7.13 Experimental Microbial Genetics

Fall 2008

CAS Agar for Pseudomonas

Note: There is no Mg(II) in the medium

1. Medium, in a 1 l flask

540 ml H_2O
1.62 g sodium succinate
1.68 g casamino acids
0.43 g Na_2SO_4
5.44 g PIPES
Dissolve, adjust to pH=6.7 (exactly!) with 5 M NaOH
9 g agar
Autoclave (with the magnetic stirrer bar still in the flask)

2. CAS solution, in a 100 ml flask

30 ml H_2O
35 mg chrome azurol S
Dissolve, while stirring add
6.2 ml 1 mM $FeCl_3$ (made in 10 mM HCl)

3. Cetrimide-CAS solution

24 ml H_2O
47 mg cetrimide
Dissolve, then add the CAS solution slowly while stirring.
Autoclave

4. CAS plates

Cool medium to 50˚C
Add Cetrimide-CAS solution slowly while stirring.
Pour for low iron CAS plates.
Add 1/1000 vol. of 100 mM $FeCl_3$ (made in 10 mM HCl) to a final conc. of 100 μM for high iron CAS plates.

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Expected result for *Pseudomonas aeruginosa* strain PAO1 and *fur* mutants on high and low CAS medium plates