

An Inquiry-Based Laboratory Module to Promote Understanding of the Scientific Method and Bacterial Conjugation †

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INTRODUCTION

Conjugation, or mating, is the transfer of DNA from a donor to a recipient cell via direct cell contact (2). As a major form of horizontal gene transfer, conjugation leads to the spread of antibiotic resistance and virulence genes in bacteria. Conjugation is often induced through exposure of the donor cell to cellular stress or DNA damage, thereby increasing the chances of propagation and survival of the conjugative DNA element (1, 3). This laboratory module allows students to explore the effects of stress conditions on conjugation frequency and donor cell viability of the safe Gram-positive model bacterium, *Bacillus subtilis*. Previously, mitomycin C was shown to induce mating of the conjugative element ICEBsI of *B. subtilis* (1). Mitomycin C damages DNA, which induces ICEBsI conjugation through activation of the SOS response, a global response to DNA damage that stops the cell cycle and turns on DNA repair. Other conditions that stimulate the SOS response in *B. subtilis* are also likely to stimulate mating. It is also possible that sublethal levels of biocides and antibiotics induce conjugation of ICEBsI as seen for other microbes (3; see also Appendix 1).

A common learning goal for many courses is for students to understand the scientific method. However, most laboratory manuals focus on teaching technical skills without allowing students to design their own experiments. Over three lab periods, students apply the scientific method to determine whether their chosen stress conditions induce conjugation and cell death. In the first lab, students consult the primary literature to identify conditions that cause DNA damage, the SOS response, and/or cellular stress. In groups, students develop their hypotheses and formulate their experimental plans, including positive and negative controls. In the second lab, students subject the donor cells to the chosen stress conditions and conduct mating assays. In the final lab, students plot their collective data on

mating frequency and cell viability (Fig. 1) and discuss their results in light of the controls. This exercise would be an ideal capstone project in an undergraduate microbiology, genetics, or multidisciplinary research laboratory course to reinforce understanding of conjugation and the scientific method. This module also allows students to learn or apply several important laboratory skills including aseptic technique, serial dilutions, and spectroscopy.

PROCEDURE

Student handouts with protocols (Appendix 1) and detailed instructor notes with recipes (Appendix 2) are available as supplemental materials.

Materials

Days 1 and 3. Computers.

Day 2. *B. subtilis* donor and recipient cells (strains IRN342 and CAL419, respectively, available through the Bacillus Genetic Stock Center), LB, minimal salts agar plates, LB agar plates with antibiotics (kanamycin 5 µg/mL for donors,

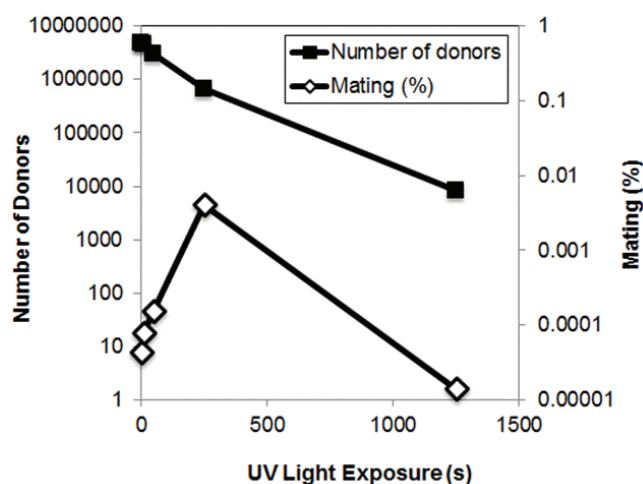


FIGURE 1. UV light induces mating and cell death of *B. subtilis*. Representative class data for % mating and donor cell viability after exposure to UV radiation for varying amounts of time. The experiment was performed as outlined in the Appendices.

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†Supplemental materials available at <http://jmbe.asm.org>

streptomycin 100 µg/mL for recipients, and kanamycin 5 µg/mL and streptomycin 100 µg/mL for transconjugants), minimal salts, shaking water bath, incubator, flasks, 50 mL conical tubes, forceps, glass beads, microcentrifuge tubes, micropipettes and tips, Nalgene filters (Fisher Scientific cat no. 09-740-30G), mitomycin C (Sigma), and experimental stress agents.

Safety issues

B. subtilis is a BSL-I organism. Gloves, goggles, and lab coats should be worn. Material Safety Data Sheets (MSDSs) should be consulted. Care should be taken when handling any biocides or DNA damaging reagents. The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories, available at www.asm.org. See also Appendices for more detailed safety information.

Activity

Day 1. Using the primary literature, students should research conditions that elicit the SOS response and cellular stress, such as DNA-damaging agents and biocides. Possible agents include ethanol, hydrogen peroxide, antibiotics, detergents, metals, heat, and UV light. Since dosage is critical, a wide range of concentrations should be tested. In groups, students should develop their protocols, determine a negative control (no treatment) and positive control (mitomycin C) for the experiment, assign tasks, and predict the trends in mating and cell viability that they expect to observe.

Day 2. Students should expose donor cells to the chosen treatments, measure the optical densities of the donor and recipient cultures, and mix donors and recipients in a 1:1 ratio. Donor and recipient cells are mated on a Nalgene filter on minimal salts agar at 37°C for two hours. Cells are resuspended from the filter and serially diluted prior to plating on various antibiotic plates. Plates are incubated overnight at 37°C.

Day 3. After the numbers of colonies are counted on the antibiotic plates, the percent mating and donor viability are calculated. Students should share results, analyze controls, and interpret their collective data. The data should be graphed (Fig. 1) and the class should discuss whether or not the observations coincided with the hypotheses.

CONCLUSION

Students are engaged and improve their critical thinking skills in laboratory courses when they have the opportunity to develop and conduct inquiry-based experiments that generate novel results. In this lab module, undergraduate

students develop hypotheses and conduct original research, while gaining insight into conjugation and conditions that cause cellular stress and DNA damage. In contrast to traditional “cookbook” experiments with predetermined results, this module is useful for introducing students to both the excitement and challenges of authentic laboratory research. This module is flexible, and could be modified to fit into the curriculum of an introductory or advanced laboratory course. Conditions that are known to induce the SOS response, such as UV light and DNA damaging agents, are likely to induce mating. Other conditions that induce a general stress response, such as heat or detergents, are more exploratory but could yield fascinating new avenues for research.

This module was field-tested in a small advanced research laboratory course at Suffolk University. Based on a number of assessments, the module enhanced student understanding of the scientific method and the process of conjugation. In addition, more than half of the students commented in the final course evaluations that they greatly enjoyed designing their own experiments.

SUPPLEMENTAL MATERIALS

Appendix 1: Student handouts

Appendix 2: Instructor notes

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