Bacterial Calligraphy: A Memento for Undergraduate Research Students

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INTRODUCTION

The research project is a major assignment for final-year undergraduate students. As part of the project’s learning outcomes, undergraduate students are exposed to research methodology, design, and execution of experiments, as well as to data interpretation and presentation. By learning to work with other colleagues in the laboratory (postgraduate students who serve as their mentors, lab officers who help them with the logistics of research, as well as their fellow course mates), students also pick up communication skills that will prove to be useful either in the workplace or in graduate school. Given the intense nature of the research experience in time and in effort, and the close working relationships and camaraderie that develop, a memento would be fitting at the conclusion of the research project.

Various species of bacteria grow on solid agar medium with different morphologies and colors that, in addition to being aesthetically pleasing, can also be arranged into spatially defined patterns; cultures of these bacteria can be used to draw patterns on an agar surface as a memento for the students. In this communication, a method is described for the creation of such a memento in which a purple pigment-producing soil bacterium, Chromobacterium violaceum, is used as an “ink” to inscribe the initials of each student’s name on an agar plate in a process named “bacterial calligraphy.” The method is simple and can be accomplished with standard microbiological tools—incubators, Petri dishes, shake-flasks, and inoculation loops—without the need for specialized equipment.

PROCEDURE

Many species of bacteria are known to produce structurally complex pigments that span the color gamut. Given the energy-intensive process of producing these pigments, it is not surprising that they often confer benefits to the bacteria; for example, functioning as antibiotics to protect the bacteria against other microbes. Chromobacterium violaceum, a Gram-negative bacterium belonging to the β subclass of Proteobacteria, produces a violet pigment known as violacein from the conversion of L-Tryptophan through an enzymatic cascade encoded by the operon—vioABCDE—during the stationary phase of growth (1, 3, 4). Violacein is an effective antibiotic against bacteria and fungi (2, 4, 6); thus, its production and extracellular secretion provides C. violaceum with protection in its native soil environment. Besides its ability to synthesize and secrete a colored pigment, another advantage for using C. violaceum as a patterning agent is that it does not engage in swarming motility—that is, the colonies remain circular and do not spread out—when grown on solid agar surface, unlike colonies of Bacillus subtilis and Pseudomonas aeruginosa (5, 7). C. violaceum is able to form spatially defined patterns that are stable on an agar surface over time. Another important consideration is the need for adequate color contrast between the pigment and the agar medium—which, apart from specialized agar and chromogenic medium, is usually light beige in color—so that the inscribed pattern can be clearly seen and photographed. Together with the color stability of the violacein pigment and well-defined round colony morphology, C. violaceum is a good choice for a pattern-forming agent on an agar medium (Fig. 1).

C. violaceum (ATCC 12472) was grown aerobically in Difco’s LB Lennox medium (composition in g/L: Tryptone, 10.0; Yeast Extract, 5.0; NaCl, 5.0) using a shake-flask culture format from a glycerol stock culture stored at –70°C. After 24 hours of cultivation at 30°C and 230 rpm, the culture should have reached stationary phase—characterized by a distinct violet color—with sufficient cell density for subculturing on a solid medium. At this point, a sterile 10 μL inoculation loop was used to aseptically withdraw an aliquot of the culture in a Class II Biological Safety Cabinet and used as inoculum for a LB Lennox agar plate (composition in g/L: Tryptone, 10.0; Yeast Extract, 5.0; NaCl, 5.0; Agar, 15.0). An inoculating loop was used to “write” the student’s initial (or another suitable pattern) on the surface of the agar plate. During the “writing” process, the application of the inoculation loop onto the agar surface had to be gentle in order not to pierce the agar. After inoculation, the agar plate was incubated at 30°C for 24 hours in an inverted position, after which the purple-colored pattern was clearly visible against the beige agar

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background; in the cases shown, the pattern was the initials of each student’s name (Fig. 2). As it would not be safe for the students to store the agar plates—with cultured bacteria—in their homes, the lids of the agar plates were removed to prevent light reflections and photographs of the plates were taken (inside the biological safety cabinet), both individually and as a group, and distributed to the students to serve as “mementoes.”

As the thickness of the streak lines depends on the size of the inoculation loop as well as on the cell density of the inoculum, a 1μL inoculation loop could be used to create thinner streak lines and achieve greater spatial control. Other chromogenic species of bacteria that produce colored extracellular metabolites can also be employed in “bacterial calligraphy,” so long as they produce a water-insoluble pigment. Unlike C. violaceum, however, where the violacein pigment adheres to the cells (8), the greenish yellow pyoverdine siderophore of Pseudomonas fluorescens and P. aeruginosa tends to diffuse non-controllably through the agar medium and distorts the desired pattern (personal experience).

FIGURE 1. Well-defined round colonies of Chromobacterium violaceum on LB Lennox agar after 24 hours of growth at 30°C. The purple violacein pigment remained associated with the colony and did not diffuse through the agar medium, making this bacterium useful for creating calligraphy or drawings on agar surfaces.

FIGURE 2. Individual agar plates inscribed with the initials of each student’s name using the method of “bacterial calligraphy.” Slight imperfections such as thick streak lines, imperfect coverage as well as extraneous colonies and lines highlighted the level of care needed to master the calligraphy technique that uses bacterial cells as “ink.”
CONCLUSION

Research, at its core, is an experience in life. In addition to seeking nature's truth and fathoming life's mysteries, the research project can also bring about camaraderie between students with a shared experience. In many areas of chemical and microbiological research, the subject matter of the students' studies—inorganic materials or microorganisms—are not suitable for keeping as mementoes due to safety, health, and toxicity concerns. This short article describes a simple method for using microorganisms—ones that secrete pigmented metabolites—as "ink" for the formation of patterns resulting from a calligraphy or drawing on an agar medium, whereupon a simple photograph serves as a permanent timestamp for a period of time in the students' lives when, in addition to picking up research skills, they also developed strong, life-long friendships with their fellow students.

The method is not restricted to Chromobacterium violaceum, but for good control of the pattern formation process as well as for clear legibility of the streak lines, a couple of prerequisites of the bacterium-pigment system must be satisfied: (i) the bacterium should not engage in swarming motility when grown on agar, (ii) the pigment should not have high diffusivity in the agar medium and preferably should adhere to the cells, and (iii) there should be good color contrast between the agar medium and pigment. Besides its use in making a memento, the "bacterial calligraphy" method can also be used in other pattern formation applications both in research and teaching.

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.

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REFERENCES