# 7.13 Experimental Microbial Genetics

Fall 2008

**MOTILITY ASSAYS**

## Twitching

*Concept*

- *P. aeruginosa* cells can adhere to and move across solid surfaces by extending and retracting type IV pili, a behavior known as 'twitching'.

*Plates*

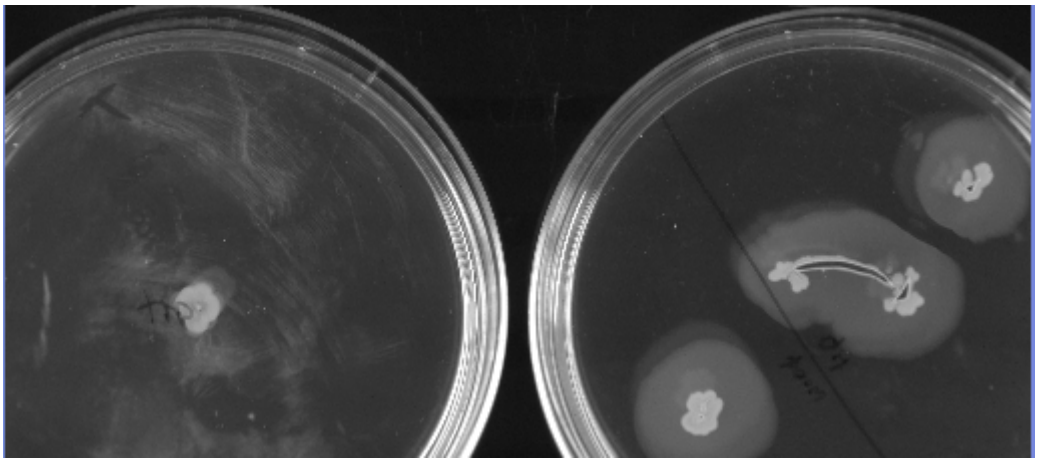
- LB + 1% bacto-agar
  - pour as shallow a layer of LB/agar as possible; this will aid in visualizing the twitching zone

*Assay*

- grow liquid culture o/n
- aspirate 2ul culture and insert pipette tip through the gel pad to the plastic/gel interface
- dispense 2ul culture into the interface
  - move pipette tip very slightly side to side until liquid volume is completely dispensed
- incubate plates for 24h at 37C

*Result*

- strains with functional pili will form a large, haze of growth surrounding a smaller, thicker colony
  - plates can be held up to light to aid in visualizing the twitching zone



***left*: PA14 pilB::MAR2XT7 (DKN# 599) *right*: PA14 (DKN# 263)**

## Swimming

*Concept*

- *P. aeruginosa* cells swim in chemotactic rings through liquid channels in semisolid agar plates. This behavior requires functional flagellum and chemotaxis machinery.

*Plates*

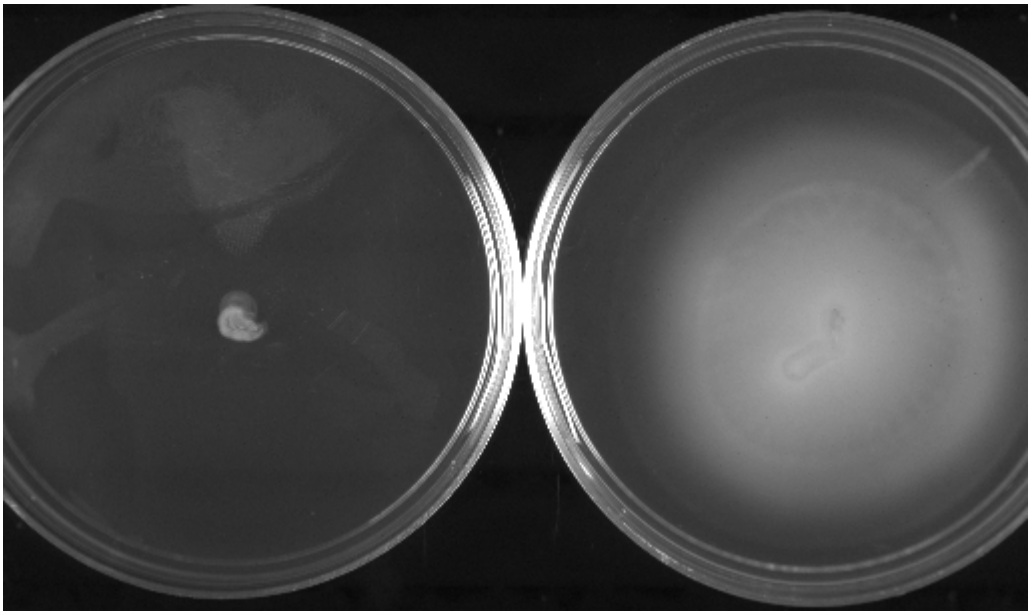
- LB + 0.3% bacto-agar
  - pour plates relatively thick to allow yourself a large area for inoculation
  - DO NOT INVERT; 0.3% agar is not sturdy and will collapse if plate is sufficiently disturbed

*Assay*

- grow liquid culture o/n
- aspirate 2ul culture and insert pipette tip into the gel pad halfway between the bottom (plastic/LB interface) and top (LB/air) surfaces
- dispense 2ul culture into the gel
- incubate plates for 12-14 hours at 30C or 24 hours at RT

*Result*

- strains with functional flagella will grow into large circular colonies while flagellar mutant colonies will be greatly reduced in diameter



***left*: PA14 flgK::tnB30 (DKN# 349) *right*: PA14 (DKN# 263)**

## Swarming

*Concept*

- Under certain conditions, *P. aeruginosa* cells move in a coordinated manner on semisolid surfaces. This activity, known as swarming, is dependent on flagella, type IV pili and production of rhamnolipids.

*Plates*

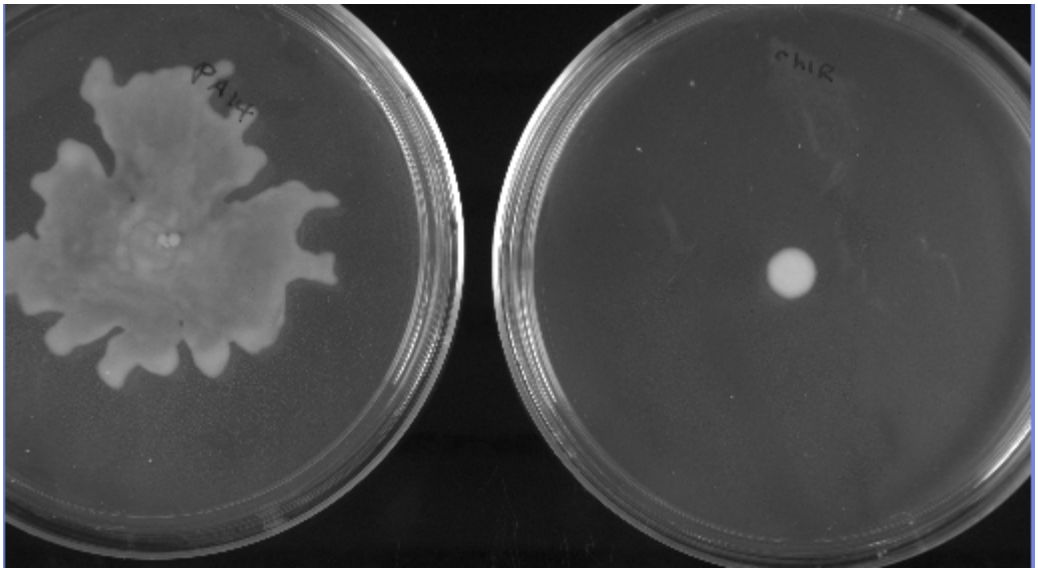
- 0.8% nutrient broth, 0.5% dextrose (D-glucose), 0.5% agar
  - plates should be dried one overnight before assay

*Assay*

- grow liquid culture o/n
- aspirate 2ul liquid culture and dispense on top of the gel in the center of the plate
- incubate at 30C for 24h

*Result*

- The complex motility pattern known as swarming involves the extension of multiple tendrils, or colony sub-groups traveling in distinct directions




***left*: PA14 (DKN# 263) *right*: PA14 rhlR::MAR2XT7 (DKN# 408)**

## KEY REFERENCES

Rashid MH and Kornberg A. Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*. PNAS. 2000 Apr 25; 97:4885-4890 (A, I, T)

Hobbs M, Collie ES, Free PD, Livingston SP, Mattick JS. PilS and PilR, a two-component transcriptional regulatory system controlling expression of type 4 filmbriae in *Pseudomonas aeruginosa*. Mol Microbiol. 1993 Mar; 7(5):669-82. (T)

Masduki A, Nakamura J, Ohga T, Umezaki R, Kato J, Ohtake H. Isolation and characterization of chemotaxis mutants and genes of *Pseudomonas aeruginosa*. J Bacteriol. 1995 Feb; 177(4):948-52. (I)

Kohler T, Curty LK, Barja F, van Delden C, Pechere JC. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. J Bacteriol. 2000 Nov; 182(21):5990-6. (A, I, T)

Semmler AB, Whitchurch CB, Mattick JS. A re-examination of twitching motility in *Pseudomonas aeruginosa*. Microbiol. 1999 Oct; 145(10):2863-73 (T)

***KEY***
A: swarming
I: swimming
T: twitching