INTRODUCTION

Regulation of bacterial processes by population density (quorum sensing) is often a difficult concept for students to grasp, and understanding how cells communicate is a core concept in introductory microbiology (2). Quorum sensing is typically a new concept for introductory microbiology students, involving complex genetic feedback mechanisms (4). Light production in bacteria, specifically the lux operon of Vibrio fischeri, is a good model system for teaching students about quorum sensing. V. fischeri is a marine bacterium that can form a symbiotic relationship with a host squid, Euprymna scolopes. Light production by V. fischeri only occurs at high cell densities, when this bacterium grows in the nutrient-rich light organ of the squid (6). Active learning has been shown to improve student learning and engagement with material (for example see 1, 5), and so we developed an interactive classroom activity where students enact the dynamics of bacterial chemical communication at low and high cell densities. We also developed a worksheet where students collaborate and use what they know about V. fischeri lux operon genetics to predict outcomes of genetic complementation experiments between mutant strains.

Approximately 40 college junior and senior students (ages 19 to 21) enrolled in a Principles of Microbiology course comprised our test audience for this activity. These activities were designed for an introductory microbiology course, with a genetics course as a prerequisite, and are best conducted toward the end of the semester as a way to integrate concepts of bacterial genetics and communication. The interactive activity and worksheet were part of a one-class lecture (75 minutes) on bioluminescence and its applications, symbiotic relationships of bioluminescent bacteria, and the lux operon of Vibrio fischeri (lecture material available upon request).

PROCEDURE

Prior to implementing these classroom activities, we registered our project with the SUNY Geneseo Institutional Review Board. During the lecture we introduced the lux operon (Fig. 1). The operon is composed of four main parts: luxI, luxR, luxAB, and luxCDE (reviewed in (3)). LuxR encodes a transcription factor that, when bound to an autoinducer molecule, up-regulates lux operon gene expression. LuxI encodes a synthase for the autoinducer molecule (acyl-homoserine lactone; AHL), and the rest of the operon encodes for the necessary components to make light. After the lecture, a short quiz was given to assess student understanding of these concepts (sample questions in Appendix 1).

After the lecture, students were moved into a large space and we ran the following activity twice, first with a small group (five students), and next with the entire class (40 students). Each student was given a small packet of labeling stickers and one index card and instructed to move randomly within the space. Stickers represented autoinducer (AHL) molecules, and students exchanged an “autoinducer” (sticker) whenever they passed each other, placing it onto their index card. Exchanging stickers was not a perfect analogy for movement of the autoinducers, and it was emphasized that bacteria do not physically exchange these molecules (they diffuse in and out of the cell and are picked up from the environment). Initially, students hand out only one sticker at a time. A student who has collected two stickers on an index card then hands out two stickers. As students accumulate stickers, they hand out the same number of stickers as are on their index card (i.e., if they have three stickers, they now hand out three stickers to each student they pass). This is analogous to upregulation of the lux operon. When students collected five stickers on their index card, they were instructed to make a beeping sound, representing emission of light. We chose to represent light production with an auditory cue, rather than a visual one, as this allowed students to assess the level of “bioluminescence” without interrupting student interactions. The activity is conducted in exactly the same manner for small and large groups, and the small-group

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activity clearly showed that at low population density, quorum sensing was not sufficient to bring about much, if any, beeping (bioluminescence). When repeated with the larger group (high population density), almost all students were beeping within approximately one to two minutes.

We then shifted to gaining a more in-depth understanding of the \textit{lux} operon. Students were given a worksheet that presented four different strains of \textit{V. fischeri}, including one wild type and three mutants (Appendix 2). Students were given four practice problems that asked them to predict bioluminescence phenotypes when various strains were streaked opposite to each other on the same plate. Before students got started, we showed a video of a similar complementation experiment from the Howard Hughes Medical Institute BioInteractive website (www.hhmi.org/biointeractive/bacterial-quorum-sensing), and we explained how two strains streaked next to each other on a plate can share diffusible molecules, such as AHLs. Students were given approximately 10 to 15 minutes to complete the worksheet in groups, and we then discussed it as a class.

**CONCLUSION**

Overall, both activities were successful at increasing student engagement with the topic and their understanding of the material. For the interactive demonstration, the difference between small and large groups was striking. A potential issue for this activity is making sure that students fully understand the analogy and are able to extrapolate back to what actually occurs in a bacterial population. To add in a quantitative aspect, students could record the amount of time it takes to for everyone to be beeping in smaller groups compared with progressively larger groups, or record the level of beeping using a decibel meter (many smartphones are capable of measuring decibels).

In larger classes, the interactive activity could be done as a demonstration with a subset of the students, or (if facilitators are available) students could be broken up into groups of 20 to 40 to complete this activity. The activity is straightforward to run, and instructors could even have students serve as facilitators for each group after a brief overview of the activity rules. The genetic complementation worksheet would work well in small or large classes. If time is limited, the interactive activity and the worksheet can be completed separately. Instructors can choose to complete either one of the activities alone based on the level of detail desired (the interactive activity emphasizes the basics of quorum sensing, while the worksheet emphasizes quorum sensing genetics), or the genetic complementation worksheet can be assigned as homework instead of being completed during class.

**SUPPLEMENTAL MATERIALS**

Appendix 1: Sample quiz or exam questions for instructors
Appendix 2: Genetic complementation worksheet

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