Wednesday 9/9/09

Media Prep for Transformation

Last transformation with *P. putida* strains, the antibiotic concentration was not high enough. Therefore, ATCC sodium benzoate plates will be made with 105 and 210 ug/mL of Chloramphenicol according to the following recipe to determine if *P. putida* is naturally resistant to these high concentrations of chloramphenicol.

<u>ATCC sodium benzoate plate recipe</u> (suggested growing conditions for P. putida mt-2 with TOL plasmid)

Autoclave following solution:

- 0.6 g $(NH_4)_2HPO_4$
- 0.24 g KH_2PO_4
- 1.0 g $NaCl_2$
- 0.098 g $MgSO_4$
- 0.1 g yeast extract
- 4 g Agar
- 180 mL DI water

After Autoclaving mix in sodium benzoate solution filter sterilized through a 0.22 um filter

- 20 mL of sodium benzoate solution
    - 1.5 g sodium benzoate
    - 50 mL DI water

When solution has cooled to 50C add antibiotics

- 105 ug/mL CHL
    - 600 uL of 35 ug/mL stock solution
- 210 ug/mL CHL
    - 1200 uL of 35 ug/mL stock solution

<u>Liquid ATCC sodium benzoate media recipe</u>

The liquid media used for growing out the overnight culture for the transformation will be the same media as above minus the agar. 500 mL of liquid ATCC sodium benzoate solution was made by multiplying the above ingredient amounts by 2.5