7.13 Experimental Microbial Genetics

Fall 2008

Generating and screening for mutants in Fur

Concept

Growth on manganese rich media has been shown in both *E. coli* and PAO1 to select for mutants with defects in iron acquisition. The majority of these mutants are in the *fur* gene. In Pseudomonas, Fur is essential for aerobic growth, so any mutants isolated are point mutations, rather than knockouts.

Media

LB

1. Add 25 g LB to 1 L deionized water. For plates, add 15 g bacto agar.
2. Autoclave for 35 min on the fluid cycle.
3. Let cool, then add required antibiotic for selective media. If making plates, pour into petri dishes, let solidify, and refrigerate.

Manganese plates:

2 g Tryptone
1.2 g NaCl
10mM MnCl2
7.5 g agar
500 ml deionized water

Autoclave and cool to 50^{o}C, then add

50 uM desferral (desferroxamine) (make and filter sterilize a 25 mM stock solution. Use 2 ul/ml)

These plates must be made fresh – use within a week.

BHI

Add 37 g BHI to 1 L water. For plates, add 15 g bacto agar.
Autoclave for 35 min on liquid cycle.

Protocol

1. Grow liquid LB culture of wt PA-14 overnight.
2. Dilute 1:10 and plate 25 μl onto manganese selection plates. You may want to play with the concentrations of cells plated to achieve optimal results.
3. Wrap plates in parafilm and incubate at 37^{o}C for 1-2 days.
4. If resulting colonies are well spaced on the plate, stamp to low and high iron CAS plates (see posted protocol), and to a BHI plate. If the colonies are close together, pick each one with a sterile stick and transfer to low and high iron CAS plates and a BHI plate. Be sure to spot a colony of wt PA-14 onto each plate as a control. If stamping, be sure to mark the orientation of the plates, so you can identify colonies.
5. Incubate at 30^{o}C overnight.
6. Screen for mutants based on the pictures provided in the CAS medium protocol.

Based on PA14 genome, design primers to amplify and sequence the *Fur* gene to determine the nature of the mutation in the putative *fur* mutants you identify.