

Observing Chemotaxis in *Vibrio fischeri* Using Soft Agar Assays in an Undergraduate Microbiology Laboratory

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INTRODUCTION

Chemotaxis, the directed movement of cells towards or away from a chemical, is both an exciting and complicated behavior observed in many bacterial species. Attempting to adequately visualize or demonstrate the chemotactic response of bacteria in the classroom is difficult at best, with good models to illustrate the concept lacking. The BSL-I marine bacterium *Vibrio fischeri* (a.k.a. *Aliivibrio fischeri*) is easy to culture, making it an ideal candidate for experiments in an undergraduate microbiology course. A number of chemoattractants for *V. fischeri* have been identified, including a variety of sugars, nucleosides, and amino acids (1, 2). Below presents how the soft agar-based chemotaxis assay can be implemented in the undergraduate laboratory. As bacterial cells migrate towards one or more attractants in soft agar, students can directly observe the chemotactic behavior of *V. fischeri* without the need to learn complicated techniques or use specialized equipment. Once the bands of bacterial cells are observed, the migration can then be disrupted by the addition of excess attractant to the soft agar, thereby visualizing what happens once cells are no longer in a gradient of attractant. In addition, soft agar plates lacking attractants can be used to visualize the random movements of bacterial cells that are non-chemotaxing. These exercises can be used in the microbiology laboratory to help students understand the complex behavior of bacterial chemotaxis.

General culture and media preparation procedures

V. fischeri ES114 (available as ATCC 700601) grows in seawater tryptone (SWT) media (0.5% Tryptone and 0.3% yeast extract in 70% artificial seawater [0.88% NaCl, 0.62% MgSO₄, 0.072% CaCl₂, 0.038% KCl]), Luria-Bertani salt (LBS) media (1% Tryptone, 0.5% yeast extract, 2% NaCl, and 20 mM pH 7.2 Tris), or minimal media with mannitol

(MM-M), (70% artificial seawater, 50 mM HEPES buffer pH 7.5, 19 mM NH₄Cl, 0.33 mM K₂HPO₄, 0.01 mM FeSO₄, and 25 mM mannitol which serves as a carbon source but not an attractant for *V. fischeri*) (2). Recommended attractants for *V. fischeri* to be added to MM-M include 1 mM final concentrations of either glucose or *N*-acetylglucosamine. A soft agar concentration of 0.25–0.4% is suggested (plates should be the consistency of soft JELL-O and not runny). *V. fischeri* grows best at 28°C (it also grows at room temperature although an increase in the timing of experiments will be needed) and can be frozen at -80°C in glycerol. Plate cultures may be maintained at room temperature for up to a week.

Plate-based chemotaxis assay description

Motile bacterial cells inoculated onto the center of soft agar containing one or more attractants for the organism can be observed to form discernible bands of migration that move through the soft agar matrix outward from the center of the plate as the microbe establishes a gradient of attractant from the consumption and subsequent diffusion of the chemical (4). Depending on the medium, one or more bands may be visible as the cells in the outer band migrate towards and consume a preferred attractant, while cells in inner band(s) are attracted to secondary attractants (arrows, Fig. 1(A)) (3). In SWT soft agar which contains multiple attractants, two distinct migrating bands of *V. fischeri* cells are typically observed (Fig. 1(A)), while in the presence of a single attractant such as in MM-M containing glucose, *V. fischeri* forms a single migrating band (Fig. 1(B)). Further evidence that a migrating band is sensing a particular attractant can be demonstrated by perturbation of the migration by addition of an excess of attractant (on SWT, typically 10 µl of 0.1–1 M concentrations of either serine or thymidine works best) at the front of the migrating band (arrow, Fig. 1(C)). For a negative control, inoculation of *V. fischeri* onto MM-M soft agar lacking an attractant can be used to demonstrate the random movement of non-chemotaxing cells that results in a fuzzy, non-concentric outward ring that is significantly smaller in diameter than a true migrating band of bacterial cells (Fig. 1(D)). Given the appropriate culture conditions, this activity can also be adapted to other bacterial species.

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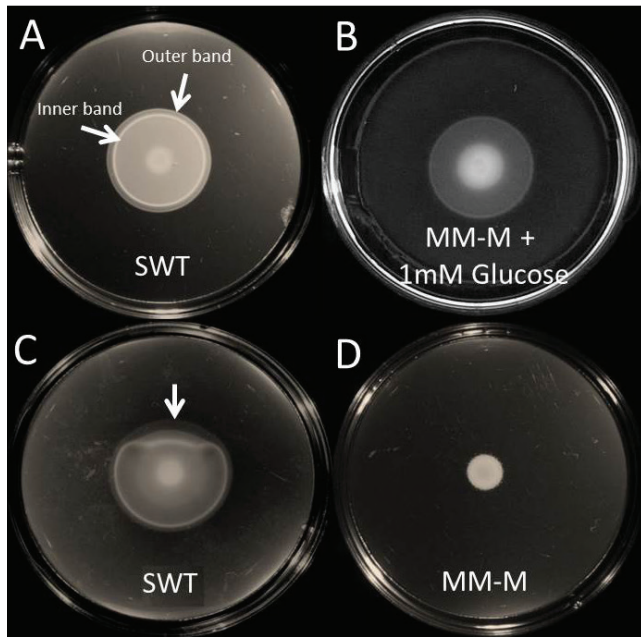


FIGURE 1. (A) 10 μ l of log-phase *V. fischeri* was dropped onto the center of SWT soft agar and incubated at 28°C for approximately 3.5 h. Both an inner and outer band of bacterial cells migrating towards attractants in the agar can be observed (indicated by arrows). (B) Bacterial cells inoculated onto MM-M soft agar containing 1 mM glucose migrate out in a single band. (C) Migration can be perturbed by dropping 10 μ l of excess attractant (2 M serine, indicated by the arrow) outside the migrating band(s). (D) Random migration of bacterial cells in MM-M lacking an attractant.

Plate-based chemotaxis assay procedure

1. Grow cultures in LBS at 28°C with shaking overnight prior to the day of the activity (LBS prevents overnight acidification).
2. Prepare soft agar plate media 18–24 h prior to the start of the activity (may also be made at least 3 h prior to the activity for the agar to set). Soft agar plates must be kept upright (not inverted) on a solid surface and handled as little as possible once poured and throughout the remainder of the experiment as the soft agar matrix is easy to disturb with excessive movement or shaking.
3. Inoculate 5 ml SWT from overnight cultures and grow at 28°C with shaking until an OD₆₀₀ of 0.4–0.6 is reached (2–3 h).
4. Students inoculate 10 μ l aliquots of 0.4–0.6 OD₆₀₀ cultures at the center of the soft agar plates and then incubate them at 28°C. 10 μ l aliquots should be carefully dropped directly onto the agar at the center of the plate with a micropipette without touching the tip to the agar (this step takes little time and may be done outside of classtime in order to allow migration to occur).

5. After 3–4 h on SWT soft agar at 28°C, students can observe the migration behavior of *V. fischeri* by the formation of two distinct bands of migrating bacteria. Students can then drop an excess of attractant(s) directly onto the agar outside the leading edge of the migrating bands and then place the plates at 28°C until a perturbation of migration is observed (1–2 h).
6. Students can observe the migration behavior of cultures inoculated onto MM-M soft-agar plates (with or without added attractants) after approximately 22–24 h of incubation at 28°C.

CONCLUSION

Chemotaxis is an exciting bacterial behavior which can be visually demonstrated in an undergraduate setting using soft agar assays. This assay provides a simple experiment that can be easily conducted in an undergraduate setting, and may help students enhance their understanding of this complex bacterial behavior.

The author welcomes any questions from instructors regarding this activity.

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