# Biofilm Formation Experiment

## *Background*

Naphthenic acids (NA's) are the major contaminant in oil sands production. It is reported that naphtheinic acids (NA's) are best degraded in a biofilm. We want to enhance the biofilm formation ability of bacteria that are capable of degrading NA's in tailing waters. To give the bacteria the ability to form biofilms we plan on introducing a plasmid with antigen 43 that will make the cells "frizzy".

For this experiment we are testing the native ability of bacteria to form biofilms (without the antigen 43 plasmid). Three strains will be tested for biofilm formation*: Pseudomonas putida* (oil sands), *Pseudomonas fluorescens* (oil sands) , *Pseudomonas putida* KT2440 and *E. coli* K12. The two Pseudomonas strains labeled oilsands were given to us and shown to grow in oil sands tailing water. We will use these Pseudomonas strains as model organisms that can degrade NAs. These Pseudomonas strains are known to form biofilms and we want to characterize the biofilm formation ability. The *E. coli* K12 and *Pseudomonas putida* KT2440 strain will be used as reference samples.

We will be modeling the adhesion assay from the following paper:

A rapid screening procedure to identify mini-Tn10 insertion mutants of Escherichia coli K-12 with altered adhesion properties. FEMS microbiology letters [0378-1097] Genevaux yr:1996 vol:142 iss:1 pg:27

and we will also be adapting our protocol from Alex's lab procedure

## *Materials*

### *Strains*

- *E. coli* K12
- *P. putida* (oil sands)
- *P. fluorescens* (oil sands)
- *P. putida* KT2440

### *Containers*

- 96 well sterile microplate
- 18-15 mL falcon tubes

### *Media*

- Approx. 12 mL LB media
    - 8 mL for overnight growth
    - 4 mL for microplate inoculation
- Approx. 10 mL bushnell-haas media with glucose carbon source
    - 6mL for overnight growth

    - 4 mL for microplate inoculation
- Approx. 3 mL of M9 minimal media with glucose
    - 2 mL for overnight growth
    - 1 mL for microplate inoculation
- Approx. 10 mL bushnell-haas media with glucose and casamino acids
    - 6 mL for overnight growth
    - 4 mL for microplate inoculation
- Approx 3 mL of M9 minimal media with glucose and casamino acids
    - 2 mL for overnight growth
    - 1 mL for microplate inoculation
- Approx. 10 mL bushnell-haas media with cyclohexane carboxylic acid (CHCA)
    - 6 mL for overnight growth
    - 4 mL for microplate inoculation
- Approx. 10 mL bushnell-haas media with cyclohexane carboxylic acid and pH adjusted to 9
    - 6 mL for overnight growth
    - 4 mL for microplate inoculation
- Approx. 13 mL of 0.1% crystal violet staining solution
- Approx 13 mL of 200 proof ethanol
- 35 mL PBS buffer for washing plate

***For media recipes see iGEM 2010 media recipes section of the notebook

## *Procedure*

1. Start an overnight culture of the following strains in 2 mL of of specified media and grow up at 30C in the shaker

| Strain | Media |
|---|---|
| E. coli K12 | LB |
| | M9 + glucose |
| | M9 + glucose +casamino acids |
| P. putida KT2440 | LB |
| | BH +glucose |
| | BH+glucose+casamino acids |
| | BH+CHCA |
| | BH+CHCA pH9 |
| P. putida oilsands | LB |
| | BH +glucose |
| | BH+glucose+casamino acids |
| | BH+CHCA |
| | BH+CHCA pH9 |
| P. flourescens oilsands | LB |
| | BH +glucose |
| | BH+glucose+casamino acids |
| | BH+CHCA |
| | BH+CHCA pH9 |

2. After cultures have been allowed to grow up for specified amount of time above, measure the $OD_{600}$ of each culture in the microplate reader
3. Using the $OD_{600}$ make a concentrated cell suspension for the microplate inoculation according to the following formula

    ***a.*** Volume for resuspension (μL) = 500 x $OD_{600}$
    ***b.*** *This should give a cell suspension w/ OD600 ~2, which will be used to inoculate the microplate.*
    ***c.*** *Following this procedure ensures that each well will be inoculated with the same number of cells*

    To make cell suspension

    1. Take 1 mL of culture
    2. Spin down at 12000 rpm for 2 minutes
    3. Pipet off supernatant
    4. Resuspend in calculated amount of media from above formula
4. Add 200 uL of media and 2 uL of cell suspension to each microplate well according to the layout below (a polystyrene microplate should be used)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | DI H20 | LB | LB | M9 glu | M9 glu | M9 glu CAA | M9 glu CAA | BH CHCA | BH CHCA | BH CHCA pH 9 | BH CHCA pH 9 | DI H20 |
| **B** | DI H20 | LB E. coli K12 | LB P. flourescens oilsands | M9 glucose E. coli K12 | BH glucose P. flourescens oilsands | M9 Glu. CAA E. coli K12 | BH Glu. CAA P. flourescens oilsands | BH CHCA P. putida KT2440 | BH CHCA P. putida oilsands | BH CHCA pH 9 P. flourescens oilsands | DI H20 | DI H20 |
| **C** | DI H20 | LB E. coli K12 | LB P. flourescens oilsands | M9 glucose E. coli K12 | BH glucose P. flourescens oilsands | M9 Glu. CAA E. coli K12 | BH Glu. CAA P. flourescens oilsands | BH CHCA P. putida KT2440 | BH CHCA P. putida oilsands | BH CHCA pH 9 P. flourescens oilsands | DI H20 | DI H20 |
| **D** | DI H20 | LB E. coli K12 | LB P. flourescens oilsands | M9 glucose E. coli K12 | BH glucose P. flourescens oilsands | M9 Glu. CAA E. coli K12 | BH Glu. CAA P. flourescens oilsands | BH CHCA P. putida KT2440 | BH CHCA P. putida oilsands | BH CHCA pH 9 P. flourescens oilsands | DI H20 | DI H20 |
| **E** | DI H20 | LB P. putida KT2440 | LB P. putida oilsands | BH glucose putida KT2440 | BH glucose P. putida oilsands | BH Glu. CAA P. putida KT2440 | BH Glu. CAA P. putida oilsands | BH CHCA P. flouresc ens oilsands | BH CHCA pH 9 P. putida KT2440 | BH CHCA pH 9 P. putida oilsands | DI H20 | DI H20 |
| **F** | DI H20 | LB P. putida KT2440 | LB P. putida oilsands | BH glucose putida KT2440 | BH glucose P. putida oilsands | BH Glu. CAA P. putida KT2440 | BH Glu. CAA P. putida oilsands | BH CHCA P. flouresc ens oilsands | BH CHCA pH 9 P. putida KT2440 | BH CHCA pH 9 P. putida oilsands | DI H20 | DI H20 |
| **G** | DI H20 | LB P. putida KT2440 | LB P. putida oilsands | BH glucose putida KT2440 | BH glucose P. putida oilsands | BH Glu. CAA P. putida KT2440 | BH Glu. CAA P. putida oilsands | BH CHCA P. flouresc ens oilsands | BH CHCA pH 9 P. putida KT2440 | BH CHCA pH 9 P. putida oilsands | DI H20 | DI H20 |
| **H** | DI H20 | LB | LB | BH glu | BH glu | BH glu CAA | BH glu CAA | BH CHCA | BH CHCA | BH CHCA pH 9 | BH CHCA pH 9 | DI H20 |

Key:

M9=M9 minimal media

BH=Bushnell-Haas minimal media

CAA=Casamino acids

CHCA=cyclohexane carboxylic acid

5. Allow the plate to incubate (WITHOUT SHAKING OR ANYTYPE OF MOTION) for 48 hours at 30C
6. Measure the OD600 on the plate reader after the 48 hour period
    a. This step will be used to normalize the faster growing cultures against the slower growing ones
7. Pipet out liquid culture with multiwell pipette

    a. Include the blank wells for all of the CV staining steps
8. Wash plate with 200 uL of PBS
9. Stain cells with 200uL of 0.1% crystal violet stain for 15 minute
10. Wash cells two more times with PBS
11. Let the plates dry completely
12. Add 200 uL of ethanol to each well, cover plate and let it sit for 10 minute
13. Briefly mix the contents of each well
14. Measure the adsorption at OD600 to quantify the biofilm formation

## *Results*

Comparing the adsorption readings of the OD600 of the cultures after biofilm growth (OD) vs the OD 600 after the crystal violet staining CV-OD600 should give us the biofilm formation comparison between the three strains. A plot of CV-OD600/OD600 vs the different strains will be made.