Motility Assay 1: Sectioned plates

Materials

per group:
2 GYE- water agar sectioned plates
2 GYE-GYE sectioned plates
slant of motile *E. coli*
slant of non-motile *E. coli*
sterile filter paper strips
forceps

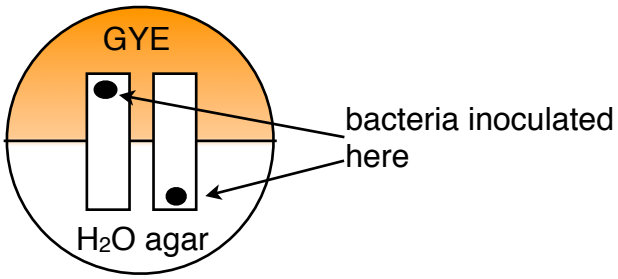
per person:
1 GYE-water agar and 1 GYE-GYE agar plate for your pet
slant of your pet

GYE agar (for 1 liter)	Water agar (for 1 liter)
10.0 g of tryptone	15.0 g of agar (1.5% agar)
10.0 g of yeast extract	
5.0 g of glucose	
5.0 g of K_2HPO_4	
15.0 g of agar (1.5% agar)	

Method

1. Pass your forceps through the flame.
2. Center 2 sterile filter strips bridging the ridge of a petri dish - see diagram below.
3. Gently press the paper strips into place on the agar.
4. Using sterile technique and an inoculating loop, place a small amount of bacteria from the slant onto opposite ends of the paper strips, so that one strip is inoculated on each side of the plate.
5. On the underside of the plate, mark the ends of the strips that were inoculated.
6. Incubate 30°C for 48 hours.

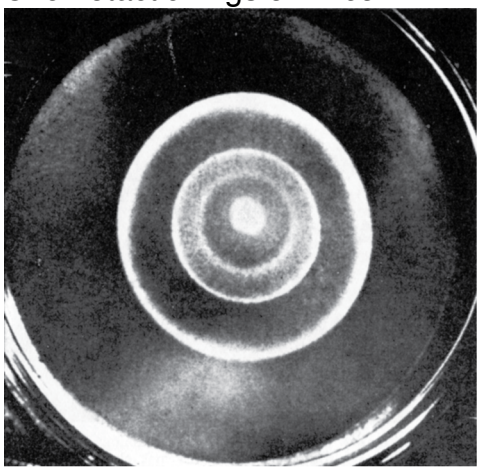
Example:



Motility Assay 2: Chemotaxis in semi-solid agar

When inoculated in the center of a semi-solid tryptone agar plate, *E. coli* will begin to consume nutrients in the tryptone, forming a pattern of concentric rings (figure on left). They first consume L-serine in the area around the point of inoculation. Some bacteria move outward in search of L-serine, forming a ring tracking L-serine. Bacteria that are left behind, where there is no L-serine, consume L-aspartate and form a second ring searching for L-aspartate. The next nutrient to be tracked is L-threonine, and bacteria searching for this amino acid form the third innermost ring. If a disk with an attractant or repellent is placed near one of the migrating rings, the ring shape will deform near the disk as the bacteria move towards or away from the compound (figure on right).

Chemotactic rings of *E. coli*



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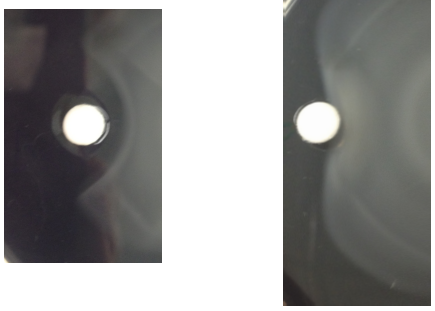
Deformation of the chemotaxis pattern by a disk of acetate, a repellent, placed outside of the L-serine ring (left) and by a disk of L-serine, an attractant (right).



Materials

semisolid tryptone agar (1% tryptone, 0.5% NaCl, 0.25% agar) inoculated with *E. coli*
sterile filter disks
solutions of potential and known attractants and repellents (acetate, sucrose, glucose, maltose, L-serine, L-aspartate, L-glutamine, L-histidine).
forceps

If you bring in a compound to test and it is not already liquid, mix it with a small amount of sterile water.

Methods

** Be very careful with the plates. Do not turn them upside-down. The agar is semi-solid, which means semi-liquid too. It can slop around and disturb the chemotactic rings.***

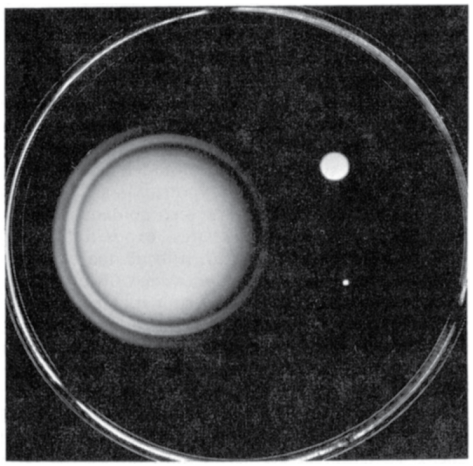
1. Place a drop of compound onto a filter disk.
2. Repeat on separate disks with each compound that you will test.
3. Flame your forceps.
4. Carefully place one of the filter disks near but outside one of the chemotactic rings formed by the *E. coli* in the plate.
5. Label the underside of the plate so you know what disk has each compound.
6. Place the plates at 35 °C and check every 15-20 min. for evidence of chemotaxis.

*** Be very careful with the plates. Do not turn them upside-down. The agar is semi-solid, which means semi-liquid too. It can slop around and disturb the chemotactic rings.***

Test your pet to determine if it is chemotactic to components of tryptone or is motile, but does not respond to the tryptone growth medium.

1. Pick a colony of your pet microbe with a sterile inoculating loop.
2. Stab the loop into the agar at the center of the semi-solid agar plate.
3. Incubate at room temperature overnight.

Growth on a semisolid tryptone plate.



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