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Increasing student interest with the Bacterial Unknown Identification Project: using mixed cultures to create real-world applications

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ABSTRACT Many introductory-level classes teach fundamental concepts, as they are prerequisites for upper-division courses. Therefore, the student body in these classes has diverse interests. To address this breadth of career trajectory, introductory-level laboratory courses often include experiments that demonstrate a wide range of microbiological techniques and processes. One of the experiments that is a standard component of many microbiology classes, the Bacterial Unknown Identification Project (BUIP), is often limited to isolated organisms or a specific environment. Here, we describe an updated method for the BUIP that incorporates the projected student career diversity through the implementation of multiple mixed cultures of microorganisms associated with different environments. This update can be utilized in any microbiology laboratory classroom. We maintained the learning objectives, including applying appropriate microbiological methods to analyze and interpret results, and effectively communicate scientific findings, while modifying the sample composition. Assessment of the modification demonstrated that upon completion of the BUIP, students felt that the project applied to their career and it did not take too much of their free time to complete.

KEYWORDS Bacterial Unknown Identification Project, mixed culture

The "Bacterial Unknown Identification Project" (BUIP) is typically the ultimate assessment used by undergraduate microbiology laboratory instructors to gauge students' hands-on laboratory skills, including microscopy, staining techniques, aseptic transfer, and isolation of microorganisms (1). After students complete their assigned task of identifying an unknown microorganism, they must then write a report or give an oral presentation describing their results (2). The BUIP is, therefore, aimed at reinforcing fundamental microbial techniques, while also emphasizing the practical applications of microbiology.

The traditional BUIP consists of undergraduate students being given a pure culture of their microorganism and informed of its identity from a list of known bacteria assigned by the instructor. However, providing students with a pure culture neglects to factor in the inherent mixed culture nature of microorganisms in the environment. Students are also left without the option to pick a microorganism that interests them, particularly one that relates to their career goals. Moreover, the traditional BUIP lacks molecular approaches, which are often used for bacterial identification (3).

Modified approaches to the BUIP have been developed that include isolation of microorganisms from the environment that necessitate using molecular techniques because the organisms are truly unknown (4–6). These approaches, while useful in small classrooms of upper-division students, are difficult to implement in lower-division courses due to the large number and diversity of students. Additionally, it can

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be challenging to include molecular techniques with the BUIP due to size and cost limitations.

Here, we describe a "mixed culture" method for the BUIP. Rather than providing students with a pure culture, they are given the option of choosing a sample from among five unique sources that each contain Gram-positive and Gram-negative microorganisms. These samples are environmental simulations that represent the fields of medicine, agriculture, food science, and veterinary science; the added microorganisms are chosen by the instructor based on the projected student career. From this mixed culture, students must streak the sample and determine the identity of one of the isolated microorganisms. Since lower-division microbiology laboratory courses consist of a broad student body with diverse interests, this modified approach enables students to pursue a sample that aligns with their major or future career. Indeed, we observed a high interest among students as they selected their mixed culture sample, and students felt more comfortable with the process of microorganism identification upon completion of their BUIP. Moreover, implementation of mixed cultures rather than pure cultures of microorganisms makes the BUIP more applicable to real-world scenarios, as bacteria are typically found in mixed environmental communities.

PROCEDURE

Intended audience

The project is designed for non-biology undergraduates whose majors include, but are not limited to, pharmacy, animal science, food science, and agriculture (Supplementary Material, Appendix 4). The BUIP occurs after students develop a basic understanding of microbiological skills, including staining techniques, microscopy, streak plating, aseptic transfer, and selective and differential media. The expected enrollment is over 200 students. Required prior coursework includes Introductory Biology and Introductory Chemistry (Supplementary Material, Appendix 4).

Instructor preparation

The microorganisms that are used for this project can be the same set of microorganisms typically used in the BUIP. Here, we report the use of biosafety level 2 (BSL-2) organisms and suggest that the appropriate safety precautions be taken, including but not limited to demonstration of proficiency working with BSL-1 level organisms, usage of proper personal protective equipment (laboratory coats, gloves, and goggle), posting of biohazard signage, and discarding all wastes according to local regulations. In the event that a laboratory is not BSL-2 certified, this method can be implemented with BSL-1 level organisms in similar mixtures, although the organisms suggested have not been tested, and should be verified before use (Supplementary Material, Appendix 2). Mixtures can be prepared in advance and provided on sterilized material. Complete instructions for instructors are provided in the Supplemental Material (Supplementary Material, Appendix 2).

General overview

The BUIP described here requires students to isolate and identify a microorganism from a mixed culture relevant to their field of study. Instead of a typical BUIP in which students are given an isolate (Supplementary Material, Appendix 5), in this scenario, students are given the choice of five sample types that represent the fields of medicine, agriculture, food science, and veterinary science (Supplementary Material, Appendix 1). Each sample contains a mixed bacterial culture of three organisms presented on life-like substrates. Working with appropriate BSL-2 practices in place, the first step students must complete is to streak the consortium onto a lysogeny broth (LB) agar plate for isolation. After the growth of their mixed culture, students pick one of the colonies from the plate and continue to isolate it. Upon obtaining a pure culture, students are provided LB broth and agar for the maintenance of the organism, as well as necessary materials

for the identification of the unknown organism. Materials include a Gram-staining kit, a microscope, one fluid thioglycollate broth tube, selective and differential media, oxidase reagent, and hydrogen peroxide. Students are also given a table containing 11 Gram-positive and 12 Gram-negative organisms and their physiological characteristics. This project was self-driven, where students worked on their own over 5–6 weeks. Each week, students had 30 minutes to 1 hour during class, as well as open-lab periods (1 hour Monday through Friday), to work on the project.

Assessment

At the end of the project, students submit a written three to five page report that includes an introduction in which they provide a hypothesis based on their selection, methods for each test, a description and explanation of the results, and a discussion of the broader impact of their results. Students are graded on the project using a detailed rubric. To determine student self-efficacy and enthusiasm, students complete a survey at the culmination of the project. Example surveys regarding student learning and enthusiasm are provided in the Supplemental Material (Supplementary Material, Appendix 2). This laboratory exercise was implemented for two semesters, for which Institutional Review Board (IRB) approval was obtained to collect the survey data under IRB 2021-1226 (Purdue University).

Results

The laboratory exercise described was implemented for two consecutive semesters (Fall 2020 and Spring 2021); 423 students completed the mixed culture BUIP. We found that students were able to correctly answer questions that are taught in the first half of the course at a high rate (~80%–90% correct response rate), whereas a question that addressed hypothesis generation had a lower correct response rate (~40%) (Table 1).

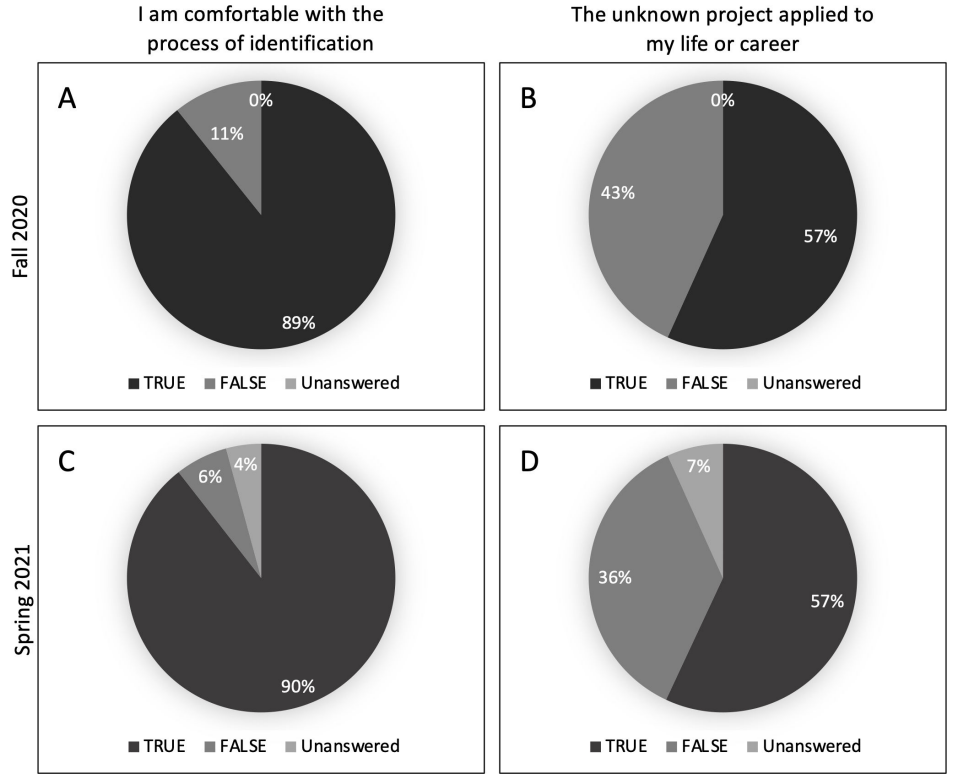

FIG 1 Student survey response to the BUIP at the culmination of the project. Students considered their comfort with the identification process during the Fall 2020 (A) and Spring 2021 (C) semesters and the relevance of the project to the student's life and career during the Fall 2020 (B) and Spring 2021 (D) semesters.

TABLE 1 Percentage of correct responses by students to evaluate student learning[a]

	Fall 2020	Spring 2021
What color would a Gram-negative organism appear after completion of a Gram stain? (Pink)	87.43	90.30
Which is an example of a good hypothesis? (My unknown organism will be sensitive to 1 of the 13 antibiotics tested)	37.70	43.04
Bacteria are typically found in (mixed) communities	86.98	87.12
Gram stain is based on chemical properties of the: (cell wall)	55.43	48.43
Unknown bacteria can be identified exclusively by: (small rRNA sequencing)	4.32	4.67

[a]Correct responses are indicated in parentheses.

While these questions were answered correctly at a rate greater than random choice (25%), a question regarding 16S rRNA gene sequencing, which was not a component of this study, had very low correct response rates (~4%) (Table 1). We take these results to mean that the BUIP reinforces core components of microbiology, and future iterations of this project can incorporate analysis of 16S rRNA gene sequences.

In the laboratory, teaching staff noticed that students had difficulty in isolating organisms from a plate containing *Proteus mirabilis*, which swarms across the entire plate if left too long. This difficulty could be mitigated by replacing *P. mirabilis* with Rhizobium or a non-swarming TnphoA mutant. Anecdotally, most students were unaware that the samples provided were organism mixtures, and many included language to incorporate the storylines indicated in the instructions into their final reports.

CONCLUSION

The diversity of interests in introductory microbiology courses warrants curriculum tailored to the student body. Allowing students to select from a range of potential sources using a mixed culture model enables students to connect the BUIP with their future careers (Fig. 1). This BUIP is useful in reinforcing key concepts taught throughout the course and can be used to teach the scientific method, beginning with hypothesis

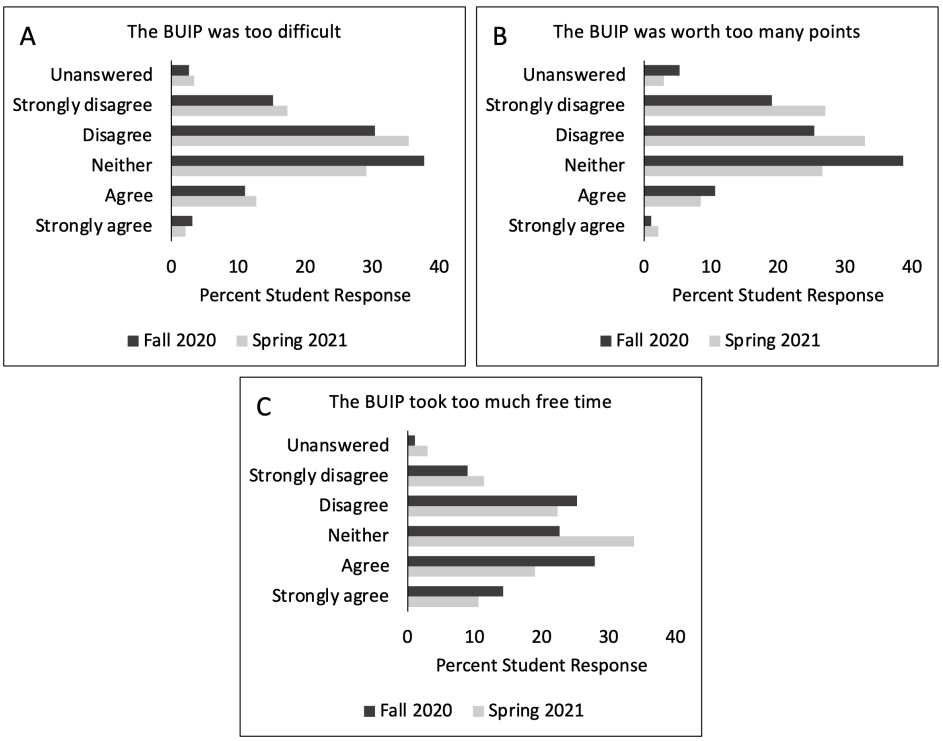

FIG 2 Student responses for three Likert scale questions on a survey at the end of the BUIP. Students reflected on the perceived difficulty of the project (A), the assigned project points (B), and the amount of non-class hours spent on the project (C).

generation (Table 1), and ending with a research report that stresses the importance of science communication. Any microorganisms used in the classroom can be thoughtfully mixed to incorporate the specific student demographics. These environmental simulations make students feel comfortable with the process of identification while limiting the effort of isolation and identification of organisms (Fig. 2). Mixed culture and thematic selections provide the opportunity to strengthen skills relevant to their future careers in a format that increases engagement with the material.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental material (jmbe00070-23-S0001.docx). Supplemental material including appendices 1 to 5.
Supplemental Table S1 (jmbe00070-23-s0002.xlsx). List of potential organisms with associated phenotypic information.

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