Patterning bacteria on agar for understanding distance mediated cell to cell signaling and metabolic exchanges

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Abstract

Microbes, while living in close proximity in biofilms, may in other cases not be closely localized in the environment. In the latter case, their interactions and communications are dependent on myriad factors such as existence of direct connections (e.g., web of hyphae from fungi) or pools of water where they could migrate. Hence, disparate groups of microbes could subsist on nutrients in their local microhabitat while maintaining weak communication links and metabolic exchanges with groups farther afield. But, how do we probe such distance dependent communications links between groups of microbes of the same or different species in the laboratory? Using a purple pigment producing bacterium for "writing" on agar, a surface patterning technique was demonstrated to be 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, which may find use in understanding possible interactions between different microbial species. Although a coarse surface patterning technique compared to more refined robot assisted patterning methods, simple spread plate deposition of different microbial species, in defined patterns, on separate areas of the agar surface, constituted a useful way forward in allowing us to delve deeper into understanding microbial interactions such as those in spatially resolved synthetic microbial community. Taking into account bacterial motility patterns, pigment diffusivity, and contrast of pigment with agar background color, deposition of patterns of bacterial cells through spread plate technique is an affordable method for lending a lens to spatially defined microbial interactions for microbial brethren unable to undergo migration on the agar surface. A synopsis of the work 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, as an open access article.
URL: http://www.asmscience.org/content/journal/jmbe/10.1128/jmbe.v13i2.414

Keywords: surface patterning, swarming motility, color contrast, cell spreading, synthetic biology, pigment diffusivity, cell signaling, metabolic exchanges, community interactions, microbial ecology,

Subject areas: microbiology, biochemistry, cell biology, molecular biology, biotechnology,

Synopsis

Bacterial cell growth on agar engenders different patterns dictated by various factors such as local concentration of nutrients and oxygen, but chief amongst which is the presence of swarming motility (i.e., collective and coordinated movement of cells) – a form of motility that would create its own colony growth patterns on agar independent of constraints on colony size and shape.[1] Hence, possibility exists in using an inoculation loop for depositing bacterial cells not capable of swarming motility onto agar, in patterns useful for understanding how different separation distance between cells impact on cell to cell communications as well as collective migratory behavior. To this end, a coarse surface patterning technique was demonstrated using cells from the *Proteobacteria* family which do not engage in swarming motility. Upon cultivation, pigments secreted by the bacteria make visible the patterns written, which in absence of significant colony expansion and motility, enables the creation of well defined surface patterns. Specifically, cells of the bacterium *Chromobacterium violaceum* were deposited on agar by an inoculation loop, which after 24 hours of incubation, reproduced sufficient cell numbers to allow the pattern to be visible. Secretion of a purple pigment (violacein) by the bacterium further improved color contrast between the pattern and beige agar. Since the process of depositing bacterial cells on agar surface closely resembles a writing process, with the inoculating loop and bacteria cells serving as "pen" and "ink" respectively, I described the process as "Bacterial calligraphy" (Ng, 2012).[2]

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 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 and possible division of labor and functions.[3 4 5] However, we know relatively little of the interactions 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. Although unable to deliver the high spatial resolution available to other surface patterning methods, 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 bacterial 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.

Whether for calligraphy or as a surface patterning method for testing research ideas, spatial resolution achievable with the technique is a key consideration for the experimenter. In general, spatial resolution of the deposited pattern depends on the experimenter's skills in maneuvering the inoculation loop, the motility mode of the bacterium, and diffusivity of any secreted pigments.

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Specifically, bacterial species such as *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Myxococcus xanthus*, engage in swarming motility and are not suitable for depositing defined patterns of microbial cells on agar. Swarming motility, in particular, destroys any intended pattern as the cells move away, as a group, from the colony's point of growth. 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); thus, enabling relatively good pattern reproduction. Additionally, bacterial inoculum is usually beige in color; thus, visual discrimination of patterned and non-patterned areas is difficult. Hence, selecting bacterial species whose secreted pigments adhere to the cell surface would help improve the resolution of pattern obtained, as colored cells helps guide the patterning process. Finally, bacterial species that secretes pigments of strong color intensity would be preferable (over those that do not) for enhancing color contrast between deposited pattern and agar (usually beige in color).

Collectively, a surface patterning technique using bacterial cells for depositing patterns on agar is described, which find ready use in understanding how different spatial localization of cells impacts on cell-cell communications and community behavior. Judicious choice of bacterial species is important for high resolution pattern reproduction, where motility mode of bacterium, as well as color intensity and diffusivity of secreted pigments determines the resolution obtainable. Finally, although the spatial resolution available from the described manual patterning technique is inferior to that of more sophisticated methods,[6] 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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New in this version

This version updates language and syntax.

Conflicts of interest

This synopsis describes a published paper written by the author.

Author's contribution

The author wrote this synopsis.

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Funding

No funding was used in this work.

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