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 fostered 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 microorganism 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", is available in the *Journal of Microbiology and Biology Education,* Vol. 13, No. 2, pp. 172-174, http://www.asmscience.org/content/journal/jmbe/10.1128/jmbe.v13i2.414 as an open access article.

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

Subject areas: microbiology; education;

Structured abstract

Background

Besides acquiring knowledge and critical thinking skills, formation of strong, life long friendships is another dividend of university education – one that may positively impact on students' life, happiness and career long after graduation. In the final year of most honours programmes, students typically participate 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 laboratory. For this, students exchange ideas and tips, and help one another in the lab; thus, strong friendships are usually forged, even between those who have not met each other prior to the research experience. Personally, it was gratifying to see my students progressing from strangers to friends.

Methods

To help my students remember the friendships fostered during their final year research projects, I developed a simple memento for them. In a process similar to writing on paper, bacteria 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 (individual and as a group) constituted the memento.

Discussion

Success of the "Bacterial calligraphy" technique where the inoculation loop and bacteria cells served as "pen" and "ink", 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 bacteria species that engages in surface spreading (i.e., coordinated movement of cells away from the point of deposition, also known as swarming motility), or secreted pigments that diffuse into the surrounding agar rather than adhering to cells. On the other hand, lack of colour contrast between pigment and agar reduce the aesthetic appeal of patterns and also hamper subsequent optical imaging. Finally, fine movement control is necessary for obtaining precise patterning of
cells on agar. Although the spatial resolution available from the described technique is typically

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lower than that of more sophisticated methods, it may nevertheless find use in niche applications where a simple (but coarse) surface patterning method would suffice in testing the feasibility of research ideas. For example, the technique can be used to probe possible interactions between bacteria species arranged in defined patterns prior to committing more resources and sophisticated instruments in a full study.

Conclusion

Collectively, utilizing bacteria 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 stages 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 completing 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 project. 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, an 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 by 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 bacteria cells on agar surface closely resembles a writing process; with the inoculating loop and bacteria cells serving as "pen" and "ink", I described the process as "Bacterial calligraphy" (Ng, 2012).[1] Photographs of the agar plates serve as mementos.

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Figure 1: Bacteria 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 obtainable. Note also that inadvertent drips of cells onto non-target areas may result in the formation of stray colonies away from the main pattern. Experiment 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 bacteria cells is also a useful surface patterning method for testing research ideas, given its ability to deposit cells in both straight and curvilinear lines. Bacteria, in 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] However, we know relatively little of the interactions[4] between species, which are mediated by myriad signaling and metabolite exchanges. Specifically, intense research efforts are focused on elucidating the mechanistic underpinnings and broader ecological significance of cell to cell interactions between and within species.[5, 6] Although unable to deliver the high spatial resolution available in other surface patterning methods,[7] the described technique can still be employed for patterning cells in preliminary studies seeking to test methodological concepts. As an example, determining possible interactions between different bacteria species separated by varying distances can be facilitated by using the "calligraphy" technique to pattern cells on agar, rather than other more complicated and costly techniques such as robot-based surface patterning of cells etc.

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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, bacteria species such as *Bacillus subtilis*,[9] *Proteus mirabilis*,[10, 11] *Pseudomonas aeruginosa*,[12, 13] and *Myxococcus xanthus*,[14] engage in swarming motility and are not suitable for "Bacterial calligraphy". Swarming motility, in particular, destroys any intended pattern as the cells move away as a group from the colony's point of growth, which is also the point of deposition. Of a different nature, pigments that do not adhere to cells but diffuse into the surrounding agar medium also significantly affect pattern reproduction. In the described method, the purple pigment adheres strongly to cells (with no diffusion into or on the agar medium, Figure 2); thus, enabling relatively good pattern reproduction. In addition, bacteria inoculum usually appears as beige colour; thus, visual discrimination of patterned and non-patterned areas is difficult. Hence, selecting bacteria species whose secreted pigments adhere to the cell surface would help improve the resolution of pattern formation, as coloured cells helps guide the patterning process. Finally, bacteria 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 bacteria 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 resolution pattern formation. Lack of swarming motility by the bacterium, and strong colour contrast between the purple pigment and beige agar also contributed to the species' utility in surface patterning through the "Bacterial calligraphy" technique. Experiment 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 for bacteria cells was used in creating a memento for

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the strong friendships fostered between students during their final year research projects. Specifically, the initials of students' names were patterned on agar by a "writing" process utilizing an inoculation loop as "pen" for depositing cells as "ink". Judicious choice of bacteria species is important for high resolution pattern formation, where the criteria are: motility mode of bacterium, and colour intensity and diffusivity of secreted pigments. Finally, although the spatial resolution available from the described manual patterning technique is inferior to that of more sophisticated methods, its simplicity facilitates its use in testing the feasibility of research ideas prior to detailed investigations utilizing elaborate techniques and instrumentation.

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Conflicts of interest

This synopsis describes a published paper written by the author.

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