Dose Dependant Swim Assay

Experiment derived from

Patricia Domınguez-Cuevas, et al. “Transcriptional Tradeoff between Metabolic and Stress-response Programs in *Pseudomonas putida* KT2440 Cells Exposed to Toluene.**”** Journal of Biological Chemistry. Volume 281, Issue 17, p 11981-11991.

Plate recipes derived from the protocol located at

http://2009.igem.org/wiki/images/9/90/Minimum_Plates.pdf

From Lin Group in Chemical Engineering

Media

M9 Sodium Benzoate Minimal Plates 3% (w/v) Agar

Autoclave:

When making 200 mL total medium for plates, autoclave 6 g agar in 100 ml deionized water.

Sterile Components added directly after autoclaving

1. 40mL 5X M9 salts
2. 200 µL 1M Mg2SO4
3. 56.6 mL deionized water

Sterile components after the agar solution has cooled to ~50 °C:

4. 2 mL of sodium benzoate solution (concentration from ATCC minimal media for p. putida (pWW0)
    - Sodium benzoate solution (total 10 mL)
        1. 3 g Sodium Benzoate
        2. 10 mL DI water
        3. Sterilize with .022 µm filter syringe
5. 20 µL 1M CaCl2
6. 1mL 40% (w/v) glucose

M9 Toluene Minimal Swim Plates .3% (w/v) Agar

Autoclave:

When making 200 mL total medium for plates, autoclave .6 g agar in 100 ml deionized water.

Sterile Components added directly after autoclaving

1. 40mL 5X M9 salts
2. 200 µL 1M Mg2SO4
3. 58.8 mL deionized water

Sterile components added after the agar solution has cooled to ~50 °C:

WARNING! – Toluene is VERY flammable; do not expose it to open flame or high heat.

4. 20 µL 1M CaCl2
5. 1mL 40% (w/v) glucose
6. Toluene (use table to determine amounts)

Final Toluene Concentration (mM)	Amount of Toluene Added (µL)
0	0
5	105
10	210

Overnight Cultures

1. 2 mL LB media for KT2440
2. 2 mL ATCC Sodium Benzoate Media

Strains

P. putida KT2440 – Located in -80 C freezer in IGEM box cell 5

P. putida pWW0 – Located in -80 C freezer in IGEM box cell 6

Toluene Dose Dependant Swim Assay Experimental Protocol

Goal: The dose dependant swim assay is designed to determine and measure the chemotactic response of bacteria to differing doses of toluene dissolved into the media. The total radius of the growth/swim distance after incubation will be measured and recorded as the swim distance of the bacteria. Good results will show a small standard deviation among similar plates and a statistically significant difference between the median growth radii of plates of differing concentrations of toluene.

Making M9 Toluene Minimal Swim Plates .3% (w/v) Agar and M9 Sodium Benzoate Minimal Plates 3% (w/v) Agar

WARNING! – Toluene is VERY flammable; do not expose it to open flame or high heat.

WARNING! – Materials coming directly from the autoclave are hot and present a burn hazard. Take the proper safety protocols into consideration. (Use the hot gloves)

1. Autoclave the agar and water needed for 1 flask of 200 mL sodium benzoate minimal media and 3 flasks of 200 mL toluene minimal swim plates in separate containers for each 200 mL quantity using the amounts listed above in the media section.
2. After removing the agar solutions from the autoclave mix the sterile components listed in the media recipe above into the solutions.
3. After allowing the agar solutions to cool to ~ 50 °C mix in the remaining sterile ingredients listed in the media recipes recorded above.
4. Pour the media into 100 mL round Petri dishes, until the bottom of the dish is covered, and let the plates cool and harden overnight at room temp.

Growing culture/colonies for plating

1. An overnight culture of the strains to be measured is taken from -80 °C fridge via pipette tip and grown up overnight at 30 °C in a solution of:
    - 2 mL of LB media
    - 2 mL ATCC Sodium Benzoate Media
3. Harvest 1 mL of cell growth from overnight culture and dilute to 1/10 and 1/100 of the original concentration using DI water to dilute.
4. Place the dilution onto a M9 Sodium Benzoate Minimal Plate 3% (w/v) agar.
5. Place ~5 sterile glass beads onto the plate and shake in all direction for approximately 2 minutes to distribute bacteria evenly over the plate.
6. Remove glass beads from plate, apply parafilm around edges of plate, and incubate overnight at 30 °C

Plating of bacteria onto swim plates

1. Use a sterile toothpick to transfer a colony of growth from a plate containing 1/100 dilution of bacterial growth onto a M9 Toluene Minimal Swim Plate .3% (w/v) Agar.
2. Wrap the edges of the swim plate with parafilm and incubate overnight at 30 °C for 14-16 hrs.

Recording Results

1. Photograph the swim plates after removing them from incubation.
2. Calculate the area of the halo of growth.
3. Average the area of the halo for the plates containing the same amount of toluene.
4. Compare the area of the halo among plates containing differing amounts of tol.

7-10-09

Toluene Concentration Dependant Swim Assay

Ann and I started 2 overnight cultures, one containing the KT2440 strain, and one containing the pWW0 strain using the growth of colonies for plating protocol listed above. We also made M9 Sodium Benzoate Minimal Plates 3% (w/v) Agar and M9 Toluene Minimal Swim Plates .3% (w/v) Agar using the protocol listed above. Note: I measured the amount of agar incorrectly and as a result the swim plates have a 1.5% (w/v) agar instead of the prescribed 3%. This could influence experimental results so that the swim distances appear larger than other previously done experiments. We plated the agar solutions, of which there were 4 different types, 0 mM Toluene, 5 mMol Toluene, 10 mMol Toluene, and the sodium benzoate. We let these sit out on the bench overnight to cool and harden.

7-19-09

Ann and I taped the toluene plates up and left them on the bench. We also made 1:10, 1:100, and 1:1000 dilutions of the overnight cultures of both strains and plated them onto the benzoate plates. We taped the inoculated plates and left them in the incubator overnight.

7-20-09

I photographed the plates and did a quick analysis using excel. See below for results. The low number of observations makes the results very inconclusive, but we did see enough similarities and significant results to justify the inclusion of the Domınguez-Cuevas paper in our overall bioremediation design.

Average Area (mm)

pWW0		stdev	KT2440		stdev
10mM	0.335	0.0027496	10mM	0.507	#DIV/0!
5mM	0.550	0.0064158	5mM	0.528	0.00091654
0mM	0.168	0.0082794	0mM	0.104	0.0047404