Curriculum

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Bootleg Biology: a Semester-Long CURE Using Wild Yeast to Brew Beer

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Laboratory exercises for undergraduates that involve authentic discovery and research have been shown to increase student learning and engagement. To bring these advantages into the microbiology curriculum, we developed a semester-long course-based undergraduate research experience for a laboratory based on brewing beer with wild yeast. This set of lab exercises uses many of the same protocols found in traditional microbiology lab curricula—isolating and maintaining pure cultures, staining and microscopy, use of aseptic technique, PCR, gel electrophoresis, and media preparation—and integrates them into a novel and exciting project that enables students to be active participants in the scientific method. Students are assessed on their ability to brew beer successfully and to stain and visualize microorganisms; they are also assessed for knowledge gains in the traditional portion of the course, their ability to use their brewing knowledge in other settings, and their attitudes about science. After completing the course, students showed gains in general microbiology knowledge and their engagement with science.

KEYWORDS CURE, fermentation, brewing, microbiology

INTRODUCTION

The past decade has seen a large overhaul in how biology courses and especially laboratories are taught to undergraduate students (1). In response to nationwide calls for reform, short "canned" labs have been phased out in favor of longer, investigative projects where the outcome is unknown to both the instructor and students (2–4). While multiple models now exist for introductory courses (5–7), and senior-level independent research has long been a part of many curricula, there are fewer options for instructors of elective and intermediate-level courses. We sought to bridge this gap by designing a lab course suitable for an intermediate (200- or 300-level) undergraduate microbiology class. There are a number of skills common to many microbiology lab curricula, including sterile technique, media preparation, and staining and viewing of specimens under a microscope (3), as well as molecular biology skills such as identification by PCR and Sanger sequencing. There are also many different ways to incorporate these items into a cohesive curriculum, rather than a series of unrelated 1-week "cookbook" exercises. For that reason, we have developed a course designed to maximize student interest and engagement by isolating wild yeast and using it to brew small batches of beer.

Studying the process of brewing is a relevant exercise, given that brewing (and other forms of fermentation) is intricately tied to microbiology (4) and real world experiences, as can be seen from the recent explosion in the craft brewing and distilling scene. College courses have started to utilize fermentation in their curricula (8). In addition, a number of universities, such as Cornell University, University of California Davis, and University of the Sciences in Philadelphia, have set up classes and even certificates designed specifically around brewing sciences. This lab has been set up and implemented in a microbiology lab at La Salle University, a private liberal arts school in Philadelphia. In 2015, Sato et al. (6) published a protocol and exercises to brew beer in a standard microbiology lab. We have refined and expanded that 3-week protocol into an entire semester's worth of activities and learning. Students now capture, identify, and culture wild yeast; they also change and alter their brewing recipes over the semester to brew better-tasting beer each time.

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The authors declare a conflict of interest. J. Mello is the founder of Bootleg Biology, which sells kits to perform some of the protocols described in the paper (https://bootlegbiology.com/).
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Three of the five scientific thinking skills (demonstrate an ability to formulate hypotheses and design experiments based on the scientific method; use mathematical reasoning and graphing skills to solve problems in microbiology; effectively communicate fundamental concepts of microbiology in written and oral format) and five of the seven laboratory skills (properly prepare and view specimens for examination using microscopy; use pure culture and selective techniques to enrich for and isolate microorganisms; use appropriate methods to identify microorganisms; use appropriate microbiological and molecular lab equipment and methods; practice safe microbiology, using appropriate protective and emergency procedures) are included in some form in our course (5). Additionally, by using precourse and postcourse tests, poster sessions, and a final exam based on a manuscript reporting contamination in a brewery, students (i) increased their engagement in science, (ii) used laboratory techniques to brew beer, (iii) used the scientific method to successfully brew beer, and (iv) demonstrated improvement in learning as measured by the Microbiology Concept Inventory (MCI). While not a direct goal of the project, students also reported an improved ability to read and understand scientific literature after completing the course.

Intended audience and prerequisite knowledge

This CURE has been developed for and used in an upper-level course, BIO303 Microbiology, at La Salle University. The course enrollment contains primarily third- and fourth-year students; around 50% of the class is usually 21 years old or older. Any undergraduate student, including lower-division biology majors and nonmajors, can accomplish the initial brewing procedure, characterization of the final beer, and even capturing and culturing wild yeast. However, to understand the biological processes behind the experiments, the students should complete their school's introductory sequence of courses for biology majors. Other work, such as genomic DNA prep, PCR, and sequencing analysis, is done in some introductory labs and so can be used as reinforcement of those techniques or alternatively as a first introduction. Knowledge of liquid handling and aseptic technique greatly increase the chance of success and are taught in the introductory labs at our institution.

Learning time

This CURE has been designed to run for an entire 15-week semester. At La Salle University, the microbiology course meets twice a week in 2-h blocks; lab and lecture time are distributed on an as-needed basis. The original module was designed for three 1-week labs (6); our course is therefore divided into loose groupings of 3 weeks each. The first week is when the brewing actually occurs, the second is used for adding sugar (for carbonation) and transferring the material to a new container, and the third is for testing and tasting. The procedures in week 2 only take a few minutes, so that is when complementary experiments in the module (staining, DNA purification, PCR, Sanger sequencing) are performed. For faculty that only wish to incorporate some of this lab, the following groups of experiments found in Table 1 can stand alone: brewing beer, sessions 1 to 3; capturing and isolating yeast, sessions 2, 4, 5, and 6; genomic analysis of yeast isolates, sessions 7, 9, and 10.

Learning objectives

The learning objectives for the course are shown below. Of these, #1 and #3 contain elements of the brewing lab, while #2 and #4 are entirely based around brewing.

1. Students will be able to describe how yeast metabolize wort into beer that contains alcohol and CO_2.
2. Students will be able to create, keep, and maintain a laboratory notebook.
3. Students will be able to organize, analyze, and present data from their own experiments.
4. Students will be able to perform standard microbiology lab techniques and apply them to the process of brewing beer with wild yeast.
5. Students will experience and gain an appreciation for their ability to perform scientific research.

Objectives 1 to 4 are assessed and graded as part of the course. Objectives 1 and 4 are also assessed with the MCI (7); objectives 3 and 5 are assessed in the CURE survey (www.grinnell.edu/academics/areas/psychology/assessments/cure-survey) (9).

PROCEDURE

Materials

Brewing ingredients can be bought online at sites like Northern Brewer or Amazon, or at a homebrew store if one is nearby. Specific ingredients used in our implementation of the module included the following: (i) yeast (Muntons Premium Gold, Nottingham Brewing Yeast, and Safbrew WB-06), 1 package each for the semester; (ii) hops (Centennial hop pellets, Chinook hop pellets, Fuggle hop pellets), 1 1.5-oz package of each for the semester; (iii) malt extract (dry malt extract for extra light, wheat, amber, and extra dark), sold as 3- or 5-lb bags of dehydrated powder, 6 lb of each are required for the semester.

Dry malt extract can be stored at room temperature as long as it is kept dry. Yeast packets and hops should be stored in the refrigerator. Nonconsumable equipment includes sterile 200-mL glass bottles (2 to 3 per group), 600-mL beakers (1 to 2 per group), Bunsen burners or hot plates (1 per group), scales (3 to 6 per class), sterile stirring rods (2 per group), ice buckets (1 per group), hydrometers and hydrometer test jars (3 to 5 per class; e.g., beer and wine triple-scale hydrometer from Northern Brewing), an inoculating loop for each group, and pH paper.

Equipment for subsequent experiments includes the following: methylene blue and/or Gram stain reagent packs (1 bottle of each for every 2 to 3 groups), 1 500-mL bottle of bacteriological-grade agar and sterile petri dishes (3 to 4 plates per group for

TABLE 1
Sample 14-week syllabus for Bootleg Biology[a]

Lab session no.	Exercise title	Protocol used	Skills
1	Beer brewing introduction	Protocol A	L4, L5
2	Beer carbonation	Protocol B	ST1, L4, L5
	Yeast taming introduction	Protocol D	
3	Beer analysis and tasting	Protocol C	ST2, ST3, L4, L5
4	Making agar plates for yeast	Protocol E	L2, L4, L5
5	Yeast taming analysis, streaking	Protocol F	L2, L4, L5
6	Yeast isolation and purification	Protocol E part II	L1, L2, L3, L4, L5
	Staining and microscopy		
7	Colony PCR	Protocol F	L3, L4, L5
8	Brewing with your yeast	Protocol A	ST1, ST2, L2, L4, L5
9	Gel electrophoresis and purification; carbonation	Protocol B	L3, L4, L5
10	Sequence analysis	Protocol F	L3
11	Local yeast beer tasting and analysis	Protocol C	ST2, ST3, L4, L5
12	Brewing a new beer with your yeast	Protocol A	ST1, ST2, L2, L4, L5
13	Carbonation	Protocol B	L4, L5
14	Final beer analysis and tasting	Protocol C	ST2, ST3, L4, L5

[a]Skill abbreviations: ST1, demonstrate an ability to formulate hypotheses and design experiments based on the scientific method; ST2, use mathematical reasoning and graphing skills to solve problems in microbiology; ST3, effectively communicate fundamental concepts of microbiology in written and oral format; L1, properly prepare and view specimens for examination using microscopy; L2, use pure culture and selective techniques to enrich for and isolate microorganisms; L3, use appropriate methods to identify microorganisms; L4, use appropriate microbiological and molecular lab equipment and methods; L5, practice safe microbiology, using appropriate protective and emergency procedures.

the semester), one bottle of yeast nutrient, 500 mL of 33% sucrose, mason jars (1 to 2 jars per group), cheesecloth, a yeast genomic DNA prep kit (1 per class), primers (1 set per class), master mix (500 μL), and an account for Sanger sequencing. Yeast genomic DNA was purified using a yeast colony PCR protocol (10) modified for this lab, and internal transcribed spacer (ITS) DNA was amplified using the SC1 and SC2 primers (11). Samples were purified with the GeneJet PCR purification kit according to the manufacturer's instructions and sequenced at Eurofins Genomics.

Student instructions

Students received a lab manual in the form of the initial module (originally published by Sato et al. [6]) and the materials presented in the supplemental material. Students were also given a blank lab notebook on the first day of class and were expected to keep detailed notes on their work throughout the semester.

Faculty instructions

Students are given a lecture reproduced from that of Sato and colleagues (6) at the start of the lab to introduce the brewing process. The student lab manual contains all needed instructions to analyze beer at the end of each brewing session. On brew days, it is important to set up multiple scales in the lab as well as one hot plate per group. Malt extract and hops can be stationed near the scales if the room allows, as students tend to get slightly off-schedule from one other during the course of brewing. On tasting and analysis days, it is helpful to first give a demonstration of the calculations for calories and alcohol. Finally, if the instructor has an experienced beer palate, they can offer critiques and suggestions of student brews. Of note, if the student population is mixed between underage and over-21 adults, it can be helpful to pair students so that each group has at least one member who can legally taste their own beer.

Suggestions for determining student learning

Assessment of student learning outcomes relating to the introductory material has been published previously (6). New assessments stemming from the capture and use of wild yeast in our are a combination of practicums and scientific communication. Student progress is measured via virtual posters, i.e., files submitted to a learning management system in lieu of traditional lab reports. These posters are graded on specific elements (e.g., quality of Gram stain images, inclusion of information key to brewing such as alcohol content and calorie calculations) and also for visual appeal and clarity. Rubrics and example submissions can be found in the supplemental materials. Students are also expected to keep and maintain a detailed lab notebook

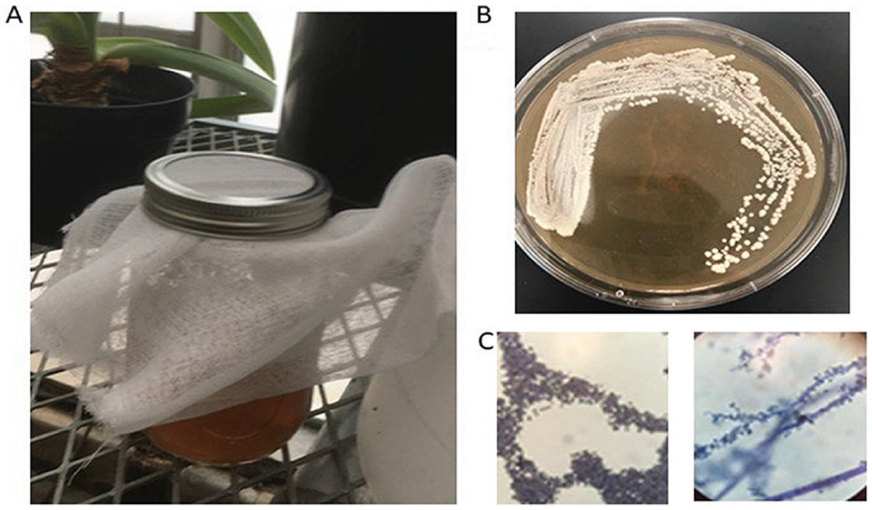

FIG 1. Examples of student data: wort in an open container to capture wild yeast (A); one example of a pure culture on student-made plates (B); and bright-field images of yeast cells stained with a Gram stain protocol and a simple stain with methylene blue (C).

throughout the course. This is assessed in an open lab book quiz at the midpoint of the semester and also by turning in their notebooks at the conclusion of the course. Notebooks are graded off a combination of having detailed entries each session and for specific spot checks of data; for example, having an alcohol by volume calculation for a test day. Finally, while not a formal assessment, lab pairs compete with each other during the third tasting and analysis lab. Each table of 4 to 6 groups sample each other's beer and have to come to a consensus winner to scale up as a larger group for their final project. This final batch is larger (approximately 1 liter of beer); the batch and a companion poster are presented at the school's end-of-semester symposium, in which any student of age can sample all three finalists and vote for a winner. The results of the competition are not directly factored into any grade, but the friendly competition is provided to help motivate students. Students are typically challenged but excited by the scale-up aspect of this competition. Each group is responsible for submitting a "grain bill" (a list of ingredients and amounts) for their new, larger recipe; while some were uncomfortable with having to do the calculations in the end, all the beers were successfully fermented and even tasted good.

Sample data

Examples of student data are shown in Fig. 1 and 2. Figure 1A shows the yeast "capturing" setup, with wort in a partially exposed Mason jar. Also pictured (Fig. 1B) is an example of a pure culture streak from fermented wort; Gram and methylene blue stains of the wild yeast isolate are shown in Fig. 1C. Figure 2A shows strain-level identification using PCR and traditional Sanger sequencing, and Fig. 2B shows an example of whole-genome sequencing. Examples of sample data can also be found in the supplemental material in the examples for each of the three poster assignments.

Possible modifications and extensions

As stated above, individual modules in this lab can be run in as quickly as 3 weeks. Therefore, instructors have the ability to import some ideas into their own lab curricula if they do not want to commit to an entire semester. The basics—capturing wild yeast, culturing it, and brewing a bottle of beer—could also be conceivably worked into existing lab syllabi, as only "brew days" take up the entire lab time. By the same token, this flexibility also means that there is room for many additional experiments. Protocols that are often included in standard microbiology labs (3) can be easily imported. Table 1 and Fig. 1 show a few examples: in the field testing lab, we performed both methylene blue and Gram stains on our yeast isolates. We also performed genomic DNA purification for PCR (12), agarose gel electrophoresis, product purification for Sanger sequencing, and BLAST analysis (Fig. 2). Finally, we took advantage of a new iSeq system in the department to perform next-generation whole-genome sequencing on three of the class's isolates. As seen in Fig. 2, one isolate, while very closely related to a commercial strain, also contained genetic material usually associated with wild yeasts. Groups whose isolates were fully sequenced were free to use that data in their final posters; however, since the next-generation sequencing could only be performed for 3 samples (of 11 collected across the class) and was not required, it was not formally assessed. Data such as these can be given to systems biology or genomics courses for further analysis. Our class also used the knowledge gained from laboratory exercises and applied it to questions about a research paper for their final exam, a paper that investigated spoilage bacteria in brewery settings (13).

COVID-19 and distance learning

Of importance during coronavirus disease 2019 (COVID-19), much of this work could be performed in a virtual classroom

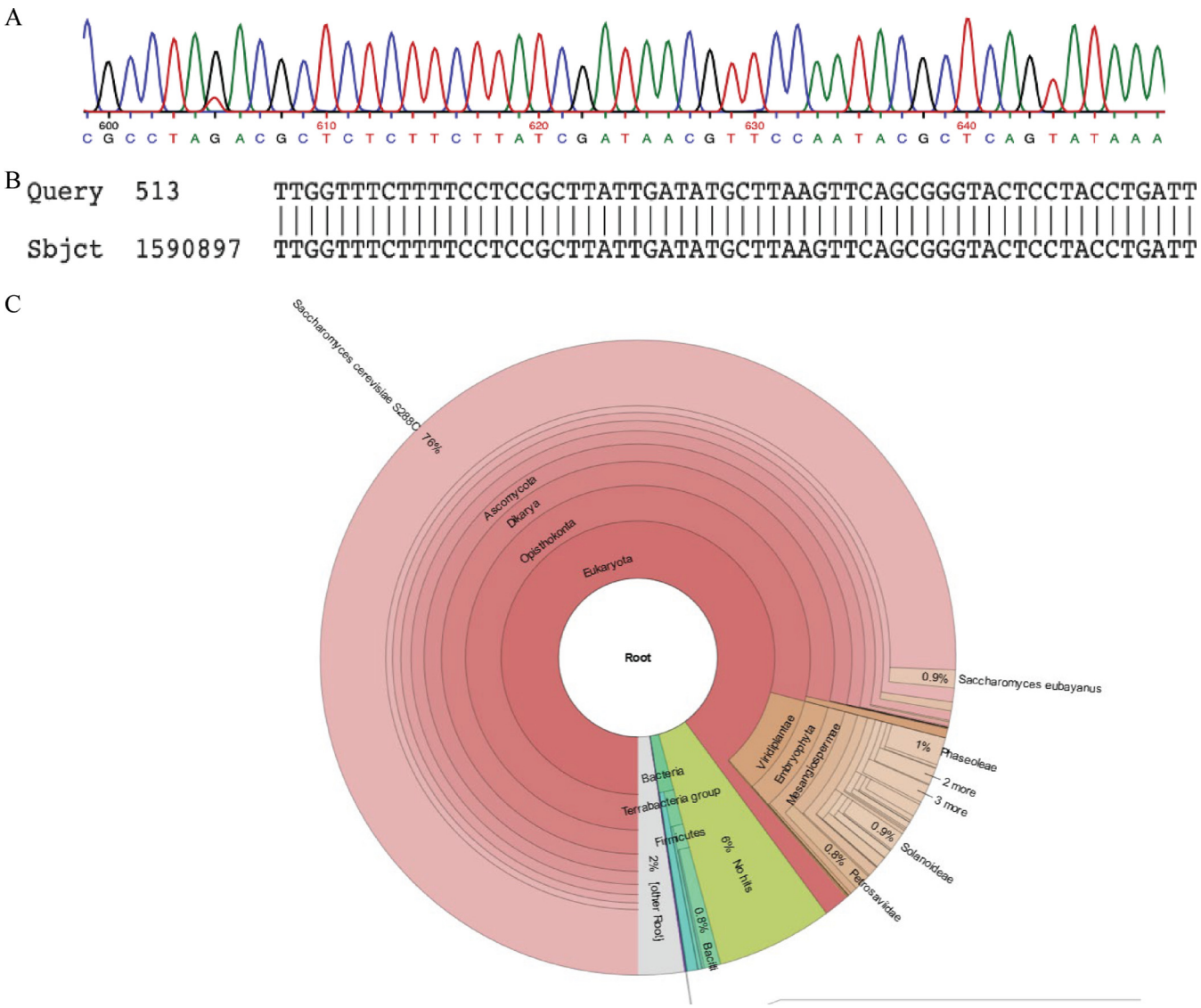

FIG 2. Sequence analysis of wild yeast isolates. (A) Chromatogram sequence from a wild yeast isolate. (B) Alignment of ITS PCR product from a student yeast with a published sequence. Data were acquired using the modified "yeast colony PCR" protocol (see the supplemental methods). This isolate was most closely related to a strain of *S. cerevisiae* used in commercial production of rice wine. (C) Whole-genome analysis of a different isolate via next-generation sequencing on an iSeq system. This strain was also *S. cerevisiae* but had a noticeable number of genes from *S. eubayanus*.

setting. Like many schools, La Salle University had to quickly pivot to all-online classes in the fall of 2020 when COVID cases rose. Students enrolled in this microbiology class were polled, and every student was either over 21 years of age or lived with an adult who could legally handle their wort once it fermented and became beer (importantly, Pennsylvania law allows anyone 18+ to purchase, possess, and brew with the ingredients in this course). Students then had the option of coming to campus to pick up supplies or ordering their own online or at a local homebrew store. While we were not able to perform sequencing, from our kitchens over Zoom the class brewed beer with commercial yeasts, captured their own wild yeast, isolated it on home-made agar plates, and brewed two batches with their cultures. Students responded favorably to the experiments, and some were even featured in a local newspaper story (14).

Safety issues

Commercial beer ingredients have no known safety issues, as outlined previously (6). The biggest risk is therefore inadvertently allowing underage drinking of alcoholic beverages. We followed the protocols outlined previously (6), where IDs were checked before all analysis labs. Fortunately, there is a myriad of data that can be collected and analyzed without tasting, such as alcohol content, calorie levels, and color analysis. Culturing unknown yeasts (usually *Saccharomyces cerevisiae*) from the environment may sound scary but has been done safely for

TABLE 2
Assessment data from the CURE survey that highlighted perceived gain in the indicated skill due to the course research experience[a]

Skill	Module CURE (mean ± SD)	National CURE (mean ± SD)
Skill in interpretation of results	3.1 ± 0.5	3.7 ± 1.0
Tolerance for obstacles faced in the research process	3.2 ± 0.7	3.7 ± 1.0
Understanding the research process	3.3 ± 0.9	3.7 ± 1.1
Ability to integrate theory and practice	3.6 ± 0.9	3.6 ± 1.0
Understanding that scientific assertions require supporting evidence	3.8 ± 0.8	3.8 ± 1.0
Ability to analyze data and other information	3.8 ± 0.8	3.9 ± 1.0
Understanding science	3.7 ± 0.9	3.8 ± 1.0
Learning laboratory techniques	3.7 ± 1.0	3.9 ± 1.1
Ability to read and understand primary literature	4.0 ± 0.7	3.6 ± 1.1

[a]Responses were collected after participation in the module on a scale from 1 (no gain or very small gain in the skill) to 5 (very large gain in the skill). Means and standard deviations are reported. For reference, national responses from students who participated in CUREs (as curated by Lopatto and Jaworski [17]) are included.

thousands of years. Most "contaminants" of beer are spoilage organisms that are harmful to the batch but not to human health (13). In addition, beer has several elements that provide protection against harmful organisms, such as the addition of hops, a low pH, and the presence of alcohol (11, 15). This makes wort and hops a functional selective medium for brewer's yeast. Early experiments with wild yeast were done under biosafety level 2 (BSL-2) conditions. Once their organisms had been identified as strains of brewer's yeast, we moved to a standard BSL-1 room; eye protection, lab coats, and gloves were worn at all times, and the bench space was disinfected before and after each session. In order to minimize any issues, the syllabus indicated that wild yeast should be isolated first and colony PCR modified using methods outlined elsewhere (10) to make sure it is a brewer's yeast isolate before any beer is tasted.

Assessment of the module and dissemination of the data were performed in accordance with the La Salle University Institutional Review Board approval, application #19-07-033.

DISCUSSION

Field testing

This research experience was developed over several years at La Salle University. The microbiology course at La Salle is a 3-credit course that meets for 2 h twice weekly. There is only one section (18 to 24 students) that runs once every academic year. The initial brewing exercise using store-bought yeast was introduced in 2017, followed by a pilot of the expanded syllabus, including the wild yeast sections, in 2018. Formal assessment of student learning and a revision of syllabi was first tested in the fall of 2019. All of the laboratory exercises presented here can be completed in a 2-h lab. After a short traditional 2-week introductory module on pure cultures and Gram stains, the rest of the semester is spent on the brewing project. By the end of the semester, all pairs of students have cultivated a wild yeast strain and used it to successfully brew a small batch of beer.

TABLE 3
Responses to the course elements survey[a]

Course element	Precourse (mean ± SD)	Postcourse (mean ± SD)
Scripted lab or project where students know outcome	4.1 ± 0.8	3.5 ± 0.8*
Lab or project where no one knows the outcome	2.5 ± 1.2	3.3 ± 1.3*
A project where students have input into process or topic	3.0 ± 1.0	4.0 ± 0.9***
Work in small groups	4.2 ± 0.7	4.8 ± 0.5**
Read primary scientific literature	3.4 ± 1.12	4.8 ± 0.6***
Collect data	4.1 ± 0.8	4.5 ± 0.7
Present posters	2.9 ± 1.0	3.9 ± 1.0**
Maintain lab notebook	4.0 ± 1.1	4.7 ± 0.6*

[a]Responses were collected before and after participation in the module and indicate a student's experience with each item, on a scale from 1 (none) to 5 (extensive). Means and standard deviations are reported. *t* tests were performed to compare precourse and postcourse responses, with *P* values indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TABLE 4
Assessment data from the CURE survey highlighting the overall course evaluation[a]

Statement	Module CURE score (mean ± SD)	National CURE score (mean ± SD)
This course was a good way of learning about the subject	4.4 ± 0.9	4.2 ± 0.9
This course was a good way of learning about the process of scientific research	4.5 ± 0.7	4.2 ± 0.9
This course had a positive effect on my interest in science	4.4 ± 0.9	4.0 ± 1.1
I was able to ask questions in this class and get helpful responses	4.5 ± 0.7	4.2 ± 0.9

[a]Responses were collected after participation in the module, with scores on a scale from 1 (strongly disagree) to 5 (strongly agree). Means and standard deviations are reported. For reference, national responses from students who participated in CUREs (as curated by Lopatto and Jaworski [17]) are included.

The different forms of assessment utilized throughout the module are centered on whether students achieve the learning objectives. The results are discussed in the following section.

Evidence of student learning

To examine the impact of the brewing module, we surveyed students on a variety of different topics, including perceived benefits from the CURE survey (16), a course elements survey (7), and a postcourse assessment from the CURE survey. Both surveys were completed by a sample of 20 students.

Table 2 highlights the relevant items from the "benefits" portion of the CURE postcourse survey that we felt aligned with our course objectives for this module. These items are intended to highlight the possible benefits participants gained from the research experience, from the student perspective. As can be seen, students reported similar gains on a number of these items after completing the beer module relative to national data from students participating in CUREs. These national data included 18,062 students who completed the survey during 2015 and 2018 with responses collected and descriptive statistics (17). Not surprisingly, there was also a number of items for which students reported gains that were not as high as those from the CURE experience. Some of this may have been due to the fact that our course is only 3 credit hours, instead of the more common 4 for a microbiology course.

We similarly took relevant items from the CURE course elements survey to identify student responses before and after completing the module. As can be seen from Table 3, student responses were significantly higher on the vast majority of the items emphasized in the module. It should also be noted that responses to the item "scripted lab or project where students know the outcome" were significantly lower. Finally, student responses to statements from the CURE survey examining satisfaction with the course were examined, and results are displayed in Table 4. Students were very positive regarding these items, with responses that mirrored national responses.

To assess how this module affected learning gains across the course, we used the Microbiology Concept Inventory, given during the first and then last week of class (Fig. 3). Similar to findings reported by Paustian et al. (7), we calculated the normalized learning gain in each question, which was calculated as follows: [(postcourse score) – (precourse score)]/[100 – (precourse score)]. We saw overall normalized gains of 0.13 across

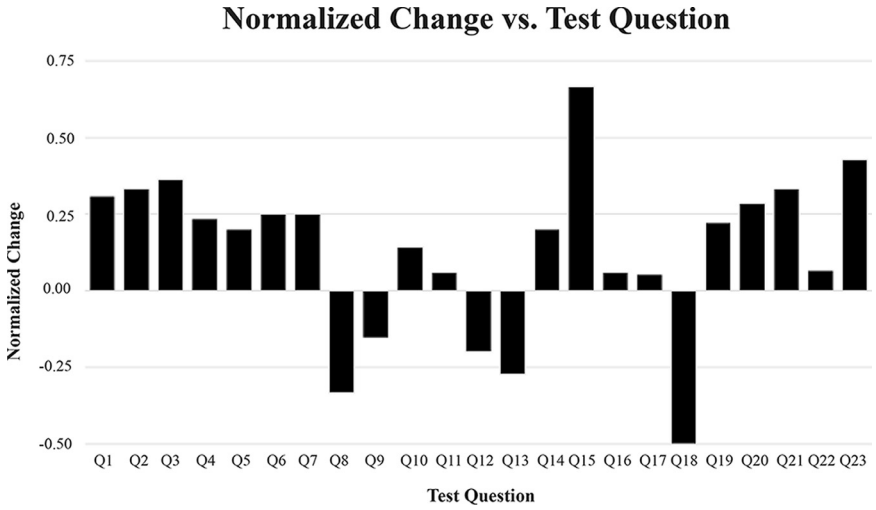

FIG 3. Assessment data from the MCI. Normalized change on the precourse test versus postcourse test performance on the MCI was examined on a per-question basis. A total of 19 students took both the precourse test and postcourse test.

all questions, similar to the published average of 0.15 (7). We also chose a subset of questions (#7, 11, 13, 16, 18, and 20) to analyze which questions were especially relevant to our lab material. Our students showed normalized learning gains that were more positive than the published average for questions #7, 11, 15, and 20 and were comparable for question #16. The results for question #11 were especially exciting, as that was one of the few questions that had a normalized loss in the published posttest data. Questions #13 and 18 in our group had negative learning gains, but this was also seen in question #13 in the published average. In our data for question #18, the pretest number was already 89% correct, and so a posttest number of "only" 84% translated into a significant normalized loss. Overall, running this lab all semester as a CURE resulted in substantial knowledge gains in most areas; these gains were most notable in some of the concepts emphasized in brewing science.

Conclusions

This set of experiments brings together multiple best practices in the field. It is a CURE in which students are in charge of their own authentic research, it ties together a number of important techniques in the field into a larger framework and, importantly, it is enjoyable and engaging for students (examples of course evaluation comments can be found in the supplemental material). Students had to capture, isolate, and characterize their own yeast isolate; they then had to develop protocols and modify reagents (ingredients) to use their yeast to make a beer that actually tasted like commercial beer. As seen in the assessment data and also anecdotally, students enjoyed working in this lab and appreciated the links to the real world, as craft brewing is a growing industry.

Students who took this version of microbiology showed gains consistent with other CUREs, as shown by the Grinnell survey data. They also showed gains in lecture on the MCI that were comparable to national averages. After participation, students reported greater interest in science and scored well for in-course assignments. In the future, we hope to "donate" yeast isolates and recipes to a nearby brewery to further strengthen connections between research, entrepreneurship, and business.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.9 MB.

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