PROTOCOL

CHEMICAL - GRADIENT MOTILITY AGAR (CGMA): A SYSTEM, DEMONSTRATING CHEMORECEPTOR MEDIATED CHEMOTAXIS.

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INTRODUCTION

The **Chemical-Gradient Motility Agar (CGMA)** is a system designed for demonstrating chemo-tactic movements of prokaryotic cells in an environment loaded with two different types of chemical gradients viz. gradient of chemical attractants (e.g. glucose) and chemical inhibitors (e.g. mercuric chloride and ampicillin). It is capable of successfully demonstrating positive chemotaxis as well as negative chemotaxis (both of which are mediated by chemo-receptors on cell surface) in a common environment, such that they are properly visualized on the Petri dishes. The analysis involving cell movement in the presence of chemo-tactic and chemo-kinetic stimuli is relevant to many different lines of basic and applied research. One of the primary uses of this technique will be for testing of various chemicals with respect to bacterial cell. Using this technique, mutations related to motility in bacteria may be studied.

MATERIALS & METHODS

- Motile Bacterial Cells i.e. cells possessing flagella e.g. Fluorescent strain of *Pseudomonas*.
- Standard *4mm* Nichrome Inoculation Loop.
- Petri plates.
- Equipments like Autoclave, Incubator and pH meter.
- Sharpened scalpel.
- Glass wares like beakers, conical flasks, Pasteur pipettes etc.
- Organism Nutrient Broth **(ONB)**.
  Its 1 litre solution can be prepared by mixing 3.6g Beef extract, 6g Peptone and 1g NaCl in 1000 ml distilled water with pH adjusted at 6.8 ± 0.3; which is then sterilized by autoclaving at 10 lbs pressure for 40 minutes at 115.2^0C in an Autoclave.
- Motility Agar Medium **(MAM)**.
  Its 1 litre solution can be prepared by mixing 5g Peptone, 3g Beef extract and 5g NaCl in 1000 ml distilled water with pH adjusted at 6.9 ± 0.3; followed by addition of 10g Agar powder. This is also sterilized in an autoclave at 10 lbs pressure for 30 minutes.
- Chemo-attractant.
  This can be prepared by making a 3% solution of any suitable substance that enhances bacterial growth (preferably a carbon source), e.g. Glucose. For that matter, concentration of this varies according to the choice of performer.
- Chemo-repellent.
  Chemo-repellent can be liquid (e.g. mercuric chloride) or solid (e.g. antibiotic) that inhibits the growth of bacteria. Concentration of liquid chemical may depend upon the choice of performer e.g. 0.1% or 0.5% etc. Standard antibiotic disc should be used in case of solid repellent.

PROCEDURE

1. Transfer about 3 loopful culture of motile bacterium into 250 ml Organism Nutrient Broth (**ONB**) with the help of nichrome inoculation loop.
2. Leave the bacterium to multiply at 37^0C in **ONB** overnight, in an incubator.
3. Now, fill all Petri plates with 15-20 ml motility agar medium such that the thickness of this medium inside the plates is approximately 4-5mm.
4. Leave these Petri plates at room temperature for solidification. After solidification you will see that the agar surface is very moist and the agar is semi-solid in nature, all these features stand perfect for motility of bacterium.
5. After motility agar i.e. **MAM** has solidified, use a sharpened scalpel for punching wells at approximate locations in the motility agar medium. First punch a long rectangular well across the agar; such that it is along the vertical diameter of the Petri plates, this is called Organism or Cell Reservoir (See **Figure 1. A.**). This well should have approximate dimensions of 12 X 0.8 cm. This organism reservoir should be punched in all **CGMA** assay systems.

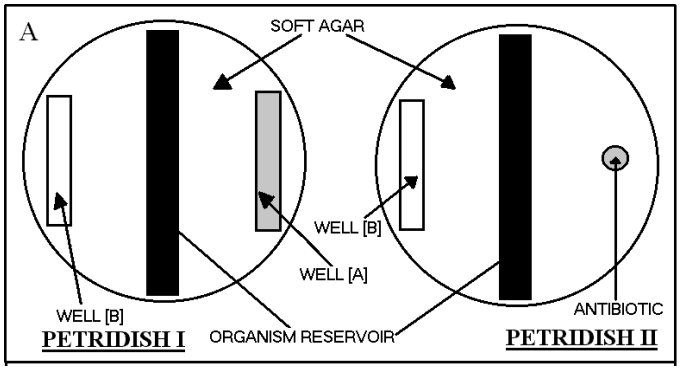

Figure 1: Schematic configuration of CGMA System:
(A). Top View of CGMA system.

{Petridish I}: It shows the Wells that were cut through the motility agar. Well [A] has inhibitory chemicals while Well [B] has attractant chemicals. Cell reservoir is the place where medium with *Pseudomonas* is poured.

{Petridish II}: Here though Well [B] and cell reservoir are intact, Well [A] has been replaced by antibiotic disc.

6. Now if your inhibitor is liquid, then punch two wells, each having identical strip or rectangular shape such that, each well is located approximately 3.5 to 4 cm on either side of Organism or Cell reservoir, along a common axis. Right side well can be labelled as *Well [A]* and the well on the left side can be labelled as *Well [B]* (See **Figure 1. A. Petri dish I.**).
7. However, if your inhibitor is solid (i.e. antibiotic disc) then only one well should be punched. This one well should be *Well [B]* on the left side of the organism reservoir. Antibiotic disc should replace *Well [A]*. This disc should be placed at a distance of approximately 2.5 cm from the organism reservoir (See **Figure 1. A. Petri dish II.**).
8. After the above set-up is complete, load respective chemo-attractant and chemo-repellent onto the Petri dishes with the help of Standard Pasteur pipettes. Load *Well [A]* of all Petri plates with 4-5 drops of chemo-repellent and *Well [B]* with same amount of chemo-attractant.
9. In case of solid repellent, simply place an antibiotic disc on agar surface at the place of *Well [A]*.
10. Leave this set-up to settle for about 1 hour to ensure formation of gradients of respective chemicals.
11. After this, add 5-6 drops of **ONB** (Organism nutrient broth containing overnight culture of motile bacteria) to the Organism reservoir of all the **CGMA** assay systems.
12. Transfer this set-up to incubator at 37^0C for about 24 hours.

RESULTS:
Results will be seen as in **Figure 2** below:

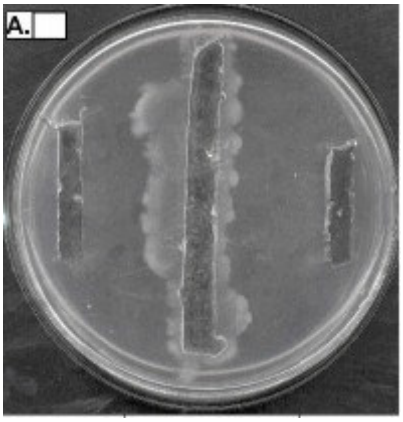

Figure 2

Results presented in **Figure 2** are sample images of the **CGMA** system in which liquid repellent was used. It can be seen that bacterial chemotaxis towards *Well A* on the right side (the well containing repellent chemical) is less, which demonstrates negative chemotaxis while on the other hand bacterial chemotaxis towards *Well B* on the left side (the well containing attractant chemical) is recognizable, which demonstrates positive chemotaxis. Similar results are obtained in the **CGMA** assay consisting of solid repellent (antibiotic disc), such that bacterial cells shows negative chemotaxis (near antibiotic disc) as well as positive chemotaxis (towards *Well B* containing chemo-attractant). The middle well in the images represents Organism reservoir. This is the site from where bacterial cells started moving outwards before they encountered the gradients of respective chemicals.

REFERENCE: Garg, A. D. & Kanitkar, D.V., Chemical-Gradient Motility Agar (CGMA): A System, demonstrating chemoreceptor mediated chemotaxis. Paper presented at *Biotechcellence '06*, 12th National Symposium at CBT, Anna University, Chennai, India on 25th February 2006.