**Procedure for the Isolation of *Pseudomonas aeruginosa* from Environmental Samples**

This procedure is adapted from Ferguson et. al. in *Infection and Immunity*, Apr. 2001, 2198-2210.

1. Mix soil sample and sterile H2O in a ratio of 1:2 (g/mL)

2. Shake for 1 hour at 300 rpm at room temperature

3. Aliquot 0.5 mL of slurry into 4.5 mL of sterile salts + acetamide solution, see solutions info page for more information on how to prepare it

4. Incubate without shaking at 42 C for 24-48 hours

5. Aliquot 0.1 mL onto plates of King’s Medium B plus centrimide (see below for important details) and incubate at 42 C for 24-48 hours

6. Check for fluorescence and transfer colonies onto King’s A Plates and check for pyocyanin production

7. Pyocyanin and fluorescein producing colonies growing on King’s A are most likely *Pseudomonas Aeruginosa* , this can be compared with a control P. A. sample growing on King’s A plates

**SOLUTIONS**

Sterile Salts Plus Acetamide:

To make 1 L, mix:

5 g NaCl

0.2 g MgSO4

1 g NH4H2PO4

1 g K2HPO4

20 g Acetamide

Add milli-Q H2O to bring total volume to 1.0 L.

This solution can be autoclaved

King’s Medium B Plus Centrimide:

To make King’s Medium B, mix:

20 g Agar

20 g Proteose Peptone No. 3

1.5 g K2HPO4 , anhydrous

15.0 g Glycerol

Add milli-Q water to bring final volume to 1.0L, pH to 7.2 +/- 0.2 at 25 C. Autoclave to sterilize and cool before adding centrimide and magnesium sulphate as follows:

Prepare a highly concentrated sterile centrimide solution by dissolving 0.30 g of centrimide solid with about 1 mL of milli-Q H2O in a microfuge. Then, transfer to another microfuge, passing it through a sterile 0.2 um filter

Add 3.2 mL of 1 M MgSO4 solution

Add centrimide solution, which corresponds to a 0.03% concentration in the final mixture.

Pour plates.