read parts 1 and 2 of the Experimental Design handout • Complete Worksheet 1, The Scientific Method • Watch iBiology videos: Experimental Research Design, Experimental Variables, Control Groups, and Replicates in an Experiment, and Don't Be Wed to your Hypothesis	• Choose a variation of the experiment to perform and discuss the hypothesis • Work on Worksheet 2, Hypothesis Development, and submit before session 2
2	Online	In breakout rooms, students will work on designing the experiment based on the standard protocol for conjugation of *E. faecalis*. Teaching team will facilitate discussions.	• Read part 3 of the Experimental Design handout • Complete Worksheet 3, Methods Design	• Discuss the materials and methods required for the proposed experiment • Work through Worksheet 4, Experimental Protocol, during class and complete prior to session 3
3	In lab	This is an optional in-lab session where students work to prepare media needed for the experiment. Alternatively, the teaching team can prep and provide the media.		• Make BHI media, pour plates, and prepare any other media needed for the experiment • Begin planning poster design
4	In lab	Students perform their designed experiments.		• Perform the experiment and continue working on the presentation design • After class (outside of class), collect and record the results prior to session 5
5	Online	Teaching team will facilitate data analysis discussions. Students will discuss results and propose needed modifications to the protocol.	• Read parts 4 and 5 of Experimental Design handout • Complete Worksheet 5, Data analysis	• Work on Worksheet 6, Protocol Modification, and complete prior to session 6
6	In lab	Students will repeat the experiment with any modifications discussed in session 5.	• Repeat experiment according to revised protocol and continue working on poster design • Collect and record results (outside of class)	• Submit a recording of their poster presentation • Peer-evaluate presentations

strong hypothesis statement based on this question. This discussion was facilitated by the graduate teaching assistants (GTAs) using the provided worksheet (Appendix S3). The breakout rooms then reconvened as a class in the main online room, where students were able to critique hypothesis statements of other lab groups and offer suggestions or comments. Table S1 (and Appendix S9) shows examples of student-generated hypothesis statements.

The second online meeting focused on SLO 2: designing an experimental procedure to test the developed hypothesis. Students were expected to read the provided handouts (Appendix S4) prior to class. GTA-led discussions guided students in developing an experimental procedure based off a general protocol for conjugation provided ahead of time (Appendix S5). During the second online meeting, students were expected to prepare a list of materials and media needed for their experiments.

The third session was an in-lab session where students were taught to make media, calculate antibiotic concentrations, pour plates, and prepare materials for the conjugation experiment. This session could be considered an optional session and media could be provided by the teaching staff, if time or room constraints are a concern.

Students performed their experiments during in-lab session four. Students worked in small teams (2 to 3 students), and each lab section (or lab room) performed the same experiment. This allowed for replicate data to be compared, although it is important to discuss the caveats of these replicates. Students collected their results outside of the scheduled class time and reported results to the group in online session five, which focused on SLOs 3 and 4: data analysis and evaluation of experimental design. If the lab space is unavailable for students between sessions, GTAs can record results and share these with the students prior to the online session. During the GTA-led breakout sessions, students discussed and interpreted their data, evaluated the experimental design components, and created alternative procedures to improve the experiment if needed (Appendices S6 and S7). Students repeated or performed a modified protocol during in-lab session six. An example of a student workflow (tested student hypothesis, protocol, and results) is shown in Fig. S1 (Appendix S10).

At the conclusion of the IBL, students created recorded video presentations of their projects and uploaded the presentations to the campus Learning Management System (LMS) for peer evaluation. Students were required to evaluate two presentations using a rubric-based critique (Appendix S8). In this portion of the module, we focused on SLOs 5 and 6: communicating and evaluating science in an oral presentation format.

Materials

Students require the following materials:

- A stable Internet connection and computer or mobile device with access to the Zoom application (alternative online platforms such as Microsoft Teams could also be used);
- Access to presentation software with the ability to record audio and video (e.g., PowerPoint, QuickTime, Kaltura);
- Access to the school's LMS (e.g., Blackboard or Canvas) to upload their recorded presentations, although public platforms such as YouTube are alternatives; and
- Basic laboratory supplies, cultures, and equipment needed for the conjugation procedure (Appendix S1).

Student instructions

A detailed outline of student expectations is presented in the timeline table (Table 1). Students work individually on preparing content for the online class discussions. Students can work individually or in teams for the in-person implementation of the experiment. Detailed student instructions can also be found in Appendices S1 to S7.

Lab notebooks

Students were expected to maintain a lab notebook. We used Microsoft OneNote as a digital lab notebook between lab partners. This allowed lab partners to record and share data among their group and to track updates, progress, and results.

Project presentation

The students were provided with a rubric (Appendix S8) to acquaint them with expectations for their project presentation. GTA or faculty feedback was facilitated during the project to guide their work on their PowerPoint presentations. Students were provided with rubrics to critique and evaluate two peer presentations to introduce them to the concept of peer review in the scientific process. Peer evaluation criteria were provided as previously described (13).

Faculty instructions

Instructors should assign students to small teams for the IBL. The instructor should prepare a basic lecture on the *Enterococcus faecalis* model to help students understand the concept of bacterial conjugation. A review of the basic components of experimental design should be prepared or the referenced iBiology videos can be used as an alternate to a prepared lecture (Appendix S1).

Suggestions for determining student learning

Student learning was assessed by monitoring progress on a pre- and post-IBL quiz which contained content-based questions. The quizzes also included a survey where students were asked to self-assess their learning (Appendix S11). Student perceptions of the characteristics of the IBL were determined with a modified Laboratory Course Assessment Survey (LCAS) at the end of the IBL (Appendix S12) (14). The results of the quizzes and survey are shown in Fig. 2 and Table 2.

Sample data

Student-generated hypotheses for the IBL are shown in Table S1 (Appendix S9). An example of a student-developed and -tested hypothesis with the experimental protocol and results is shown in Fig. S1 (Appendix S10).

Safety issues

Students and faculty should be trained in biosafety level 2 safety techniques prior to starting this IBL. Handling of human pathogens should be performed under regulatory guidelines put forth by the American Society of Microbiology Guidelines for Biosafety in Teaching Laboratories (https://asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-

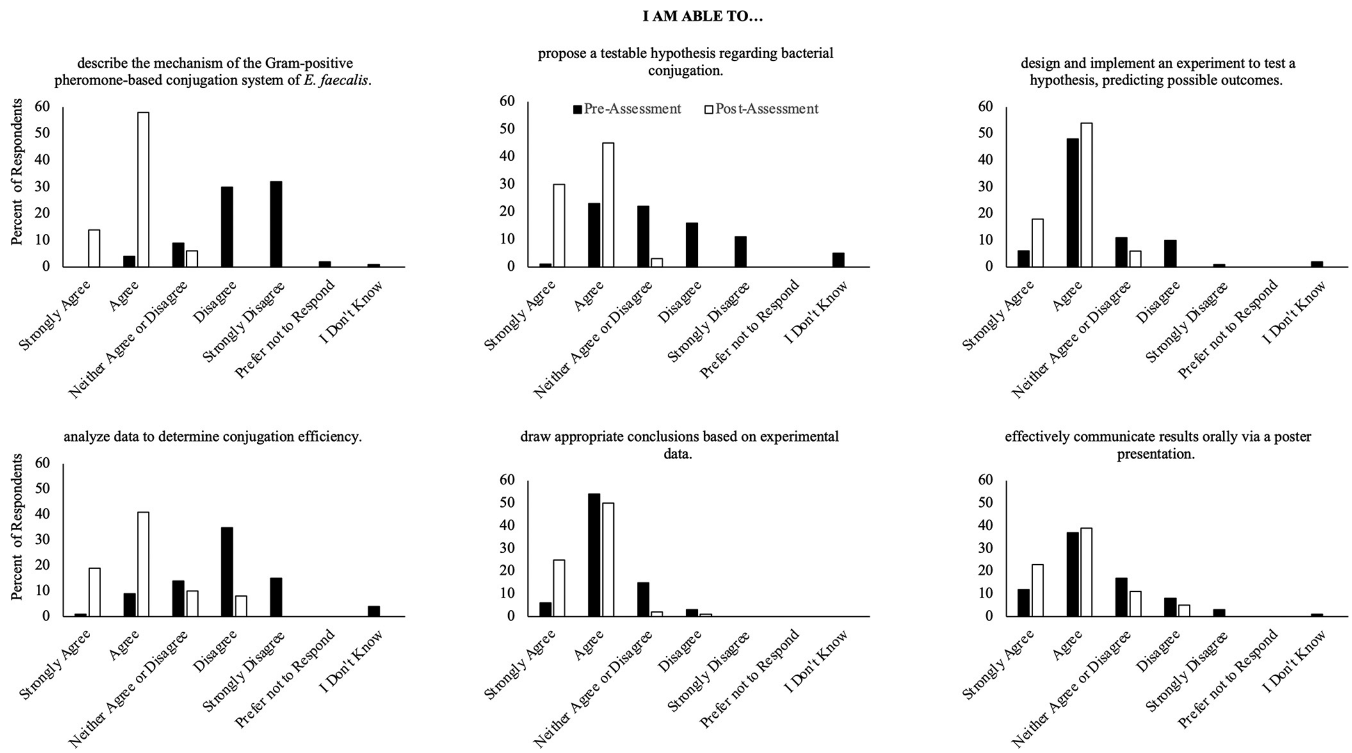

FIG 2. Student agreement to statements examining confidence in student learning objectives before and after completing the IBL. Student responses for the Spring 2021 and 2022 semesters were combined.

Laborator) and include wearing personal protective equipment (glasses, gloves, laboratory coat).

This study was approved by the University of Kansas Institutional Review Board as STUDY00146872, and subjects' informed consent to use data was obtained.

RESULTS AND DISCUSSION

Field testing

This project was developed and field tested over two semesters of an upper-level undergraduate laboratory course (Bacterial Infectious Disease Laboratory) designed for students with a prerequisite introductory microbiology laboratory course at the University of Kansas. A total of 78 students participated in this IBL during the spring semesters of 2021 (30 students) and 2022 (48 students). This course was taught by one faculty member, two graduate teaching assistants, and two (2021) or one (2022) undergraduate teaching assistants.

Evidence of student learning

To determine the effectiveness of this IBL, students participated in pre- and post-IBL assessments (Appendix S11). The pre- and post-IBL quizzes included a six-question self-assessment survey as well as content-based questions that were evaluated for accuracy. This approach allowed for evaluation of student learning in two key ways. First, we measured students' self-assessments of their ability to meet student learning objectives prior to and upon completion of the IBL. Second, we measured their improvement on SLOs with the content-linked questions.

The results of student self-assessment of learning objectives from the two semesters were combined and are shown in Fig. 2. Student's overall confidence improved in all the SLO-linked statements after completion of the IBL, with largest gains in confidence seen in describing the conjugation mechanisms, proposing a testable hypothesis, and calculating conjugation efficiency. Prior to the IBL, students had high confidence in their abilities to design and implement an experimental protocol, draw conclusions from experimental data, and communicate data in an oral presentation, although gains were seen in these areas as well. Analysis of the pre- and post-IBL quiz results showed a statistically significant increase in mean percent quiz scores of 19.8 to 20% ($P < 0.001$) over both semesters tested (Fig. 3). The quiz questions were specifically aligned to IBL learning objectives, and the increase seen in student learning agreed with the increase in overall confidence shown in the survey results (Fig. 2).

We used the LCAS (14) to provide an evaluation of the structure and characteristics of the class. The LCAS is designed to evaluate the approach of laboratory courses; courses that are more traditional "cookbook-style" laboratories would have lower scores, while more inquiry-based laboratory courses, such as course-based undergraduate research experiences, would have higher scores. By using the LCAS to survey students about (i) what they were encouraged to do, (ii) asked to

TABLE 2
Results of the LCAS

Category and statement	Mean	SD
In this module, I was encouraged to . . .		
Discuss elements of my investigation with classmates or instructors	5.63	0.56
Reflect on what I was learning	5.57	0.57
Contribute my ideas and suggestions during class discussions	5.63	0.67
Help other students collect or analyze data	5.37	0.72
Provide constructive criticism to classmates and challenge each other's interpretations	5.47	0.63
Share the problems I encountered during my investigation and seek input on how to address them	5.57	0.77
In this module, I was expected to . . .		
Generate novel results that are unknown to the instructor that could be of interest to the broader scientific community or others outside the class	4.50	1.11
Conduct an investigation to find something previously unknown to myself, other students, and the instructor	4.67	1.52
Formulate my own research question or hypothesis to guide an investigation	5.47	0.68
Develop new arguments based on data	5.17	0.95
Explain how my work has resulted in new scientific knowledge	4.80	1.13
Revise or repeat work to account for errors or fix problems	5.53	0.57
In this module, I had time to . . .		
Change the methods of investigation if it was not unfolding as predicted	5.27	0.83
Share and compare data with other students	5.53	0.57
Collect and analyze additional data to address new questions or further test hypotheses that arose during the investigation	4.80	1.24
Revise or repeat analyses based on feedback	5.27	0.83
Revise drafts of papers or presentations about my investigation based on feedback	4.93	0.94

do, and (iii) provided time to do (Table 2), we gained a better understanding of the degree to which this lab incorporated inquiry-based learning and three common themes of inquiry-based laboratories and course-based undergraduate research experiences: collaboration, discovery and relevance, and iteration. Survey results were reported on a six-point scale, with a score of 6 indicating that students strongly agreed and a 1 indicating strong disagreement (Table 2). In Table 2, we have grouped results for survey questions into the three categories, and students overall found this lesson to incorporate the three themes of collaboration, discovery and relevance, and iteration. Students were most in agreement that they were asked to "discuss elements of (their) investigation with classmates or instructors" (5.63 ± 0.56 [mean ± standard deviation]) and to "contribute (their) ideas and suggestions during class discussions" (5.63 ± 0.67). Overall, scores were highest for those related to collaboration, suggesting that this lesson was strong in this area. In the future, instructors could focus on helping students understand the novelty and value of their work, as questions related to discovery and relevance had the lowest mean scores.

Finally, this IBL teaches students about the important role scientific communication and peer review play in the scientific process by asking students to both create a presentation of their results and critique presentations of their peers. Rubric-guided evaluations (Appendices S8 and S9) were used as previously described (13). The rubrics evaluated students' presentation skills and overall presentation organization. The overall presentation grade was a combined score of the GTA review (30 points) and the student peer reviews (20 points). Results of these combined review scores for the two semesters are shown in Fig. 4. Average scores of 85 to 86% for the presentation demonstrated student proficiency for SLOs 5 and 6. As reported by Amrein and Dimond et al. (13), we found peer review grades to be in line with grades assigned by the GTAs (data not shown).

Additional benefits of hybrid IBL

We initially implemented this IBL using both in-lab and online sessions because it enabled us to meet social distancing protocols that were still in place due to the COVID-19 pandemic. However, we realized that this format fostered an environment that encouraged participation in discussions that offered several advantages to an all-in-person format. First, the online platform alleviated barriers to discussion,

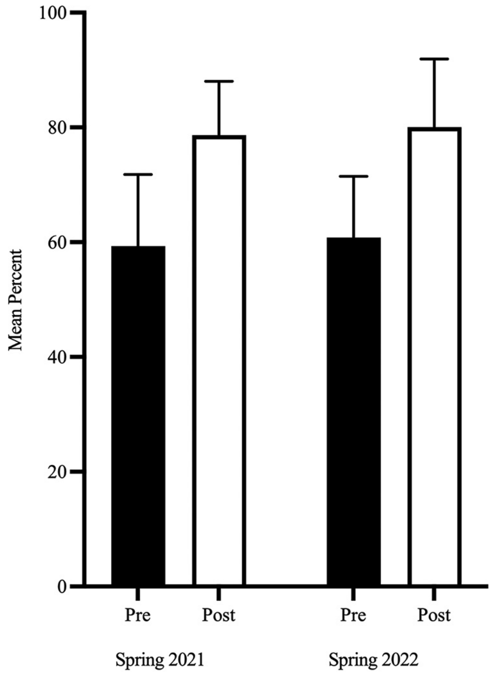

FIG 3. Mean percent comparison of pre- and post quiz scores for the Spring 2021 and 2022 semesters. A two-tailed paired *t* test was performed ($P < 0.0001$).

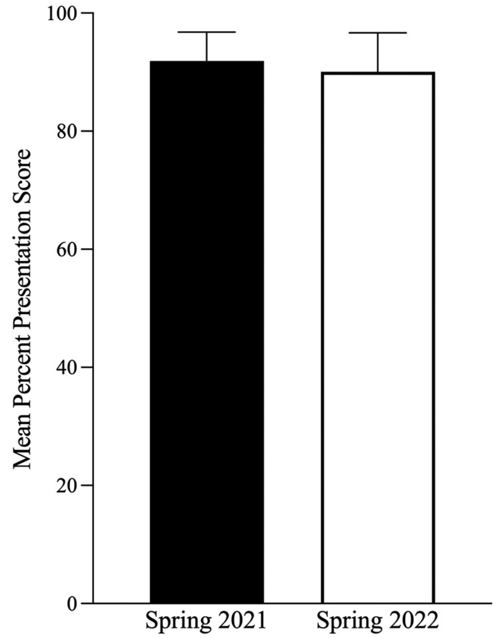

FIG 4. Presentation scores were comprised of rubric-based peer and GTA evaluations, as described by Amrein et al. (13).

such as mask wearing, and promoted an environment that was inclusive to all learners. Mask wearing, as well as loud laboratory equipment, can make group discussions challenging in the lab space, as it can be difficult to hear and understand the person speaking. This is especially true for our students with hearing challenges, as well as for international students for whom English is not their primary language. Some online platforms, such as Zoom and Microsoft Teams, allow for real-time closed captioning, which has been shown to increase accessibility for all students (15). An additional benefit of this platform is the breakout room feature. One of the hallmarks of inclusive teaching is a welcoming community where all students feel a sense of belonging and are given the opportunity contribute to classroom discussions (16). We had several concurrent lab sections which were physically separated into different lab spaces. The online platform allowed students to meet as a class and then break out into separate virtual rooms for small group discussions. While this approach is possible in a classroom setting, the online platform streamlined this process while freeing up the valuable classroom space. More importantly, the structured format of the breakout rooms and small group discussion encouraged all students to contribute their ideas, which promoted inclusivity (16). Finally, using a student's name has been shown to increase students' comfort with their instructors, promote a welcoming environment, and increase inclusivity in the classroom (17). Most online platform software offers the advantage of displaying student names and preferred pronouns, allowing the teaching team to easily identify their students and call on them by name during discussions.

This IBL also offers several opportunities to teach about the importance of failure and reproducibility in science. Allowing students to fail is often discouraged with cookbook-like protocols that have a known result or outcome. We asked students to evaluate their failures, apply improvements, and revise the protocol when repeating the experiment. This approach allowed students to use their critical thinking skills, to troubleshoot unexpected results, and to redesign their experiments, highlighting the importance of failure in research. End-of-semester course evaluations indicated a positive learning environment and experience with the IBL. One student commented that the instructional staff had "encouraged an environment where it is okay to not get the correct answer every time which is unheard of in a lab." Another student remarked that the IBL "created a very welcoming and friendly environment with students that helped to emphasize that our work was not about getting textbook perfect results but was about learning about the scientific process and how to ask questions." While we did not investigate student perceptions on the benefits of failure, it may be an interesting aspect to evaluate in the future.

Possible modifications

The *E. faecalis* conjugation model enables students to manipulate a variety of factors within the boundaries of the simple experimental procedure. Alternative strains capable of conjugation and selection could be used as a model system. If open lab times are not an option for some lab classes, GTAs could upload images of results to the students.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 2.3 MB.

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