Curriculum

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Methylothon: a Versatile Course-Based High School Research Experience in Microbiology and Bioinformatics with Pink Bacteria

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Methylothon is an inquiry-based high school learning module in microbial ecology, molecular biology, and bioinformatics that centers around pink-pigmented plant-associated methylotrophic bacteria. Here, we present an overview of the module's learning goals, describe course resources (available for public use at http://methylothon.com), and relate lessons learned from adapting Methylothon for remote learning during the pandemic in spring of 2021. This curriculum description is intended not only for instructors but also for microbial ecology researchers with an interest in conducting K-12 outreach. The original in-person version of the module allows students to isolate their own strains of methylotrophic bacteria from plants they sample from the environment, to identify these using PCR, sequencing, and phylogenetic analysis, and to contribute their strains to original research in a university lab. The adapted version strengthens the focus on bioinformatics and increases its flexibility and accessibility by making the lab portion optional and adopting free web-based tools. Student feedback and graded assignments from spring 2021 revealed that the lesson was especially effective at introducing the concepts of BLAST and phylogenetic trees and that students valued and felt inspired by the opportunity to conduct hands-on work and to participate in community science.

KEYWORDS project-based learning, course-based research experience, microbial ecology, bioinformatics, methylotrophy, community science

INTRODUCTION

Biology education has recently embraced inquiry-based learning (1–3), due to its potential to improve enthusiasm for and learning retention in sciences, technology, engineering, and math (STEM) and to increase confidence among less-prepared students or those from underrepresented minorities (4, 5). In particular, course-based undergraduate research experiences (CUREs), which allow classes to engage in research questions of interest to the community, can achieve these advances (6–8). Much published work on CUREs focuses on college students, yet high school students may also benefit from CUREs. Moreover, interaction with STEM practitioners can help to widen high school students' understanding of who can be a scientist (9) and create bridges between classrooms and community STEM opportunities (10).

Intended audience

Here we present Methylothon, a high school learning module in microbial ecology and evolution, which is similar to a CURE but is designed for 11th and 12th graders. This curriculum description is designed to help high school instructors teach

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Methylothon in their own classrooms and to inspire research laboratories to conduct outreach with high schools. Methylo-thon was originally designed as a "nose-to-tail" microbial ecology module for high school bioscience classes, in which students isolate organisms from their local environment, conduct PCR and sequencing for identification, and learn bioinformatic tools to place their isolates on the tree of life, similar to the SEA-PHAGES and PARE programs (11, 12). It includes a community science component, as isolates can contribute to original research by collaborating researchers (our coauthors). Optional final projects allow students to delve deeper into microbial diversity in the context of biotech or evolution or to focus on research design. Methylothon thus provides a scaffold for building diverse skills and experiences, including hypothesis formulation, field work, laboratory techniques in microbiology and molecular biology, bioinformatics, science communication, and literature review. It touches on several core concepts for biological literacy outlined in the AAAS 2011 *Vision & Change* report (i.e., evolution, information flow, and systems) and has potential to cover all six of the report's listed core competencies (1). It also incorporates 17 concepts and skills from the American Society for Microbiology (ASM) Curriculum Guidelines for Undergraduate Teaching (13) (see Appendix 1 in the supplemental material).

Methylothon originated as part of a research grant to study the ecology and evolution of plant-associated methylotrophic bacteria. We recognized that we could share our science with students by involving them in isolating methylotrophs. In addition to being extensively studied in the context of single-carbon metabolism (14, 15), pink-pigmented facultative methylotrophs make ideal model organisms for introducing students to microbiology. They are ubiquitous in the environment (16, 17), pose low risks to human and plant health, are straightforward to isolate at room temperature, are used in numerous biotechnological applications (17–21), and importantly, they are pink. This pigmentation makes them easy to identify on agar plates.

To develop Methylothon, the university researchers in our group used an informal network to find high school teachers in the San Francisco Bay Area who were interested in using a methylotroph lab to replace other lessons on molecular biology and phylogeny. We discussed learning goals and the context of the existing curricula before developing course materials. The methods described here are intended to be accessible to most biology laboratories in universities or well-equipped high schools, and methylotrophs can be cultured from nearly any plants in any geographic location. We encourage high schools, undergraduate classrooms, and high school–university partnerships around the world to consider implementing and adapting Methylothon.

Redesigning the module in for the 2020-2021 school year to meet the needs of remote instruction during the COVID-19 pandemic unfortunately required breaking the links between the students' original data collection and data analysis, which are a valuable part of inquiry-based learning (22). However, the final product was a more versatile module that is accessible to classes without a molecular biology lab and/or with limited computing capacity. Here, we describe the remote-learning version of Methylothon, but our online materials include resources for in-person laboratory instruction. Teachers may choose their own approach, depending on learning goals and resources.

TABLE 1
Example 1 lesson plan: 1 week, 5 days/week[a]

Day	Period	Lesson
1	Morning	Guest lecture; begin leaf press lab
2	Morning	"Intro to Methylotrophs" lecture
2	Afternoon	"Tree of Life and Phylogenetics" lecture
3	Morning	Bacterial identification lab (asynchronous)
4	Morning	"DNA Sequencing and Multiple Sequence Alignment" lecture; bioinformatics lab walkthrough
4	Afternoon	Bioinformatics lab
5	Morning	Finish bioinformatics lab; assign final project; conclude leaf press lab

[a]The Methylothon curriculum can be taught over various timetables. Shown here is one of two example lesson plans executed by our partner schools. In this class, the entire module was delivered in the course of a single week, as students met daily for lecture and additionally twice a week for lab sessions.

Learning time

The full Methylothon module is 7 sessions. The timing of delivery can easily be varied (Tables 1 and 2). Classes taught in 2021 took 1 to 3 weeks, with some classes adding a final project at the end.

Prerequisite student knowledge

Methylothon is designed for high school juniors and seniors; students should begin with awareness of microorganisms, DNA, and the central dogma. Some of our partner classes already had experience with PCR, culturing microorganisms, or conducting BLAST; for such classes, Methylothon can be a review or synthesis. For classes where all the concepts are new, teachers may use Methylothon as a scaffold for just-in-time learning and supplement with additional material to reinforce particular learning objectives. The objectives listed here correlate with 17 key concepts and skills from the ASM Recommended Curriculum Guidelines for Undergraduate Microbiology Education (13), notated by their numbers in brackets in the following list, and described in Appendix 1 in the supplemental material. Methylothon offers flexibility to emphasize the learning objectives appropriate for the class; for example, those marked with an asterisk in the following list were taught in some but not all classes in 2021.

Learning objectives

By the end of Methylothon, students should be able to:

TABLE 2
Example lesson plan 2: 3 weeks, 2 to 3 days/week[a]

Wk	Day	Lesson
1	1	Guest lecture
	2	"Intro to Methylotrophs" lecture; begin leaf press lab
2	3	"Tree of Life and Phylogenetics" lecture
	4	"DNA Sequencing and Multiple Sequence Alignment" lecture; bacterial identification lab (asynchronous)
3	5	Bioinformatics lab walkthrough
	6	Bioinformatics lab
	7	Complete bioinformatics lab; assign final project; conclude leaf press lab

[a]This is the second of two example lesson plans executed by our partner schools. This class was able to devote only 2 to 3 periods per week to the module, so the full duration was 3 weeks.

a. Describe the ubiquity and diversity of microbes in the environment [ASM 20, 27]
b. List 3 applications for microbes in biotechnology [ASM 23, 26]
c. Describe what methylotrophs are and what distinguishes them from other microbes [ASM 11, 12, 13]
d. Define what rare earth elements (lanthanides) are and identify the role they play in microbiological processes* [ASM 11, 12, 13]
e. Describe 3 methods used to identify microbes [ASM 34]
f. Explain how selective medium is used to culture methylotrophs [ASM 33]
g. Demonstrate standard methods for culturing microorganisms on agar plates and analyze the effects of methodological changes (the roles of temperature and moisture, etc.)* [ASM 33, 36, 37]
h. List the steps of PCR and explain the function of each ingredient [ASM 36]
i. Describe the role of the 16S rRNA gene in the study of evolutionary relationships [ASM 4, 5]
j. Run an NCBI BLAST analysis [ASM 34]
k. Describe what multiple sequence alignment is and how it is used for understanding relatedness [ASM 5]
l. Analyze a phylogenetic tree in the context of sample metadata to explore scientific questions [ASM 5]
m. Formulate and evaluate hypotheses, describe experimental procedures, and discuss sources of uncertainty for a microbial ecology experiment* [ASM 28, 29, 30, 38]

PROCEDURE

Methylothon consists of lectures, homework assignments, and virtual labs and, optionally, an in-person leaf press lab (Fig. 1); all materials are available at http://methylothon.com. An optional element, but one that was included in all our 2021 lessons, is an initial guest lecture by a practicing microbiologist. Students prepare for the guest lecture by reading a blog post (23), profile, or a scientific article written by the researcher (24) and formulating questions. Guest lectures discuss current research in methylotrophy (e.g., the role of rare earth elements) or microbial ecology and biotechnological applications more generally. Material covered in the guest lecture is not required for the remainder of the module; rather, the primary goal is as an entry event to spark interest and provide an opportunity to interact with a practicing scientist.

After the guest lecture, the first lesson of the module covers the motivation for working with methylotrophs and an overview of the process of sampling, isolating, and sequencing isolates, presented either as preparation for the leaf press lab (for classes that include the lab) or as background information on the origin of the sequences that students will soon analyze (for classes that omit the lab). A "how to make a leaf press" video (available at http://methylothon.com) is provided to students as a guide for techniques such as the use of gloves, handling leaves, and parafilming a culture plate.

For partner schools that include the leaf press lab (see Appendix 2 in the supplemental material), it is assigned as asynchronous work due by the following day. In the lab, students collect plant leaves from their neighborhood, press them briefly onto selective media (see Appendix 3 for the recipe), and incubate the plates, wrapped in parafilm, at room temperature in their homes. In 2021, our team provided teachers with culture plates, gloves, and parafilm to distribute to students with instructions for handling. To explore the finding that some methylotrophs depend on rare earth elements for growth (24), each student used two plates: one containing lanthanum and one without. Incubation periods may last 5 to 10 days, depending on class schedule, though better results are obtained after >1 week. At the end of each lesson, plates with colonies can be returned to a partner lab (in 2021, the Martinez-Gomez lab at UC Berkeley), where isolates can be further characterized. Alternatively, classes carrying out in-person instruction may conduct colony PCR (see Appendix 4 in the supplemental material) and Sanger sequencing. Students are required to complete online survey forms to submit photographs of plants and culture plates (Fig. 2) and record observations, including location, host plant identity (we encourage use of the iNaturalist Seek app, https://www.inaturalist.org/pages/seek_app), and any colony growth observed.

The second lecture covers the 16S rRNA gene and its role in microbial phylogeny. Students read two brief articles from the microBEnet website before lecture (25, 26) and review the homework via an interactive comment board, such as Google Jamboard. The lecture then discusses the tree of life and the use of 16s rRNA as a standard for measuring the relatedness of bacteria and archaea and reviews how to interpret relationships from phylogenetic trees, with practice problems and breakout rooms to check understanding. The third lecture covers DNA sequencing and multiple sequence

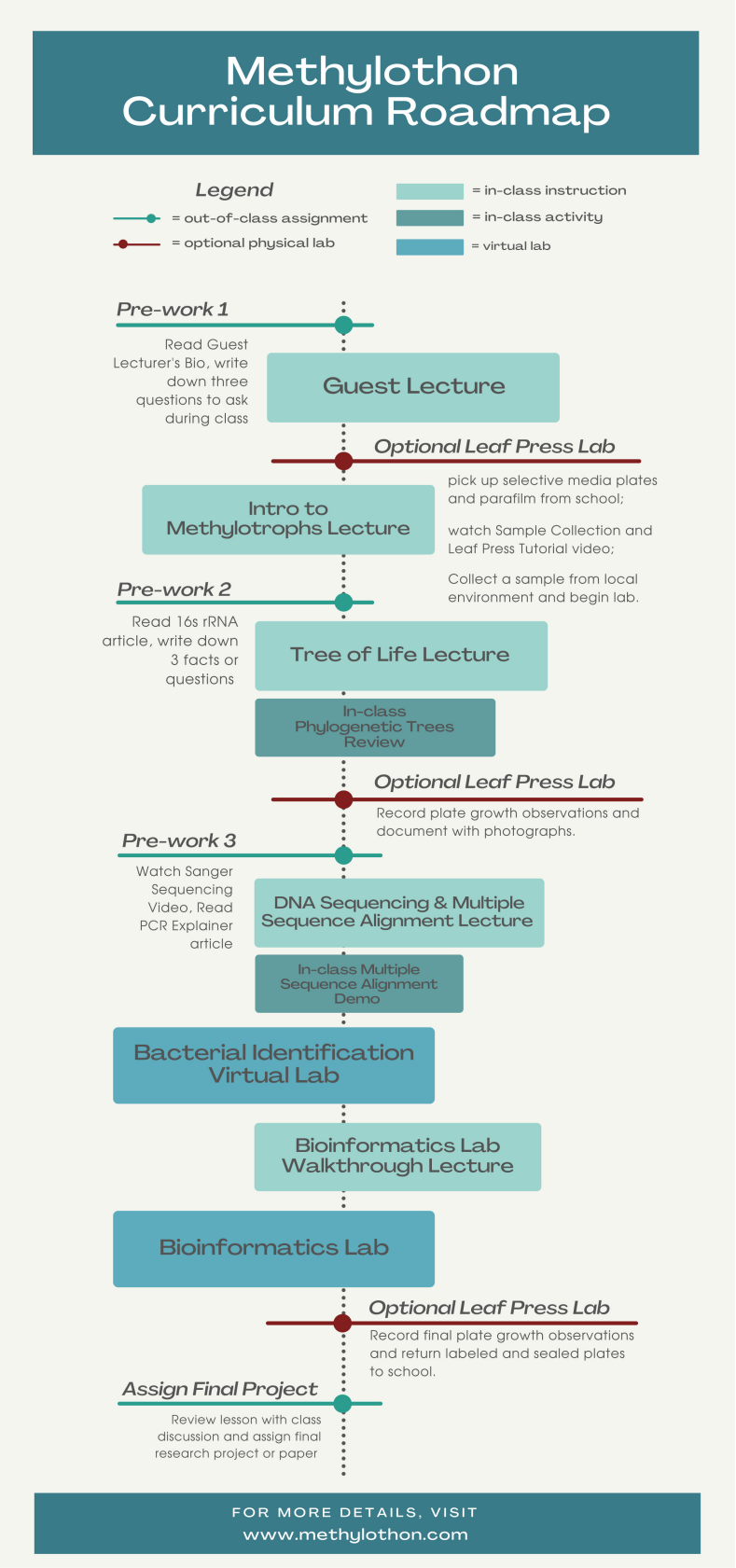

FIG 1. The components of Methylothon.

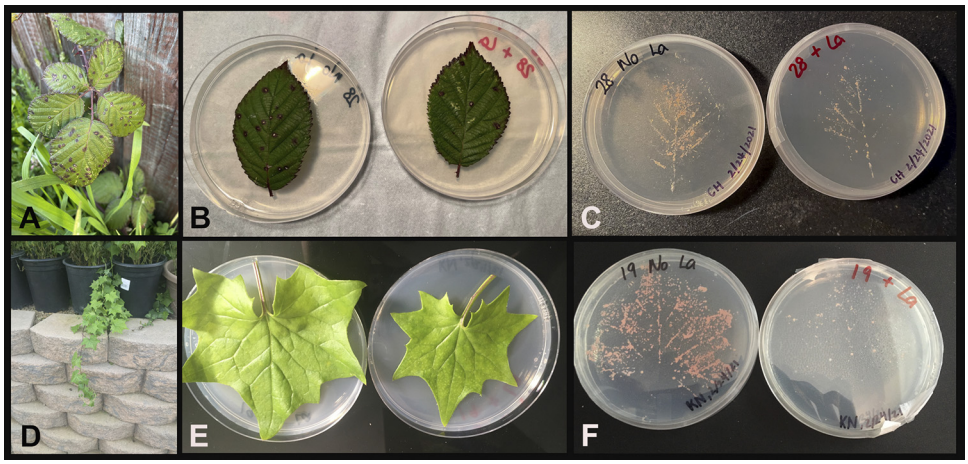

FIG 2. Selected photos of host plants and leaf press plates from Methylothon 2021. Both were collected in students' backyards in San Francisco. (A to C) Plant, leaf press, and colony growth from host, identified as brambles (likely Himalayan blackberry, *Rubus armeniacus*). (D to F) Plant, leaf press, and colony growth from host, identified as Cape Ivy, *Delairea odorata*.

alignment (MSA). Preassignments include short readings and a video on PCR and sequencing, reviewed in class using a Jamboard. The lecture covers interpretation of Sanger sequencing chromatograms and MSA and includes an in-class exercise on the underlying concepts of sequence alignment.

In place of the PCR and sequencing that would be done in lab during in-person instruction, students next complete a virtual lab on bacterial identification produced by Howard Hughes Medical Institute Biointeractive (https://www.biointeractive.org/classroom-resources/bacterial-identification-virtual-lab). The simulation begins with collecting a bacterial colony from a culture plate and ends with a "mini-BLAST" of the sequenced DNA. We provide worksheets to reinforce learning for either asynchronous independent or synchronous group work settings.

In the final lecture, we walk through the bioinformatics lab that the students will ultimately use to identify a mystery methylotroph. This includes NCBI BLAST and free online tools for sequence alignment and phylogeny. For the bioinformatics lab, students receive "mystery sequences," which are FASTA files of 16S rRNA gene sequences from methylotrophs isolated by previous classes. They also receive a file containing reference sequences of other bacteria for context. The students perform BLAST analysis on their sequences for an initial identification, then perform MSA and construct a phylogenetic tree with the mystery sequences and reference sequences. Although each student has a unique sequence, they work in small groups in online breakout rooms for peer support. Students complete a worksheet during the lab, and each student ultimately uploads an image file of their phylogenetic tree to a class slideshow. The final worksheet questions require students to interpret their own and their classmates' trees from the slideshow.

Suggestions for determining student learning

The main formative assessments for Methylothon are the handouts completed by students during the virtual labs, and a final project constitutes the summative assessment. Other components of the module, including submission of photos and metadata for the leaf press lab, can be used for judging participation. During virtual lab group work, instructors circulate among the breakout rooms, providing additional opportunities for informal assessment of learning and evaluation of the module itself.

In 2021, teachers developed final projects based on their class' specific learning goals; examples are provided on our website. For instance, students in one biotechnology class wrote a 2-page individual lab report summarizing the leaf press lab, including a literature search on a question of their choice related to microbes in biotech (see Appendix 5 in the supplemental material). In an international baccalaureate (IB) biology class, students wrote group reports describing what they could and could not conclude from their leaf press lab results, practicing IB international assessment rubric guidelines (see Appendix 6). That class subsequently assigned students to write 5- to 6-page individual essays combining Methylothon and a previous unit on evolution, to make an evidence-based argument and design an experiment demonstrating evolutionary principles. Another biotechnology class combined Methylothon with a human ancestry unit and asked students for a report in their chosen format (video, slideshow, essay, graphic novel) on the process of identifying biological samples using PCR, sequencing, and phylogenetics (see Appendix 7). Pairing modules thus can emphasize the universality of these methods and principles.

Sample data

Methylothon generates both work that can be used for evaluating learning and sample metadata and observations that can feed into the community science component. Summaries of sample metadata from 2021 are provided in Fig. 3A (see also Appendix 8 in the supplemental material), and examples of students' observations are provided in Table 3. Appendices 9 and

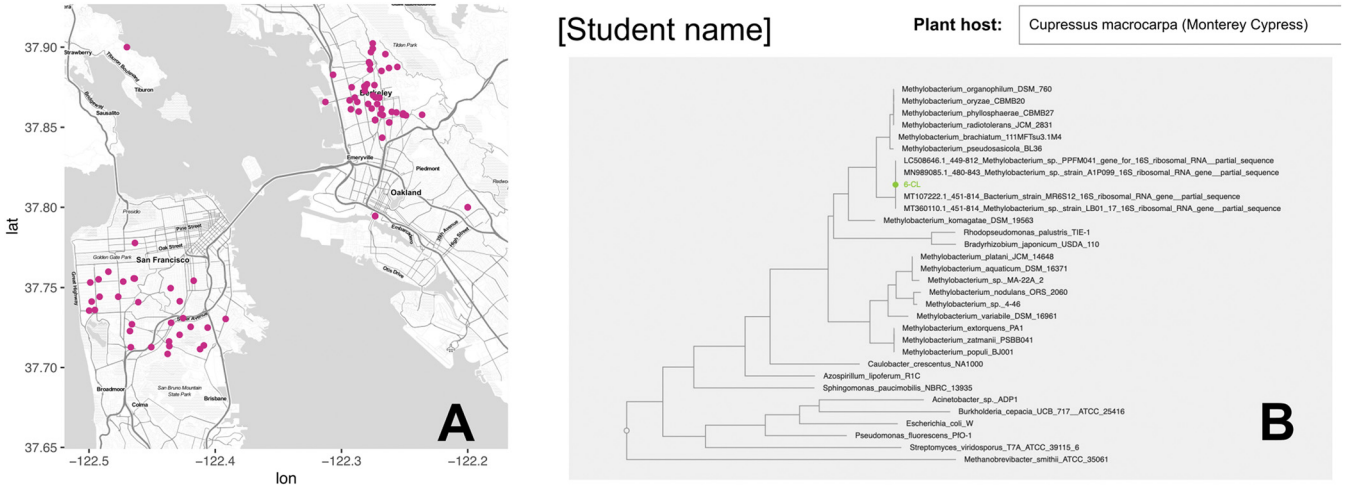

FIG 3. (A) Sites sampled for plants by the San Francisco and Berkeley high school students during 2021 Methylothon; each pink dot represents one sample site. Map tiles were obtained from Stamen Design as open source materials, and the map was generated using R v4.0.2 with RStudio v1.3.959. (B) Example of a student's phylogenetic tree, in which the name of the student's mystery sequence is in green text. Image taken from the class phylogenetic tree slide show; student's name is omitted.

10 show examples of students' individual and group work for evaluation.

Safety issues

Methylothon carries relatively few safety risks. For class-based plant sampling trips, typical field trip safety issues apply. For in-person instruction, instructors should provide lab safety training as necessary; please see the ASM Guidelines for Biosafety in Teaching Laboratories (27) and the "Addendum for biosafety considerations regarding at-home or DIY microbiology kits," with the caveat that there is no commercial kit provider to assume liability. Students at home do not use ethanol, flame, or other components of sterile technique that typically introduce laboratory hazards. Moreover, students do not reopen parafilmed leaf press plates, so the risk of contact with bacterial cultures is low.

One unavoidable hazard is the cycloheximide in the culture medium; we have found its inclusion to be necessary for inhibiting fungal growth. No occupational exposure limit has been established for cycloheximide, but it is mutagenic and teratogenic. Exposure for students is low because they do not handle concentrated chemical, but they must wear gloves when handling culture plates. Safety information on cycloheximide is included in the lesson materials, and instructors should emphasize to students not to touch the agar even with gloves, to leave plates always sealed with parafilm, and to wash hands after the experiment. For remote instruction, students must return culture plates and gloves to instructors for appropriate disposal in lab.

Because sample metadata uploaded to http://methylothon.com will be made publicly available alongside sequence data, student privacy should also be taken into account. Student names are collected so instructors can track participation, but they are never published. If students sample at their homes, they are allowed to list GPS coordinates from nearby that do not identify their home addresses.

DISCUSSION

Field testing

The in-person version of Methylothon was delivered in two consecutive years (fall 2018 and 2019) to an Advanced Biotechnology class (seniors) at Abraham Lincoln High School (ALHS) in San Francisco, CA. When many schools moved instruction online during the COVID-19 pandemic in the 2020–2021 school year, we modified Methylothon for remote learning and simultaneously expanded to new schools. Our spring 2021 partners included ALHS, three sections of IB Biology (seniors) at Berkeley High School in Berkeley, and four sections of Biotechnology (juniors and seniors) at Galileo Academy of Science and Technology in San Francisco. Together, this entailed approximately 220 students and 55 h of synchronous class time, with substantial variation in learning goals and student experience level. COVID-related institutional policies also varied, such as the balance of synchronous versus asynchronous learning and the regulation enforced by some schools that students not be required to download software or register for online user accounts.

Evidence of student learning

Each of the 2021 Methylothon classes assessed students differently. One of the biotechnology classes (3-week schedule) used formative assessments during the sequence on PCR and bioinformatics and summative assessments (analysis of phylogenetic trees and a final "abstract" writing assignment) at the end (see Appendix 5). Students were evaluated

TABLE 3
Examples of metadata submitted by students for the leaf press lab[a]

Sample code	Description of sample location	Other observations	Where in your house did you incubate your plate?	What is the general appearance of the colonies?	Plant identification	Approximately how many colonies are there on the lanthanum side of the plate?	Approximately how many colonies are there on the non-lanthanum side of the plate?
CH 2242021	The sample was taken in my backyard, which faces the ocean. My plant was surrounded by a grassy area with minimal flowers. Today was a relatively sunny day and the area where I sampled my leaves from gets a lot of sunlight.	The plant leaves have many thorns on the back and stems so when placing the leaves on the agar plate, the thorns pierced the agar. The rest of the process went smoothly.	On my desk in my bedroom	The colonies are light pink, salmon-colored, and are crowded around the veins of the leaves. Overall, they are about the same size, but there are some that stick out more than others. On the plate without lanthanum, the colonies are hard to distinguish from each other because they are so packed together.	Brambles	100	500
KN 2/24/21	The sample location was my backyard, which resides in a considerably chilly area of San Francisco, in the Excelsior district. The terrain is mountainous, and we grow chili peppers as well as numerous other things. There are many weeds and leaves of sorts. The amt of sunlight received within the area is decent, especially early afternoon. The sample grew from the ground and not in a pot.	Might have cracked the agar, but not entirely sure. Can clearly identify the outline of the plant on the agar, however. Process went smooth, in terms of sampling and identifying the plant. One of the leaves was a bit too big for the plate but somewhat worked.	I incubated my plate in my room, besides the window. It protects the plates from excessive sunlight but still allows some to shine in, therefore the agar would not dry out. The area is bit chilly and moist.	On the 19 No La plate, the colonies are all pink, some lighter in color than others. They take the form and shape of the leaf, and do not spread out too much. The colonies are very tightly packed and it is difficult to distinguish among them. On the other hand, the 19 + La plate has a few white colonies that are opaque, and the rest are light pink, much lighter than the colonies on the 19 No La plate. They are very spread out, compared to the 19 No La plate.	Cape Ivy *Delairea odorata*; Genus: Delairea, Family: Asteraceae; classified within tribe Senecioneae. Also known as German ivy in other parts of the world	>30	>300

[a]This information is a subset of the metadata accompanying the samples shown in Fig. 2; it was entered by students in the online form as part of the leaf press lab. School name, sample date, and GPS coordinates have been omitted.

for their understanding of methylotroph biology, communication of methods, interpretation of results, and elements of the scientific method, such as research question formulation and use of references (see the scoring guide in Appendix 5). More than 70% of students showed early mastery of concepts related to microbial culturing, PCR, DNA sequencing, and DNA databases. Fewer demonstrated mastery of bioinformatics core concepts, particularly the use of BLAST and phylogenetic trees, with most difficulties relating to the purpose of intermediate steps of the analysis pipeline. We attribute this to the fact that it was the students' first exposure to these concepts; future implementations might include additional background learning and/or practice opportunities to supplement Methylothon.

The IB Biology class (1-week schedule) assigned a summative assessment in which teams of 4 synthesized their work in a collaborative essay, including a hypothesis they had formulated and tested during the leaf press lab. Students were evaluated on their ability to incorporate background research to develop their research question and hypothesis, summarize their procedure, analyze their data quantitatively and qualitatively, develop a conclusion supported by evidence, and evaluate their experimental design (see the assignment and grading rubric in Appendix 6). The area that proved most challenging was the analysis of nonprocedural sources of error and limitations of the experimental designs. Furthermore, because students were given only 1 to 2 days to develop their hypothesis, many did not have time to find and analyze scientific articles, but rather they developed hypotheses based on background knowledge and information from the unit. However, all teams demonstrated achievement of core objectives, with 100% of the 21 teams receiving a grade of a 3.5 (out of 4) or higher and 16 teams receiving a 4. The same class completed an additional assessment asking students to design an experiment using MSA, PCR, and BLAST to demonstrate microbial evolution. Over 90% of students successfully utilized the information from Methylothon, and many even featured bacteria outside of the methylotrophs. We recommend that classes consider incorporating experimental design in summative assessments for Methylothon, so that students may continue developing these essential skills.

In addition to school-specific assessments, we gathered feedback from all classes via an anonymous survey sent 5 to 8 weeks after the final lesson. We asked two questions: what did students remember most from the lesson, and what did they consider the most valuable takeaway. We received 79 responses, which we grouped into 7 categories based on main topic (Fig. 4). Students reported remembering and valuing concepts relating to phylogenetic trees most often, followed closely by BLAST. We are encouraged by this result—most students remembered the lesson's central goals! Additionally, several students connected BLAST and building phylogenetic trees in their answers. To a lesser extent, students remembered intermediate steps, such as DNA sequencing and the use of databases to retrieve information.

In addition, students who participated in the leaf press lab (48 of 79 respondents) responded positively to conducting in-person lab work; 25% of those students reported that they

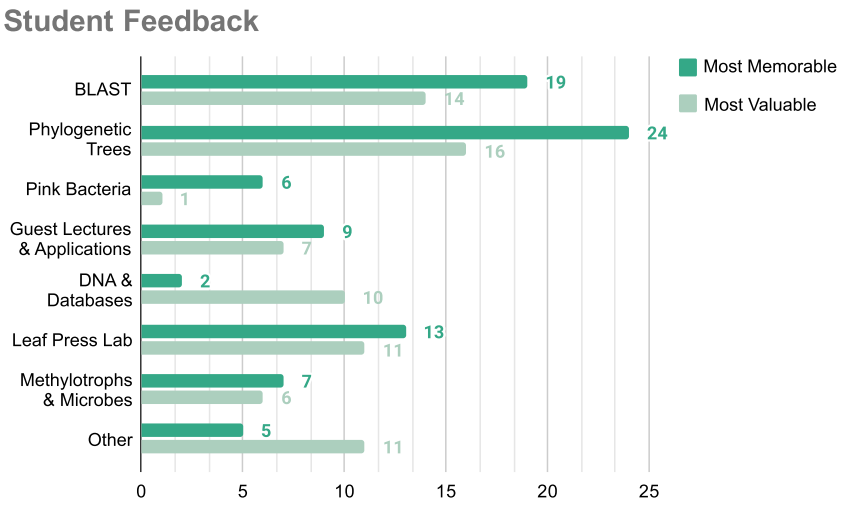

FIG 4. Topics mentioned in student responses collected from an anonymous survey that asked "What's the one thing you remember most about the lesson?" and "What did you find most valuable about the lesson?" Some responses incorporated multiple topics and were therefore counted more than once. Responses not fitting a predefined category were classified as "Other."

most remembered the opportunity to do a nonvirtual lab, and nearly 20% reported that the lab was what they found most valuable about Methylothon. Finally, several of the students who did the lab mentioned that *Methylobacterium* are pink, highlighting the memorable nature of this phenotype. Some other facets highlighted by students as particularly helpful included the opportunity to speak to scientists about their background and research interests and the chance to "experience what it is like to be a real scientist" (Box 1).

Possible modifications

Teaching Methylothon to several partner schools helped us identify multiple areas for improvement. One major pivot we made in later lessons was to change the file extensions of the DNA sequence FASTA files. FASTA is a format for molecular sequence data in a plain-text file; the .FASTA file extension can remind users of the format and help sequence analysis programs recognize the file. However, most students did not have the necessary underlying knowledge of file formats, extensions, and how to use files without viewing them, leading multiple classes to lose lesson time. We therefore changed the file extensions to .txt.

Additionally, we found some students struggled to translate concepts from our initial phylogeny lecture to their interpretation of the trees they generated, including the significance of the reference sequences. We therefore added a walkthrough of tree interpretation immediately after the students had shared their phylogenetic trees in the class slideshow and before they began interpretation. We found this review helped students approach interpreting their own results with more confidence. Relatedly, we found that some classes might do better with a lesson structure based on "just-in-time" learning. When Methylothon was stretched across multiple weeks, the elapsed time between lectures and hands-on practice in the bioinformatics lab allowed some concepts to be forgotten before they

BOX 1
Selected responses from the anonymous student survey

- "What I found most valuable about the lesson was honestly just being back in a lab oriented setting to a certain extent. Since COVID-19 has affected us in not doing many labs, I believe this lab was something that made me feel a bit more interested and motivated again."
- "[What I valued most was] seeing the other student's [phylogenetic tree] results to compare."
- "[What I valued most was] getting to experience what it is like to be a real scientist."
- "The lesson I found most valuable was being able to conduct the expt, even if it was virtual, I think that really fun and engaging even over [Z]oom."
- "[What I valued most was] the chance to watch as pink bacteria grew on my petri dishes in the shape of the leaves, knowing that my data may potentially be used in a real scientific paper!"
- "I found the most valuable part of the lesson being learning about what is actually being researched right now in the field of biology."
- "I found the introduction of BLAST the most valuable. It was my first time hearing of this term, and being taught how to utilize this program is a great skill I could carry with me in the future."
- "The thing I found most valuable about the lesson was how easy it is to go outside and collect a sample to learn more about."

were practiced. One partner teacher related that they would consider breaking up the bioinformatics lab so that individual steps can followed immediately after the relevant lecture.

The unique challenges of executing lab work in a remote learning environment included the question of how to provide a valuable scientific experience for the students, without being able to identify the methylotroph isolates. In one class, students formed hypotheses that could be evaluated based on colony growth alone and then discussed limitations in the experimental setup (e.g., the number of plates available, lack of controls, short incubation time) in their written report. For students who had experience with fast-growing organisms such as *Escherichia coli*, we had to moderate expectations by emphasizing methylotrophs' slower growth rates.

Adoption by other programs

With Methylothon we aim to provide a framework for a microbiology unit that is adaptable and accessible to diverse high schools and microbiology research laboratories. For most programs, the most challenging implementation issue will likely be making the leaf press lab culture medium. Interested groups are welcome to contact the authors for help with the C7 metals mix, the most complex component of the medium.

With schools returning to in-person instruction, two of our participating 2021 schools chose to repeat Methylothon in spring 2022 and continued to adapt and expand it. One school that had not previously carried out the leaf press lab added it, and the other added a new ecological statistics exercise based on methylotroph biogeography in order to incorporate Methylothon into their ecology unit (whereas it had previously been part of an evolution unit). We foresee ample opportunity for further adaptations. As the name Methylothon implies, it was originally intended to incorporate an element of competition. For classes able to devote more time, students' isolates could be cultured by partnering research laboratories and "competed" in a wide variety of phenotypic assays to introduce concepts of ecological niche and evolutionary adaptation, while providing a chance for every isolate to "win" at something. As more lessons are developed and more methylotroph data are gathered each year, we will continue to expand the offerings on the Methylothon website.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 2.5 MB.

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We declare no conflict of interest.

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