Bacteria as “ink” for writing the initials of names on agar

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Abstract

Mementos encapsulate memories and serve as triggers for their recollections. By using a purple pigment producing bacterium as “ink” for writing on agar, a picture memento depicting the initials of students’ names was created, to help them remember the strong friendships that they had fostered with their fellow course-mates during their final year research projects. Besides the fun activity of “Bacterial Calligraphy,” the surface patterning technique can also serve as a simple and relatively low-cost tool for testing the feasibility of research ideas; for example, depositing cells in both straight and curvilinear lines on planar substrates for investigating possible interactions between different microbial species. A synopsis of the work and a structured abstract can be found in the accompanying PDF file, while the original article, “Bacterial Calligraphy: A Memento for Undergraduate Research Students,” has been published in the *Journal of Microbiology and Biology Education*, Vol. 13, No. 2, pp. 172-174, and is available at http://jmbe.asm.org/index.php/jmbe/article/view/414 as an open-access article.

Keywords: educational research; surface patterning; *Chromobacterium violaceum*; violacein; swarming motility; colour contrast; solid medium; cell spreading; inoculation loop; pigment diffusivity;

Subject areas: microbiology; education;

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Structured Abstract

Background

Besides acquiring knowledge and critical thinking skills, formation of strong, life-long friendships is another dividend of university education – and one that may positively impact on students' life, happiness and career long after graduation. In the final year of most honours programmes, students typically partake in a research project whose intensive nature requires devotion of substantial time, energy and effort – beyond those required of taught classes in earlier parts of the curriculum. For wet-lab projects, students typically spend much time conducting experiments and gathering data in the lab. And given the social nature of research where students exchange ideas and tips, as well as help one another, strong friendships (buttressed by understanding derived from close working relationships) are usually forged, even between students who have not met each other prior to the research experience. In particular, it was a gratifying experience watching my students progressing from strangers to friends.

Methods

Thus, an idea occurred to me of developing a simple memento for helping my students remember the friendships fostered during their final year research projects. Specifically, in a process akin to writing, bacterial cells were deposited from an inoculation loop onto agar medium for patterning the initials of the students' names. After overnight incubation where multiplication of cell numbers and secretion of a purple pigment helped gave form and colour contrast to the pattern, photographs of the agar plates – individually and as a group – constituted the memento.

Discussion

Success of the "Bacterial calligraphy" technique where the inoculation loop and bacterial cells served as "pen" and "ink," respectively, hinged on the motility mode of the bacterium, as well as colour intensity and diffusivity of the secreted pigment. Specifically, poor pattern reproduction would result from bacterial species that engages in surface spreading (i.e., coordinated movement of cells away from the point of deposition) or, pigments that diffuse into the surrounding agar rather than adhering to cells after secretion. Lack of colour contrast between pigment and agar, on the other hand, would reduce the aesthetic appeal of patterns and also hamper subsequent optical imaging. Finally, fine movement control is necessary for achieving precise patterning of cells on agar. Although the spatial resolution achievable by the described technique is typically lower than those of more sophisticated methods, it may nevertheless find use in niche applications where a simple (although coarse) surface patterning method would

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suffice in testing the feasibility of research ideas – for example, probing possible interactions between bacterial species arranged in defined patterns – prior to committing more resources and sophisticated instruments in a full study.

Conclusion

Collectively, utilizing bacterial cell growth and pigment secretion for conferring form to desired patterns, a simple and low-cost manual surface patterning technique was shown to be useful for inscribing both straight and curvilinear lines on agar – which, in addition to the fun activity of creating a memento, also holds potential for preliminary testing of methodological concepts in research.

Synopsis

Mementos are physical embodiments of special occasions or time-points in life - for example, graduation from university – and also serve as memory jots for recollecting specific events in the distant and recent past. Final year undergraduate students typically undertake a research project as a major assignment prior to completion of a four-year honours degree, during which they invest significant amount of effort and energy in delivering a quality project. Often assigned to work in a laboratory with other course-mates in separate projects, the intensive nature of the experience help fosters a strong sense of camaraderie between students who may not have met each other in university prior to the research experience. From the vantage point of a graduate student mentor guiding the students' research work, it was especially gratifying to see students form strong friendships and bonds with their fellow course-mates - and thus, the idea came to my mind of creating a memento to help my students remember this particular moment in their lives - and the friendships fostered.

Specifically, cells of the bacterium, *Chromobacterium violaceum*, were deposited on solid agar via an inoculation loop, which, after 24 hours of incubation, reproduced sufficient cell numbers to make visible the patterns (i.e., the initials of the students' names). Secretion of a purple pigment (violacein) by the bacterium further improved colour contrast between the pattern and beige agar background (Figure 1). Since the process of depositing bacterial cells on agar surface closely resembles a writing process - with the inoculating loop and bacterial cells serving as "pen" and "ink," respectively - I described the process as "Bacterial Calligraphy." (Ng, 2012)[1] Photographs of the agar plates - individually and as a group - serve as the mementos. Due to biosafety concerns, the agar plates with the inscribed patterns were decontaminated and disposed of as biological waste after photo-taking.

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Figure 1: Bacterial cells as "ink" for pattern formation. Cell growth and secretion of pigment along curvilinear and straight lines traced by an inoculation loop ("pen") made visible the pattern. In addition to the bacterium's motility mode and the diffusivity of secreted pigments, the experimenter's skill in "calligraphy" also plays an important role in determining the spatial resolution achievable. Note also that inadvertent drips of cells onto non-target areas may result in the formation of stray colonies away from the main pattern. Experimental details: *Chromobacterium violaceum* (ATCC 12472) incubated for 24 hours at 30 ^{o}C on LB Lennox agar. (Adapted from Ng (2012), *JMBE*, Vol.13, No. 2, pp. 172-174)

Beyond the fun activity described above, the technique of "writing" patterns with bacterial cells is also a useful surface patterning method for testing research ideas, given its ability of depositing cells in both straight and curvilinear lines. Bacteria, under both laboratory and natural environments, associate with cells of the same or different species in complex multicellular assemblage exhibiting intricate architecture[2] and possible division of labour and functions.[3] Nevertheless, we still know relatively little of the interactions[4] – mediated by myriad signalling and metabolite exchanges – between species. In particular, intense research efforts are focused on elucidating the mechanistic underpinnings and broader ecological significance of cell-cell interactions between and within species.[5, 6] Although unable to deliver the high spatial resolution achievable by other surface patterning methods,[7] the described technique can nevertheless be employed for patterning cells in preliminary studies seeking to test methodological concepts; for example, determining possible interactions between different bacterial species separated by varying distances - prior to executing a more detailed study employing more sophisticated techniques and instruments.[8]

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Spatial resolution of the inscribed pattern depends on the experimenter's skills in maneuvering the inoculation loop, the motility mode of the bacterium, and diffusivity of any secreted pigments. Specifically, bacterial species such as *Bacillus subtilis*,[9] *Proteus mirabilis*,[10, 11] *Pseudomonas aeruginosa*,[12, 13] and *Myxococcus xanthus*,[14] that engage in swarming motility (i.e., coordinated movement of cells away from the point of deposition) are not suitable for "Bacterial Calligraphy." Similarly, pigments that do not adhere to cells but diffuse into the surrounding agar medium would also significantly reduce the fidelity of pattern reproduction. In the described method, the purple pigment adheres strongly to cells – with no diffusion into or on the agar medium (Figure 2) – thereby, enabling relatively good pattern reproduction. Additionally, given that bacterial cells are typically translucent, visual discrimination of patterned and non-patterned areas is difficult; thus, selecting bacterial species whose secreted pigments adhere to the cell surface would help improve the resolution and fidelity of pattern reproduction, as coloured cells helps guide the patterning process. Finally, bacterial species that secretes pigments of strong colour intensity would be preferable – over those that do not - for enhancing the colour contrast between the pattern and agar background (usually beige in colour).

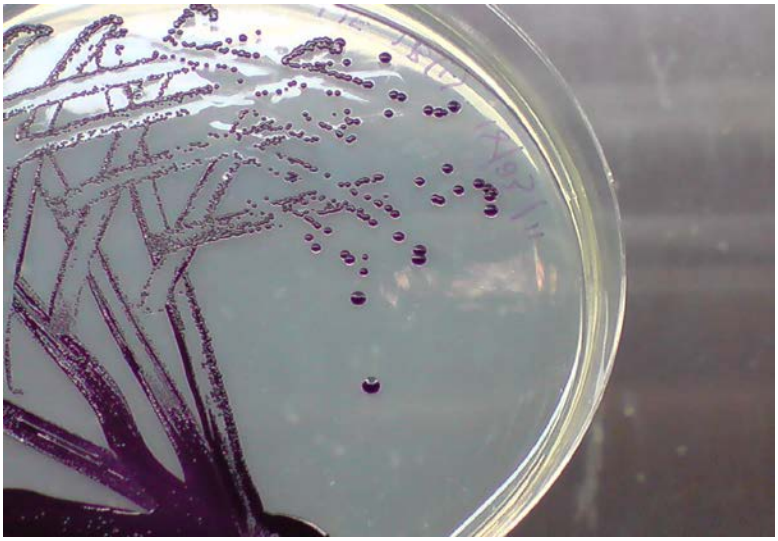

Figure 2: Distinct well-defined bacterial colonies are essential for good pattern reproduction. Adherence of the purple violacein pigment to cells of *C. violaceum* ensured the formation of well-defined colonies essential for high fidelity pattern reproduction. Lack of swarming motility in the bacterium, as well as strong colour contrast between the purple pigment and beige agar also contributed to the species' utility in surface patterning via "Bacterial Calligraphy." Experimental details: *Chromobacterium violaceum* (ATCC 12472) incubated for 24 hours at 30 ^{o}C after spread-plate inoculation on LB Lennox agar.

Collectively, a surface patterning technique, named "Bacterial Calligraphy," was used in creating a memento for the strong friendships fostered between students during their final year research

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projects. Specifically, the initials of students' names were patterned on agar via a "writing" process utilizing an inoculation loop as "pen" for depositing cells as "ink." Judicious choice of bacterial species concerning their motility mode, as well as colour intensity and diffusivity of secreted pigments is critical to high fidelity pattern reproduction. Finally, although the spatial resolution achievable by the described manual patterning technique is inferior to that of more sophisticated methods, its simplicity may afford its use in testing the feasibility of research ideas prior to detailed investigations utilizing elaborate techniques and instrumentation.

The article is available at *Journal of Microbiology and Biology Education*, Vol. 13, No. 2, pp. 172-174, http://jmbe.asm.org/index.php/jmbe/article/view/414 as an open-access article.

Conflict of Interest

This synopsis describes a published paper written by the author.

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