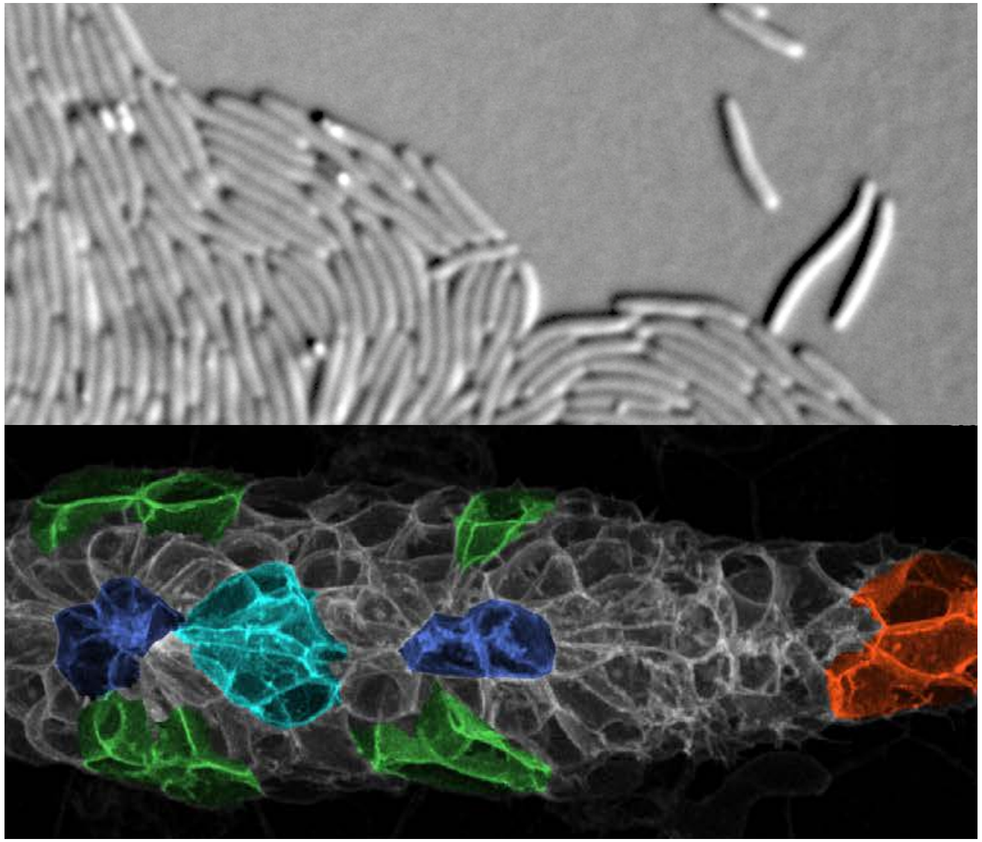
Cellular Motility



Course 5: Collective motility in bacteria

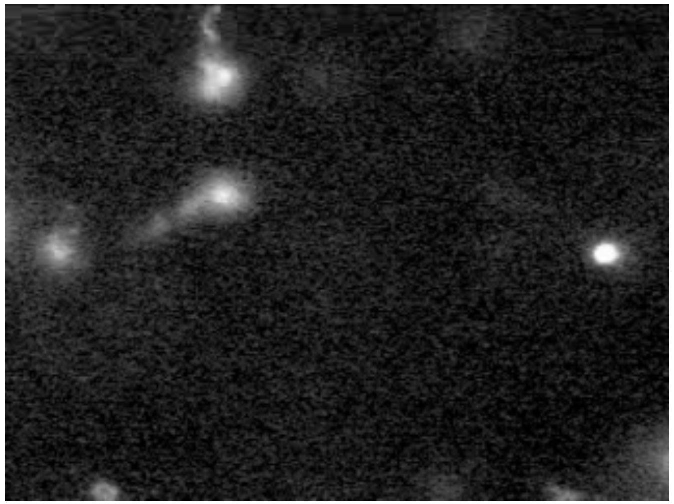
Thomas Lecuit

chaire: Dynamiques du vivant

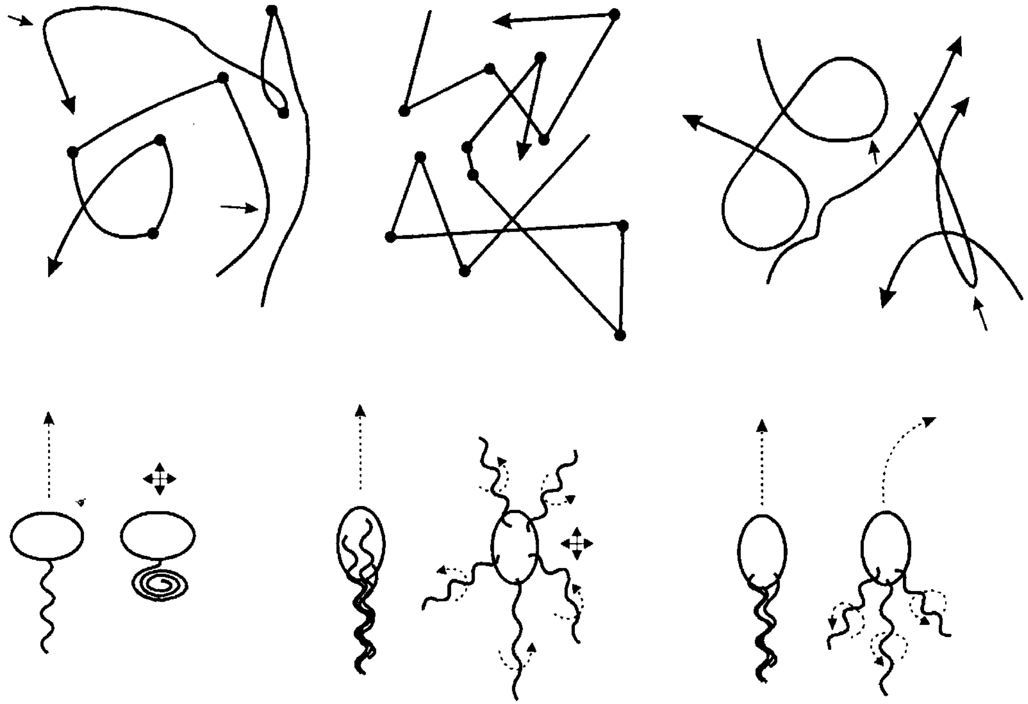
COLLÈGE DE FRANCE
1530

Bacteria swim, propelled by flagella

Variations on the theme of flagella rotation in different bacteria



Howard Berg
http://www.rowland.harvard.edu/labs/bacteria/movies/ecoli.php



Rhodobacter sphaeroides *E.coli* *Sinorhizobium meliloti*

- single flagellum rotates in single direction
- motor stops and flagellum relaxes and coils

- CW rotation of motor/flagellum in bundle
- slow rotation causes flagella to separate

J. Armitage and R. Schmidt *Microbiology.* 143, 3671-3682 (1997)

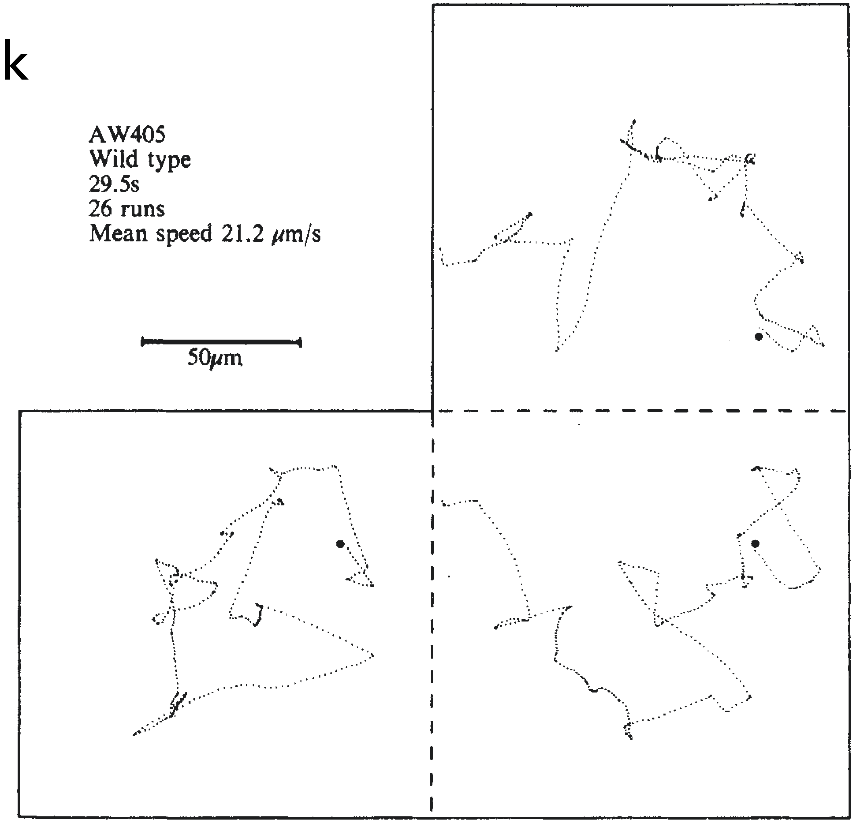
The swim of the bacterium *E. coli*

–Random walk

- **Cells follow a succession of runs and « tumble »**

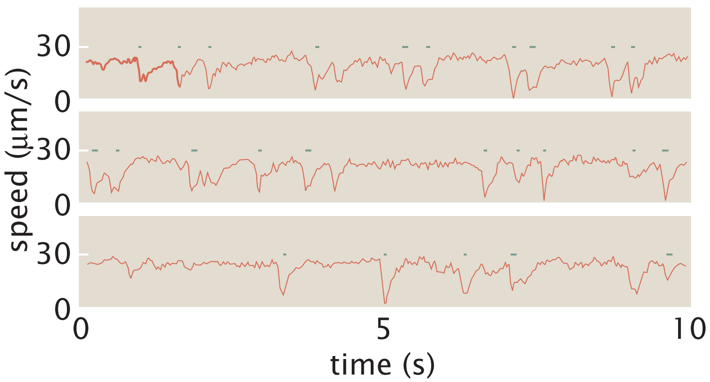
Runs are at average velocity 14µm/s and the mean run length is about 1s.
During tumbles, cells are immobilized.
Tumbles length is about 0.1s

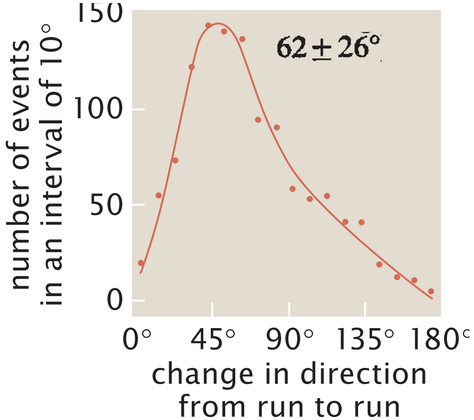
As cells resume movement, they adopt a new, nearly random trajectory (62±26°)



- **Runs and tumbles occur at random (Poisson statistics)**

For a given organism in a given environment, the probability per unit of time to stop a run or a tumble is a constant



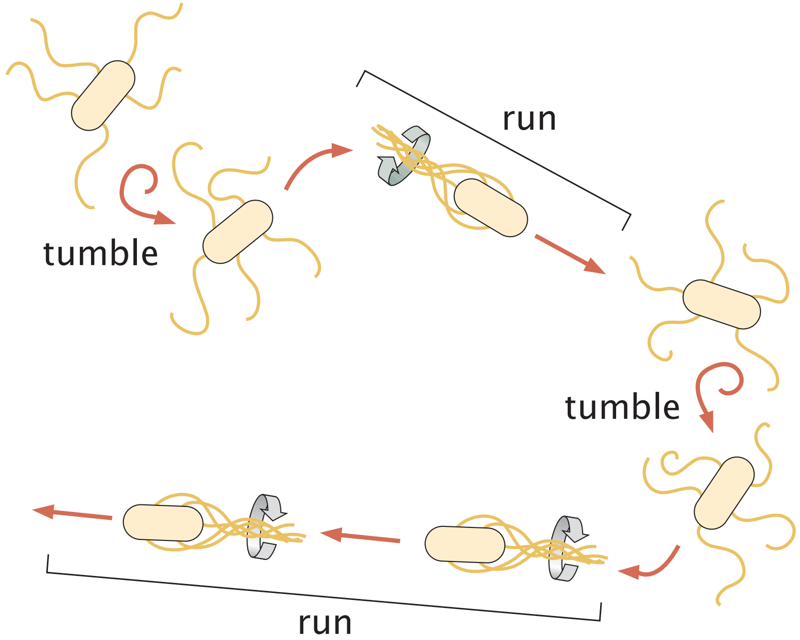


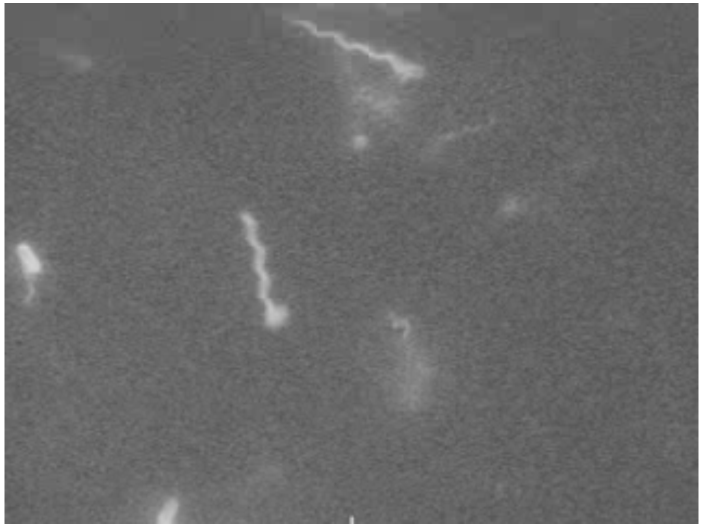
Howard Berg and Douglas Brown. 1972, *Nature* 239, 500-504

The swim of the bacterium *E. coli*

Without chemoattractant

- 6 flagella bundle when they rotate counterclockwise (CCW)
- Bundles rotate and propel *E. coli* along runs
- Runs are followed by tumbles due to CW rotation of flagella which are no longer bundled





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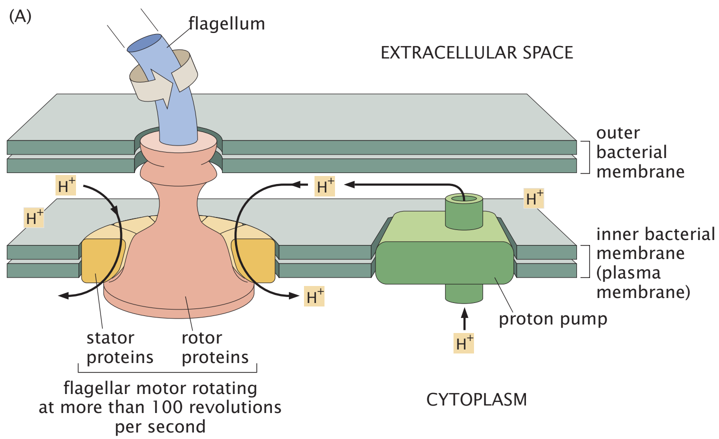
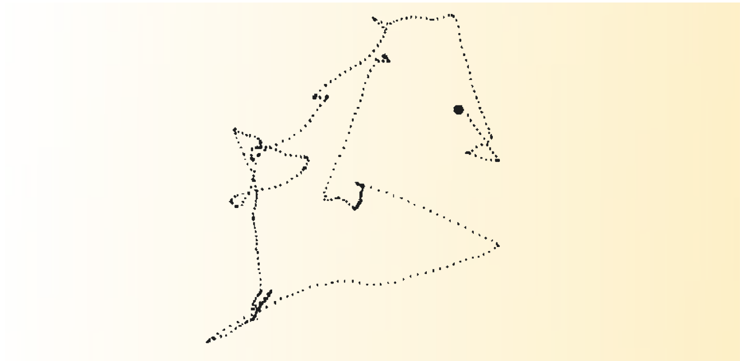
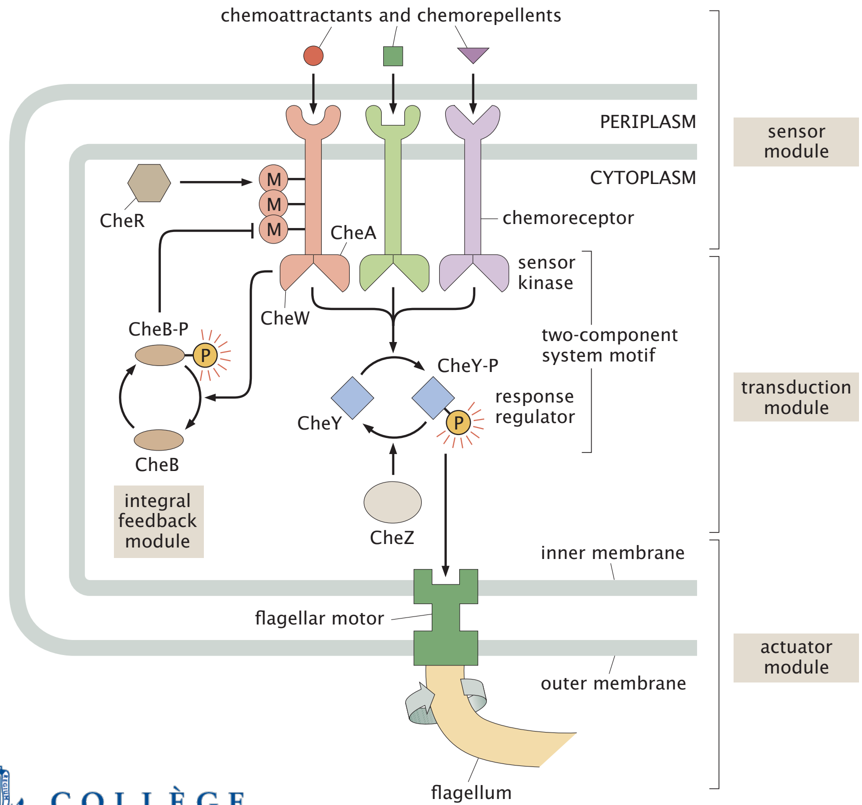


Howard Berg and Douglas Brown. *Nature* 239, 500-504 (1972)

The swim of the bacterium *E. coli*



- Biased random walk in a spatial gradient
- Temporal gradient sensing
- Memory



Key properties of chemotactic network

- Sensitivity - Gain : output/input ratio
- Adaptation: reset after input
- High amplitude range

Bacteria are social

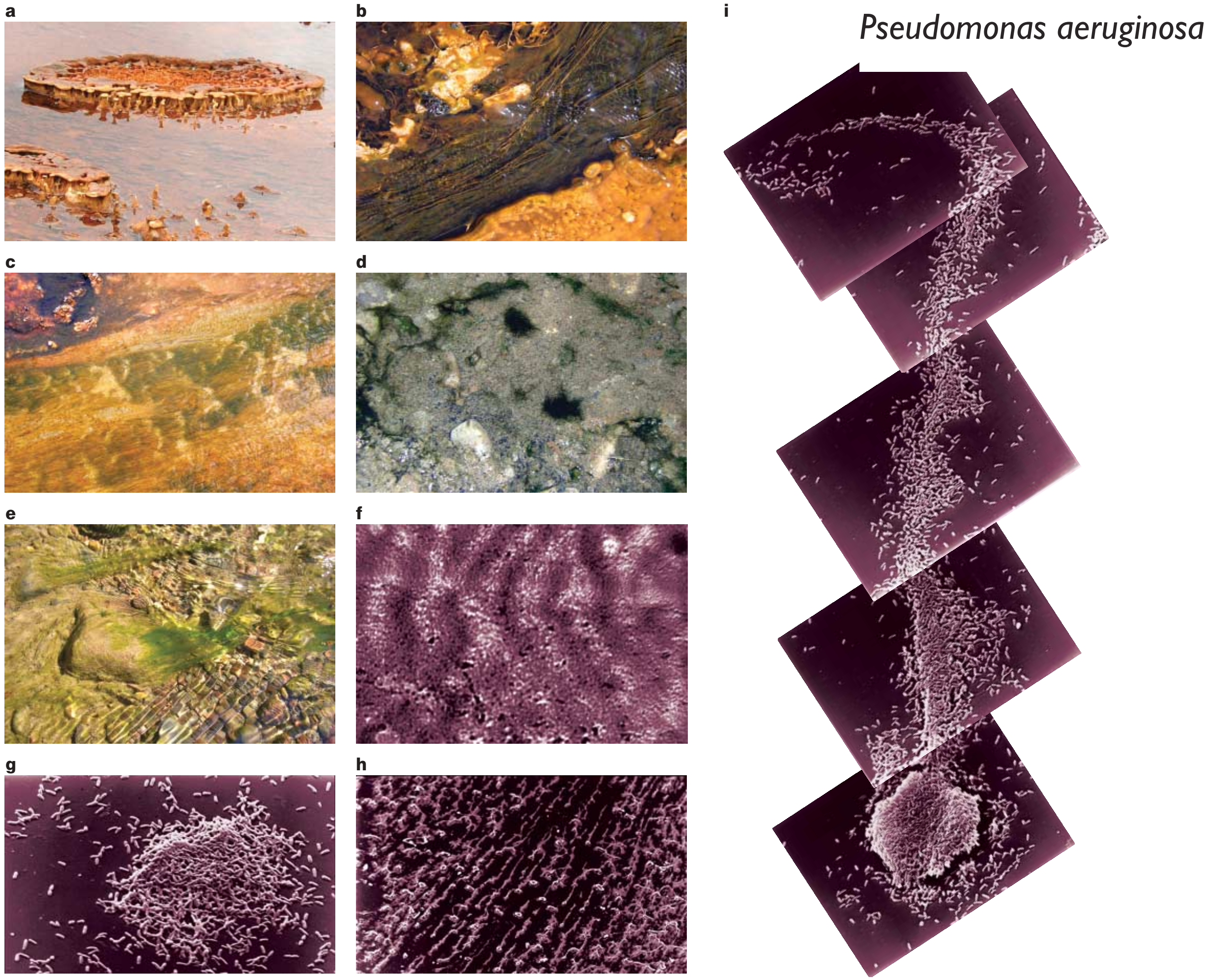
Bacteria colonize various surfaces (in organisms and external environments)
Structural similarity of biofilms growing in hydrothermal hot springs, freshwater rivers and laboratory flow cells.

Hydrothermal hot springs (**a**–**c**)

Biscuit Basin thermal area, Yellowstone National Park, USA

Biofilms growing in freshwater rivers (**d,e**)

Gardener River, Yellowstone National Park, USA (**d**), Hyalite Creek, Bozeman, Montana, USA (**e**),

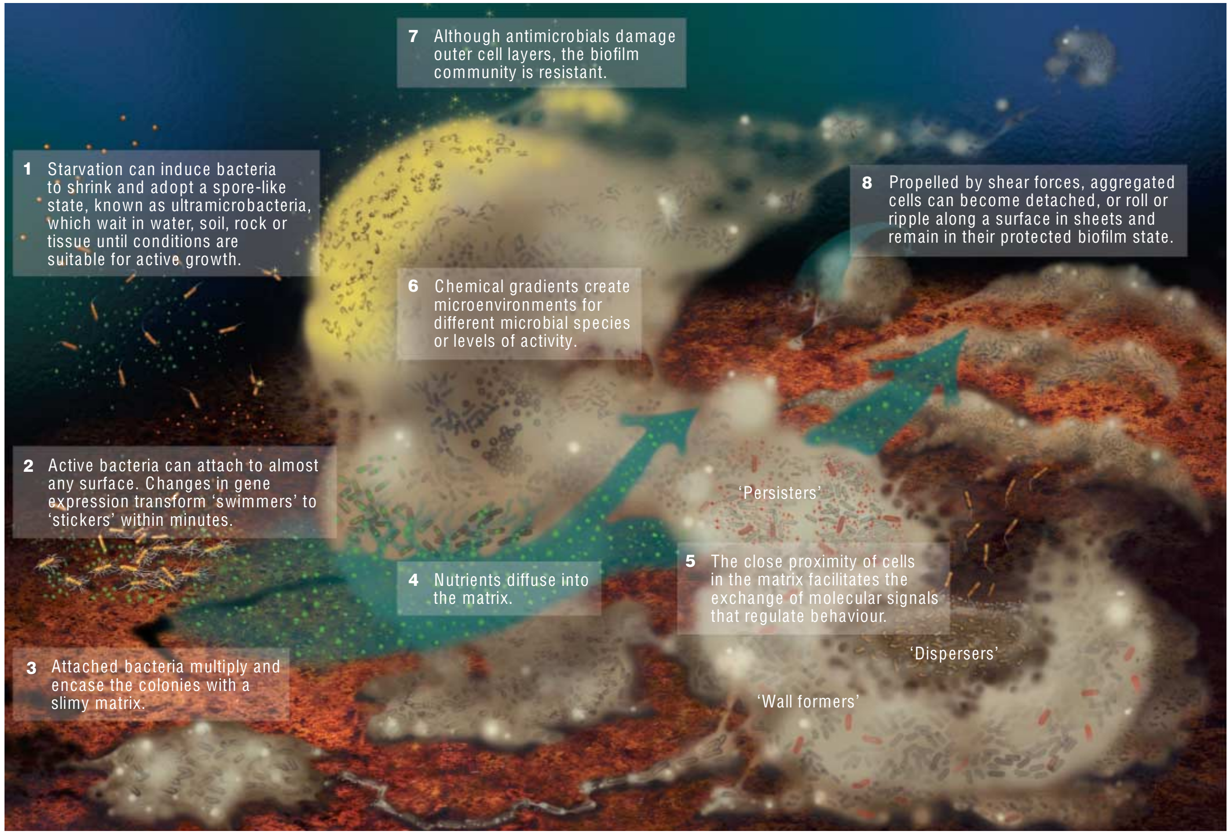


Hall-Stoodley L, Costerton JW, Stoodley P. *Nature Reviews Microbiology* 2:95–108 (2004)

Bacteria are social

- First sign of life on earth ~ 3.5B years ago

Stromatolites

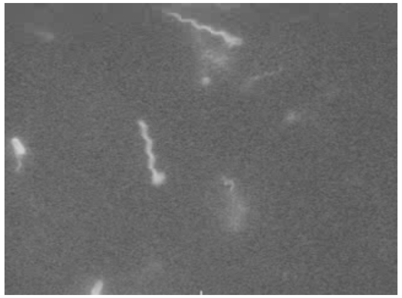


Hall-Stoodley L, Costerton JW, Stoodley P. *Nature Reviews Microbiology* 2:95–108 (2004)

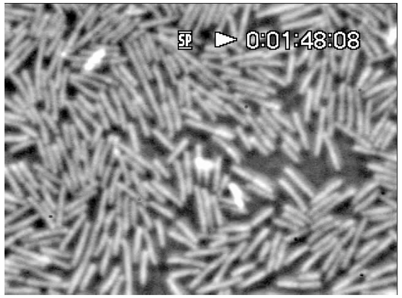
Swarming bacteria

- When swimming bacteria are in contact with a soft surface (eg. <1% agar), they differentiate and move at high density in 2D: they swarm

Swimmer (3D)



Swarmer (2D)



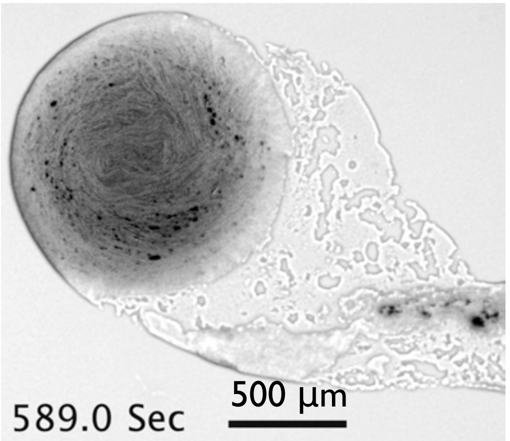
E. coli

Swarming bacteria

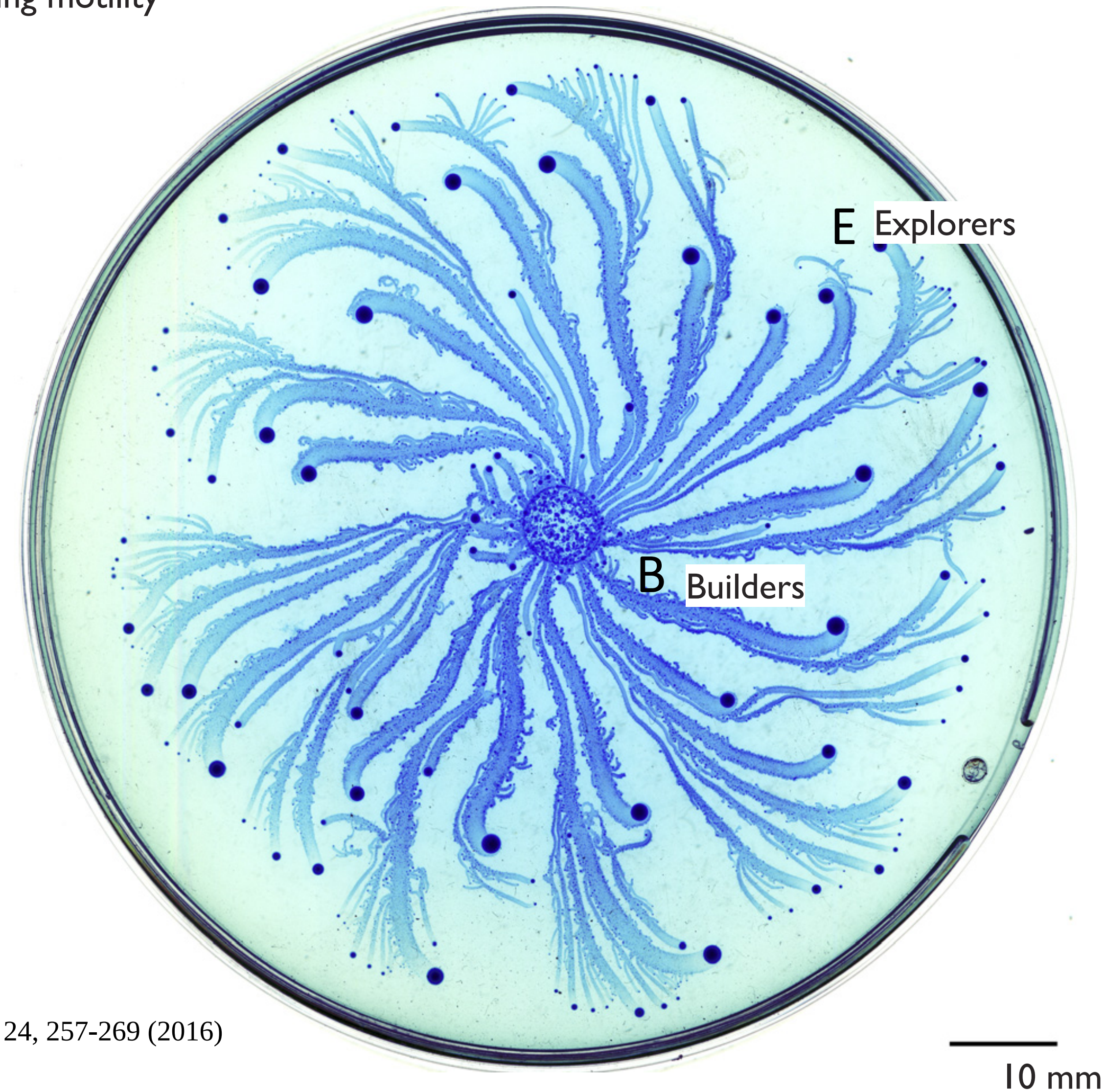
- When swimming bacteria are in contact with a soft surface (eg. <1% agar), they differentiate and move at high density in 2D: they swarm
- The bacteria *Paenibacillus vortex* has swarming motility

Builders grow and divide but are not motile

Explorers are highly motile colonies that spins out into new territories



Ingham, C.J. et al. *PNAS* 108, 19731–19736 (2011)

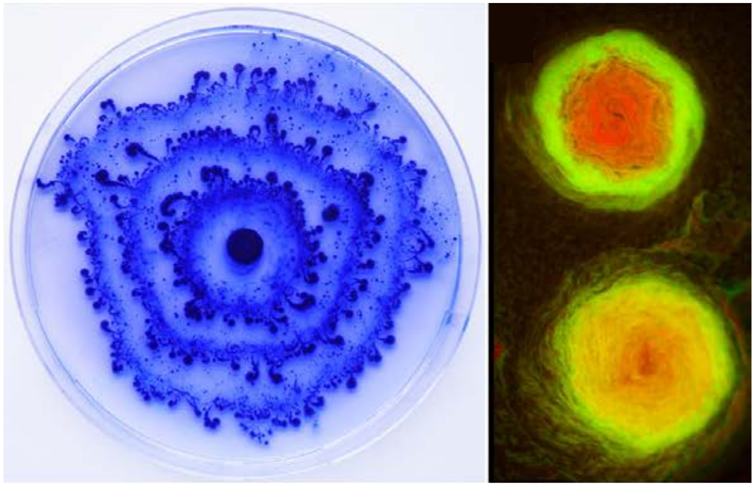


E. Ben-Jacob et al. *Trends in Microbiology*, 24, 257-269 (2016)

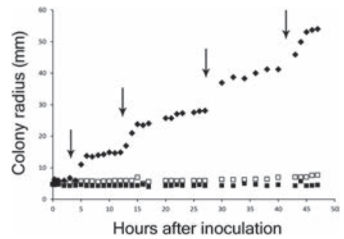
Social benefits of swarming - species interactions

—Transport of antibiotic resistant bacteria by swarming antibiotic sensitive bacteria

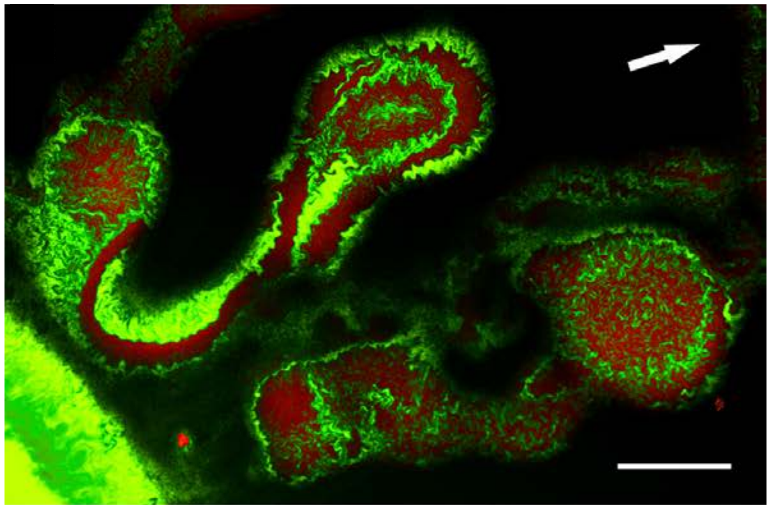
- *Paenibacillus vortex* is sensitive to Ampicillin
- An *E coli* strain resistant to Amp (Amp^R) survices but cannot expand in presence of Amp
- Swarms of *P. vortex* transports Amp^R *E. coli* and both bacteria form an expanding cooperative colony where *E. coli* detoxifies the environment.
- The colony forms concentric rings with phases of expansion under low Amp, followed by phases of slower expansion when Amp is at higher concentration.
- Bet-hedging strategy: *P. vortex* sub colonies can explore new territory without *E. coli* to avoid competition of hitchhiker. Or expand more slowly with detoxyfier bacteria.



E. coli (green), *P. vortex* (red)



- Co-transport of *P. vortex* (red) and Ctx^R bacteria *Enterobacter aerogenes* (green) in presence of Ctx (cefotaxim).

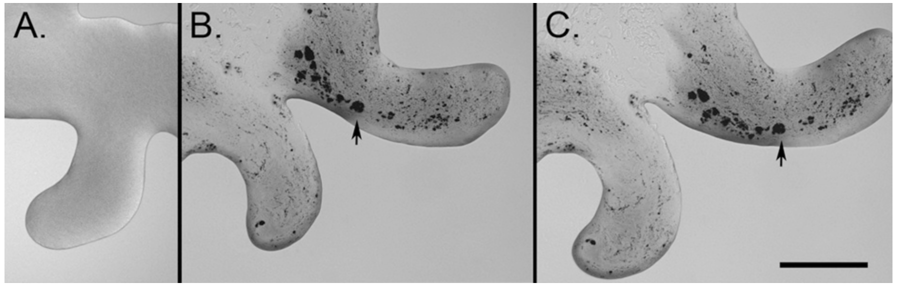


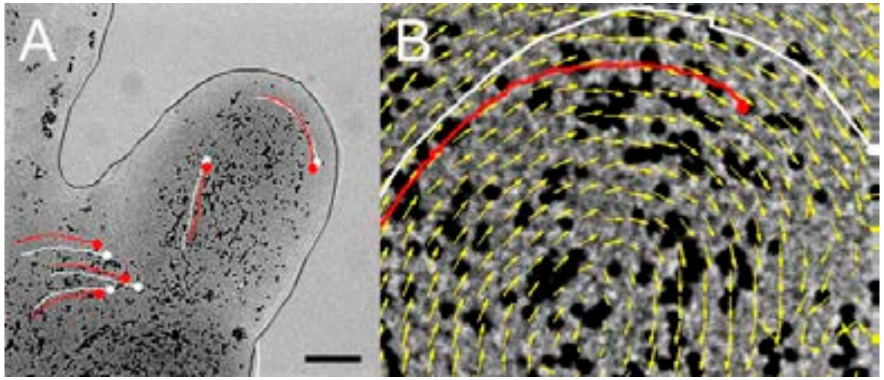
Finkelshtein, A. et al.*MBio* 6, e00074-15 (1–10) (2015)

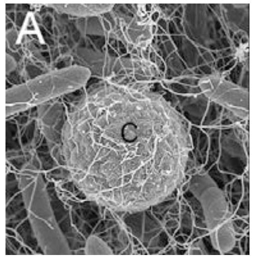
Social benefits of swarming - species interactions

—Dispersal of fungi spores by swarms of bacteria in the soil

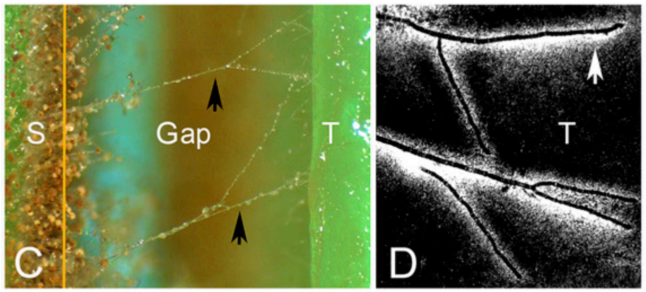
- Heterogeneous environment of the plant root (rhizosphere) comprises bacteria and fungi
- The bacteria *Paenibacillus vortex* has swarming motility
- *P. vortex* transports/advects spores of the fungus *Aspergillus fumigatus* over >30cm at speeds of 3μm/s and can move them away from sites of adverse growth conditions (eg. anti fungal molecule)
- *A. fumigatus* spores are specifically attached/recognized by bacteria flagella.







- In return, fungi filaments from tracts for the dispersal across gaps of bacteria swarms



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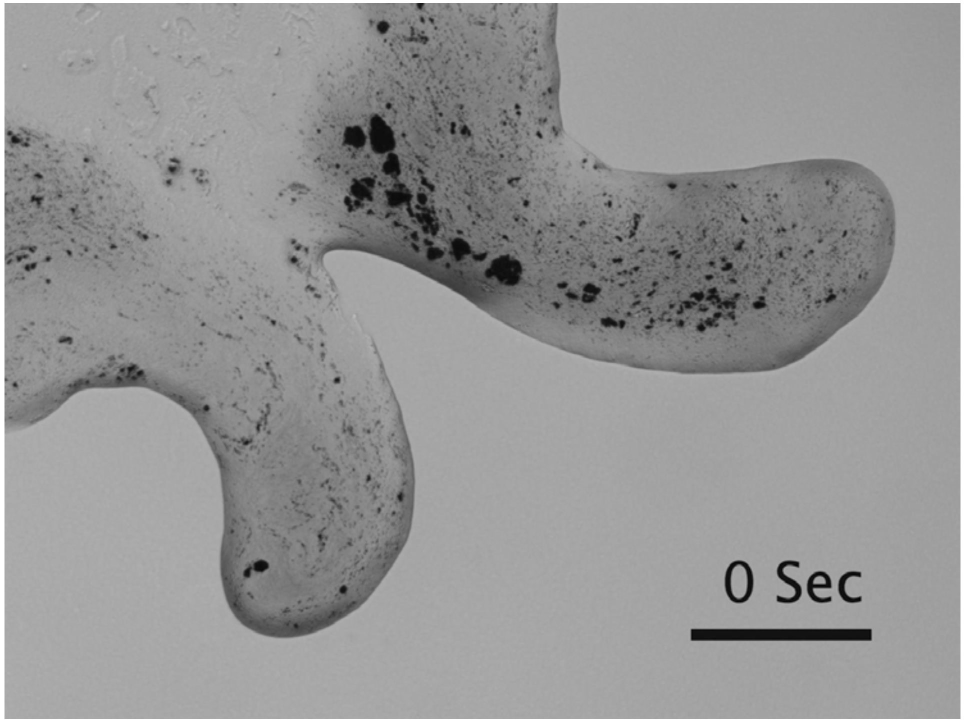
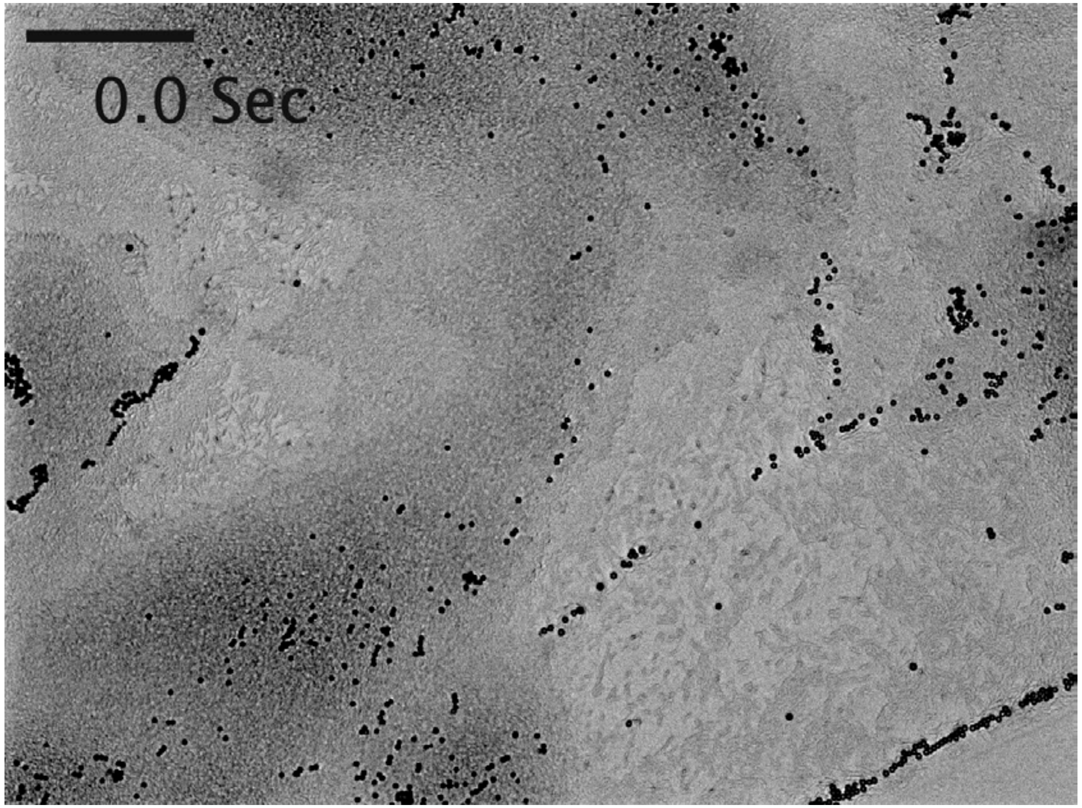




Swarming mass of *P. vortex* transporting hundreds of ungerminated conidia imaged 4 h after inoculation and 31 mm away from the coinoculation point. (Scale bar: 300 μm.)

Swarming mass of *P. vortex* transporting hundreds of ungerminated conidia. Not all conidia are in motion. (Scale bar: 100 μm.)

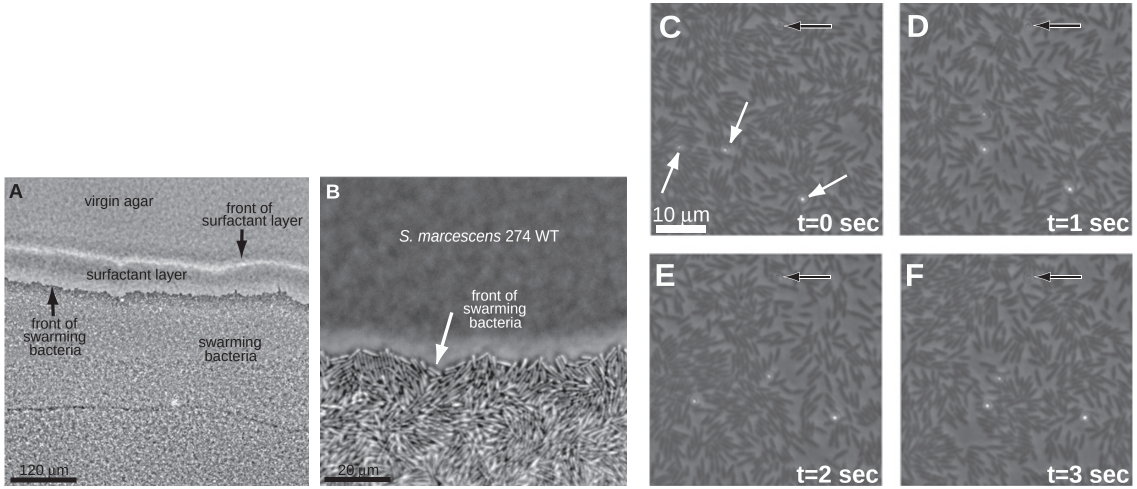
Ingham, C.J. et al. *PNAS* 108, 19731–19736 (2011)

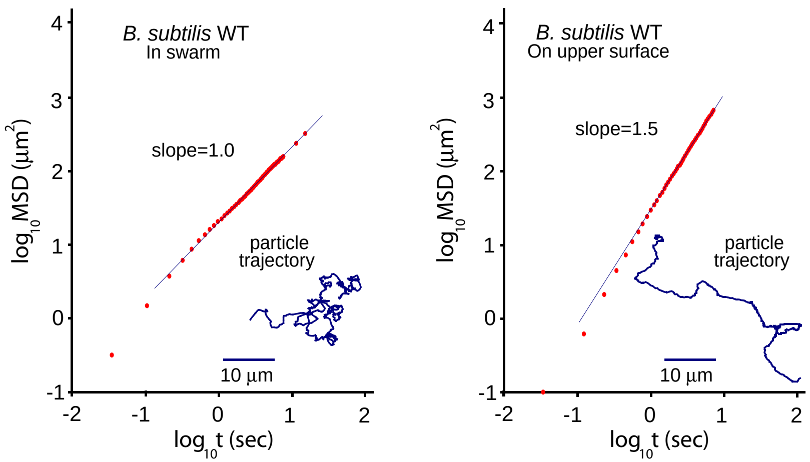
Social benefits of swarming - interaction with environment

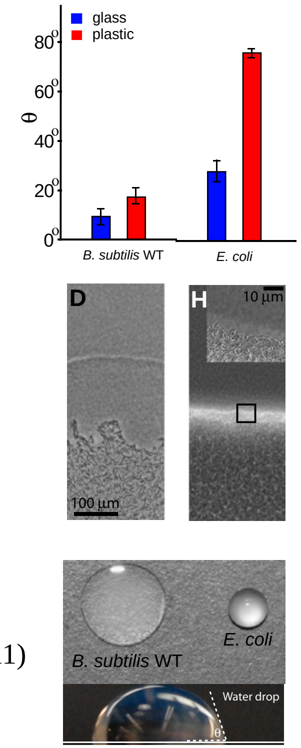
—Superdiffusive transport at swarm upper layer

- The MgO particles on swarm surfaces migrated faster than mobile particles inside the same area of the swarm and faster than particles that migrated on the upper surface of strains with a lower surfactant activity.
- Superdiffusive behavior of particles at the upper surface in contact with surfactant layer.
- **Origin of superdiffusive behavior** not clear: heterogeneity in surfactant concentrations (eg. Marangoni flows)?

- **Functional benefits?**
  - Long range communication by transport of signaling molecules (compared to Brownian motion)
  - Flow of nutrients/oxygen from edges to inner swarms, temperature regulation





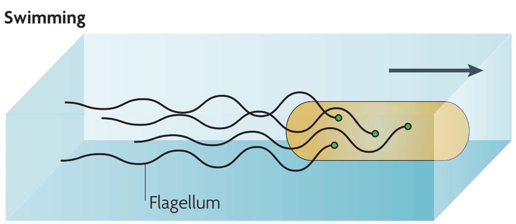


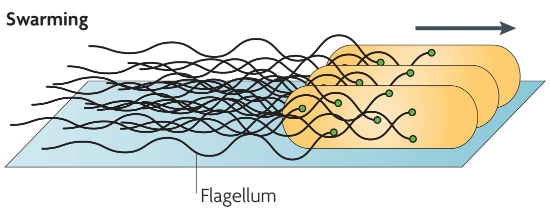
A. Be'er and R. Harshey *Biophysical Journal* 101(5) 1017–1024 (2011)

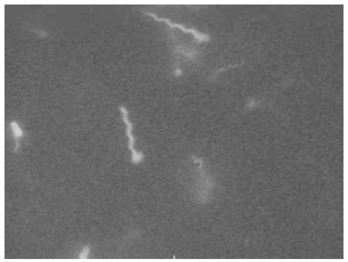
Swarming response: surface translocation of bacteria

- Swimming bacteria that contact a soft surface (eg. Agar) switch to a swarm behavior
  — swarming: multicellular movement across surface powered by rotating flagella for swimming bacteria such as *E. coli* or *Salmonella.*
  — This induction is reversible (if cells are placed back in solution, they de-differentiate and swim)
  — It occurs within 30 min

  —In some bacteria, induction of flagellar genes and **increase in number of flagella per cell**
  Flagella cover the entire cell (peritrichous flagella)
  Peritrichous flagella bundle together when they rotate to increase the effective flagellar stiffness and make force generation more efficient in viscous liquids



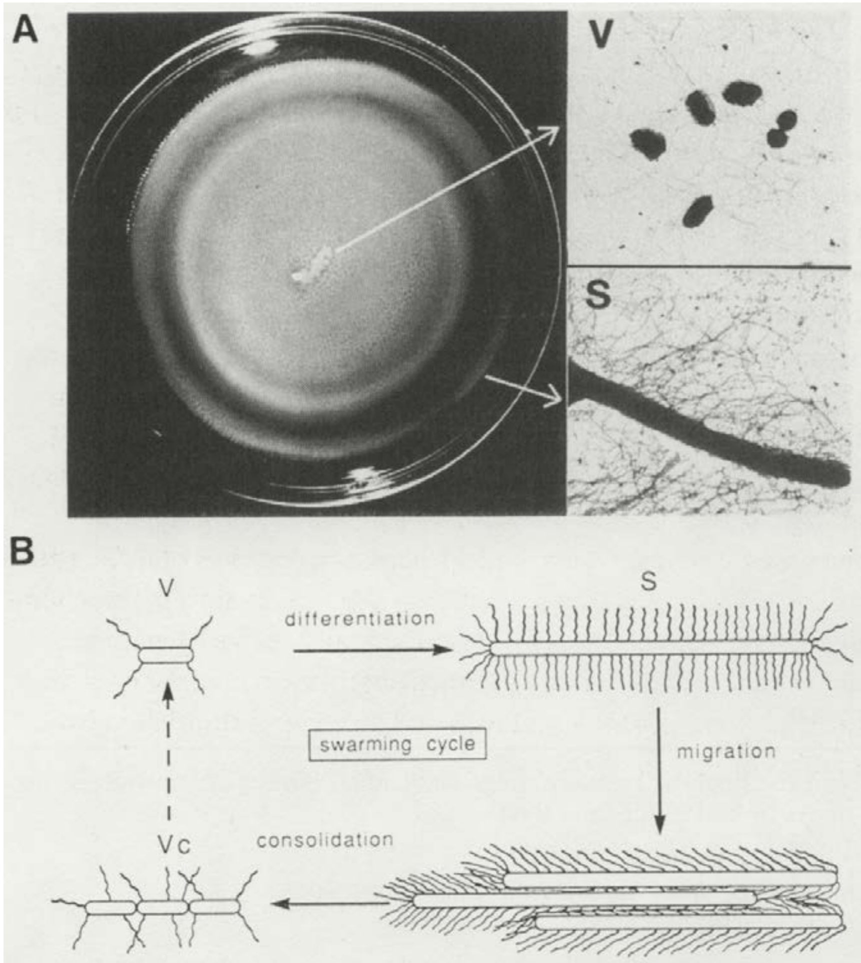




Historical origins: swarming in *Proteus*

Differentiation of cells in contact with a soft surface (eg. Agar)

- *Proteus mirabilis:* Gram negative enterobacteria.
- Commensal of digestive tract in animals
- Present in soil and water
- Responsible of infections of urinary tract in humans
- Swarming behaviour of *Proteus mirabilis:*
    - Cycles of vegetative and swarming states gives rise to a characteristic concentric colony morphology
    - Cells become longer, filamentous due to multi nucleation (cessation of septation)
    - Development of many (~50) peritrichous flagella which allows better adhesion to substratum and motility



John J. Farmer - CDC public health image library

Verstraeten N, et al. *Trends in Microbiology* 16:496–506 (2008)

Allison, C, and Hughes, C, *Science Progress* 75: 403-422 (1991)

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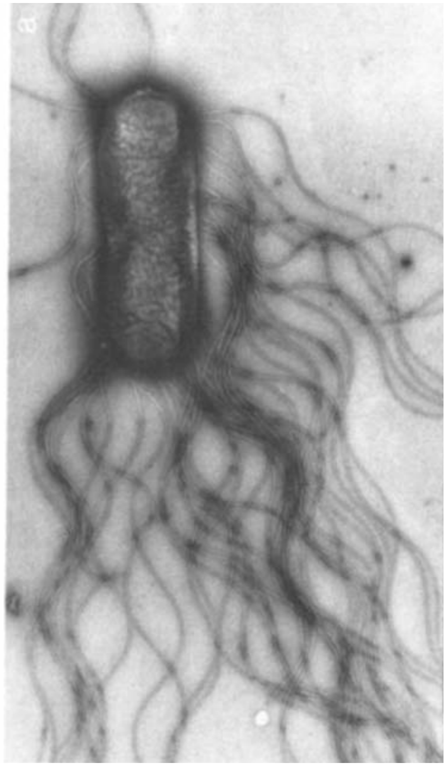
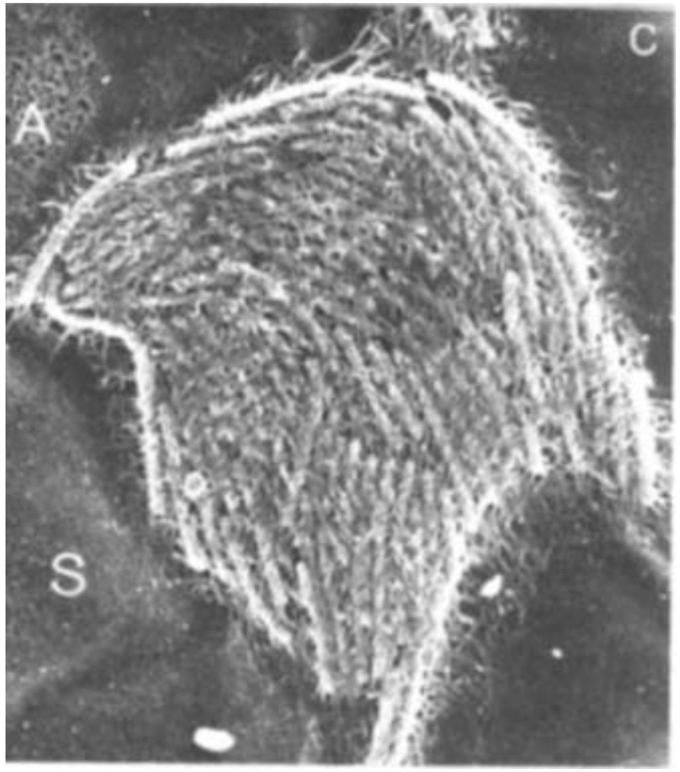


Proteus mirabilis
Swarm cell (50 flagella)



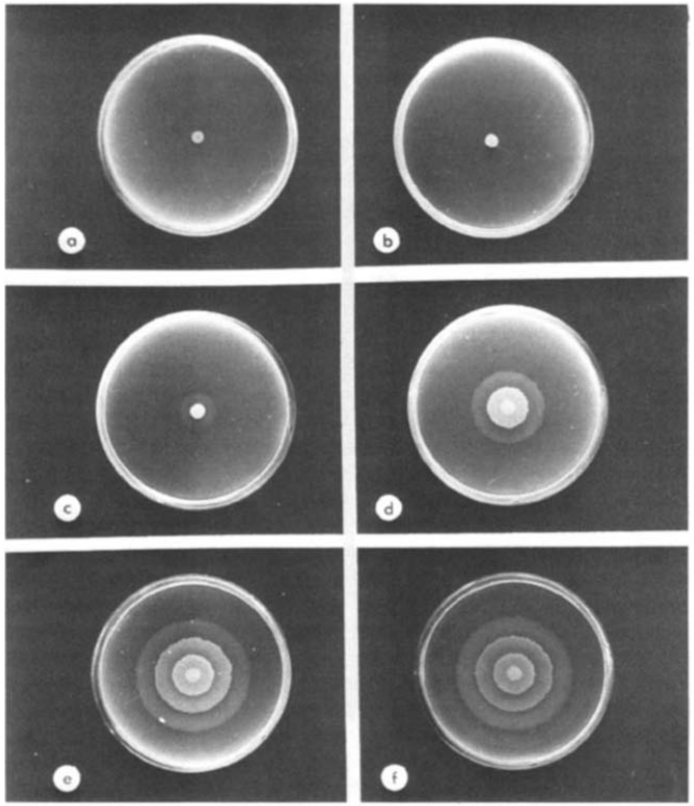
slime-covered agar (S). and agar without slime (A).

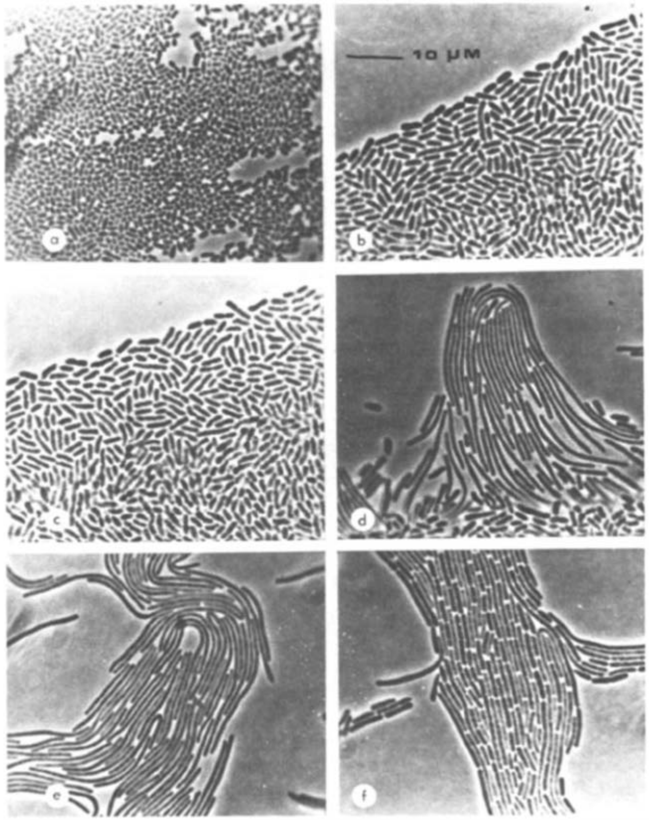
F.D. Williams and R.H. Schwarzhoff. *Ann. Rev. Microbial.* 32:101-22 (1978)

Historical origins: swarming in *Proteus*

— Cell differentiation associated with swarming behavior

- Formation of multinucleate filaments (long bacteria)
- Growth of multiple flagella (peritrichous)





Cell growth and filamentation at the swarm edge

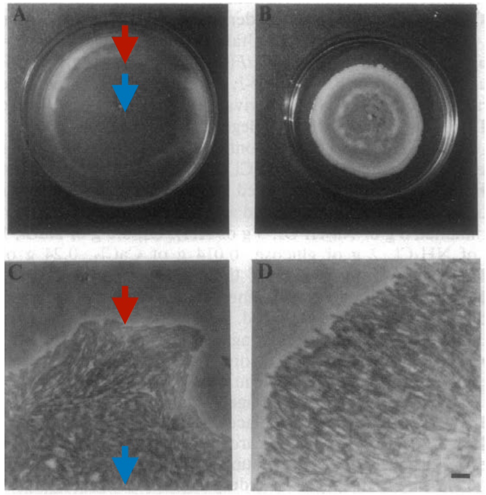
Consolidation

F.D. Williams and R.H. Schwarzhoff. *Ann. Rev. Microbial.* 32:101-22 (1978)

Swarming behaviors in other bacteria

— Cell differentiation associated with swarming behavior
- Formation of multinucleate filaments (long bacteria)
- Growth of multiple (peritrichous) flagella
- Occurs on soft media (0.5-2% Agar)
- Speed of cell front expansion: 2-10µm/s and up to 30-50µm/s

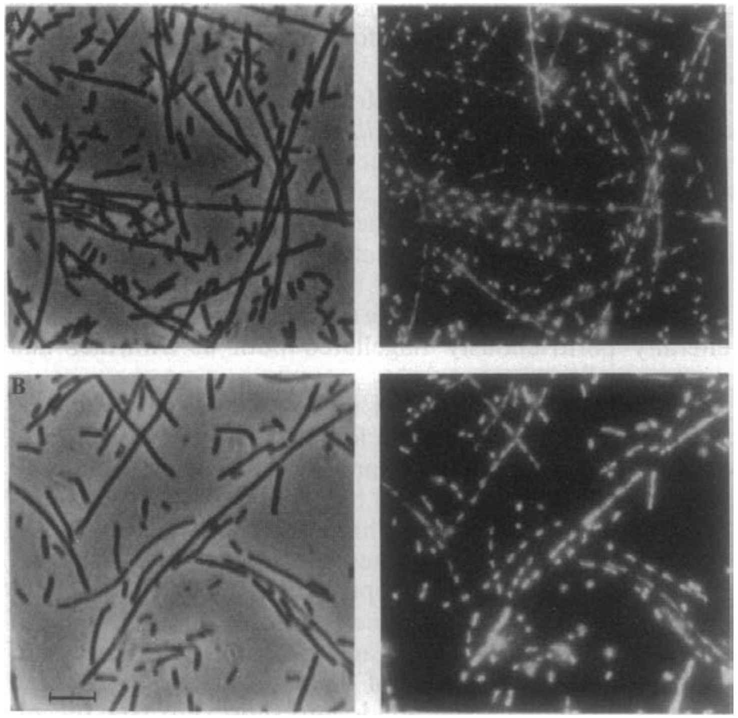
E.coli   *Salmonella typhimurium*



Nuclear staining

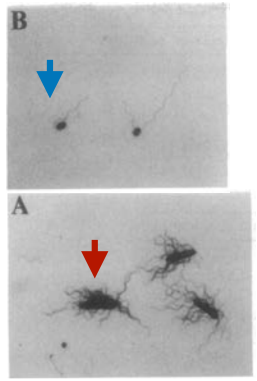
E.coli

S typhimurium



Center

Edge



Harshey RM, Matsuyama T. *PNAS* 91:8631–8635 (1994)

Diversity of swarming behaviors

— Cell differentiation associated with swarming behaviour
- Formation of multinucleate filaments (long bacteria)
- Growth of multiple flagella (peritrichous)

Bacterium	Flagella arrangement (vegetative/ swarm cell)	Agar concentration permitting swarming (%)	Rate of surface translocation* (μm/min)
Proteus mirabilis	peritrichous	2.0	950
Proteus vulgaris			
Vibrio parahaemolyticus	polar/peritrichous	2.0	125
Vibrio alginolyticus			
Serratia marcescens	peritrichous	0.7–0.8	88
Chromobacterium spp	polar/peritrichous	0.6–1.2	92
Clostridium tetani	peritrichous	2.0	670
Clostridium novyi		2.0	nd
Clostridium bifermentans		1.0	300
Clostridium sporogenes		1.0	78
Bacillus alvei	peritrichous	2.0	120
Bacillus cereus			108
Bacillus subtilis		1.8	80
Bacillus megaterium		1.1	560

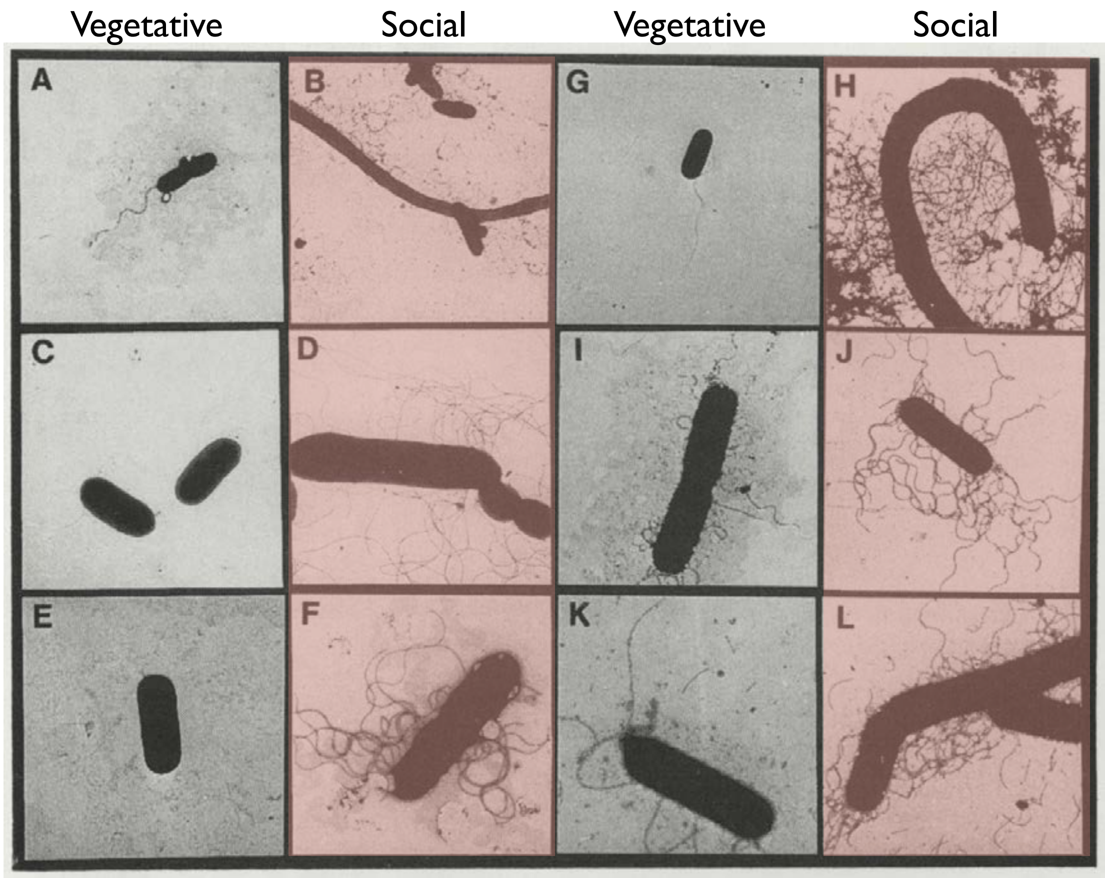


***Fig. 3.** Transmission sectron microscopy showing cell morphology of vegetative (V) and swarm cells (S) of various bacterial species. (A)* Vibrio parahaemolyticus, *V; (B)* V. parahaemolyticus, *S; (C)* Serratia marcescens, *V; (D)* S. marcescens, *S; (E)* Clostridium sporogenes, *V; (F)* C. sporogenes, *S; (G)* Clostridium tetani, *V; (H)* C. tetani, *S; (I)* Bacillus cereus, *V; (J)* B. cereus, *S; (K)* Bacillus subtilis, *V; (L)* B. subtilis, *S.*

Allison, C, and Hughes, C, *Science Progress* 75: 403-422 (1991)

Diversity of swarming behaviors

Macroscopic manifestations of swarming behaviors

The macroscopic appearance of a swarm colony:

- differs among bacterial species,
- depends on medium composition,
- agar concentration and water content,
- temperature.

D. Kearns, *Nature Rev. Micro.* 8: 634-644 (2010)

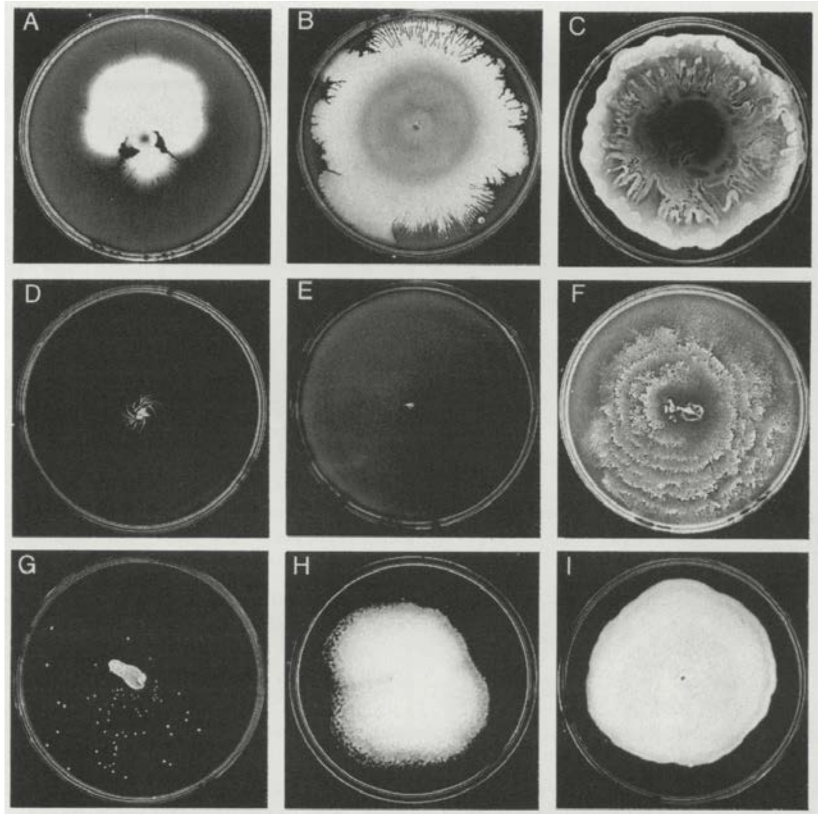
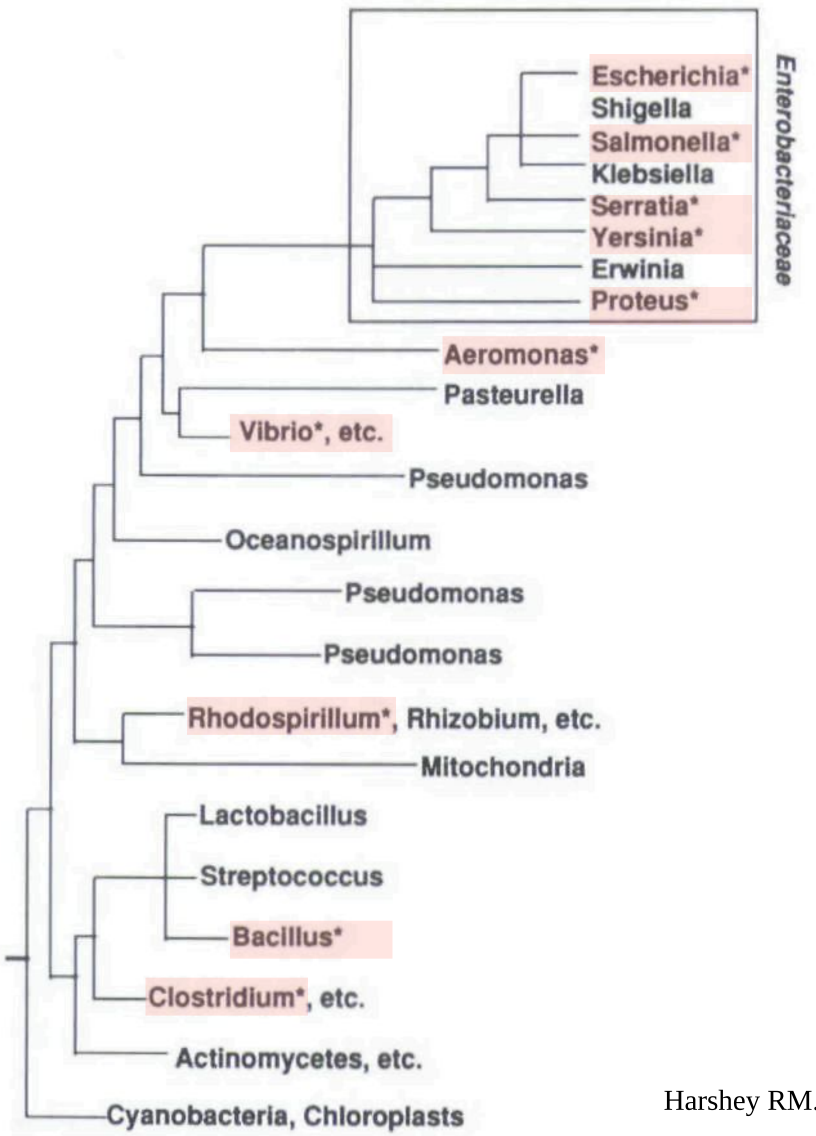


Fig. 4. *Swarming by various bacterial species on agar-containing media, (A)* Vibrio parahaemolyticus, *(B)* Vibrio alginolyticus, *(C)* Serratia marcescens, *(D)* Clostridium sporogenes, *(E)* Clostridium tetani, *(F)* Clostridium bifermentans, *(G)* Bacillus cereus, *(H)* Bacillus megaterium, *(I)* Bacillus subtilis.

Allison, C, and Hughes, C, *Science Progress* 75: 403-422 (1991)

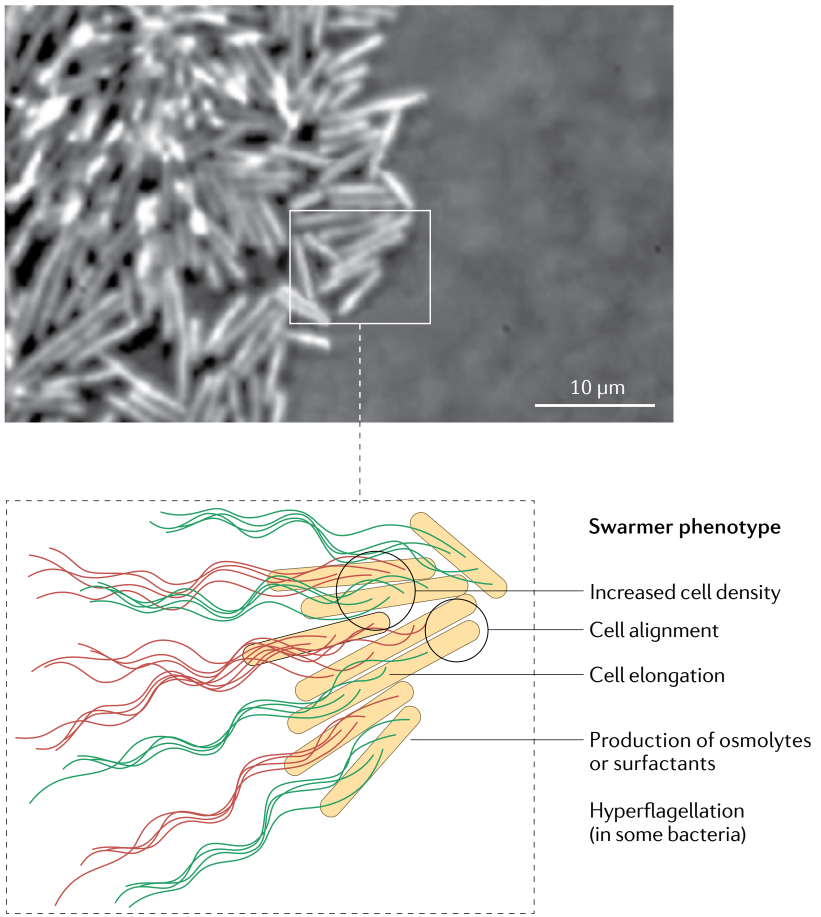
Widespread « invention » of swarming in bacteria lignages

This suggests important selective advantage of swarming behavior to colonize environments



Harshey RM. *Molecular Microbiology* 13: 389–394. (1994)

Transition to swarming state: *characteristics*

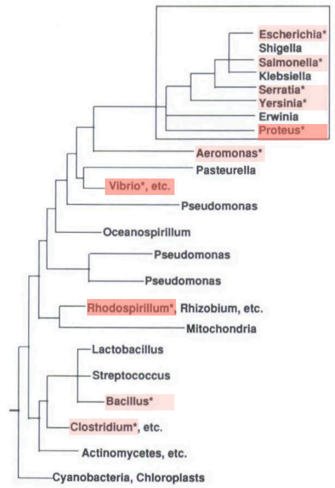


1. Robust swarmers
   - Swarm on hard agar (>1.5% agar)
   - Cell elongation
   - Hyperflagellation: polar or peritrich

   Azospirillum, *Rhodospirillum*, and *Vibrio* species , and *Proteus*

2. Temperate swarmers
   - Swarm on soft agar (0.5-0.8% agar)
   - No (or little) cell elongation
   - No hyperflagellation

   E. coli and *Bacillus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia*, and *Yersinia* species



N Wadhwa and HC. Berg. *Nature Rev. Mol. Cell Biol.* 20: 161-173 (2022)

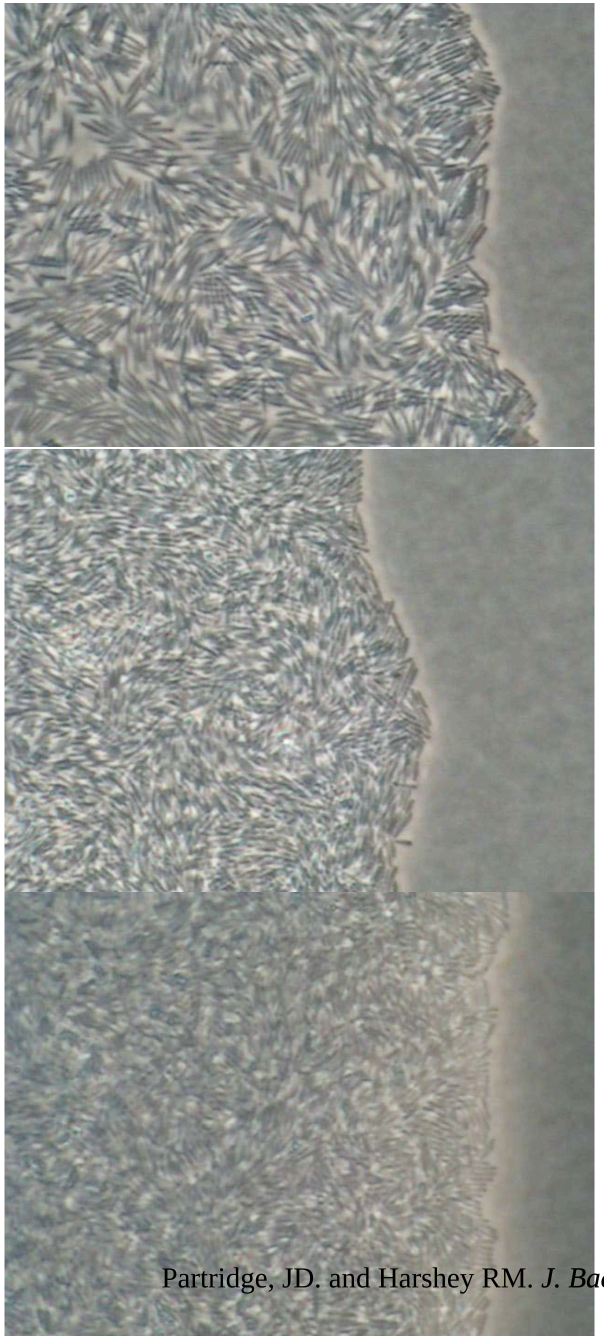
Transition to swarming state: *characteristics*

1. Robust swarmers
   - Swarm on hard agar (>1.5% agar)
   - Cell elongation
   - Hyperflagellation: polar or peritrichous

   Azospirillum, *Rhodospirillum*, and *Vibrio* species , and *Proteus*

2. Temperate swarmers
   - Swarm on soft agar (0.5-0.8% agar)
   - No (or little) cell elongation
   - No hyperflagellation

   E. coli and *Bacillus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia*, and *Yersinia* species



Bacillus subtilis

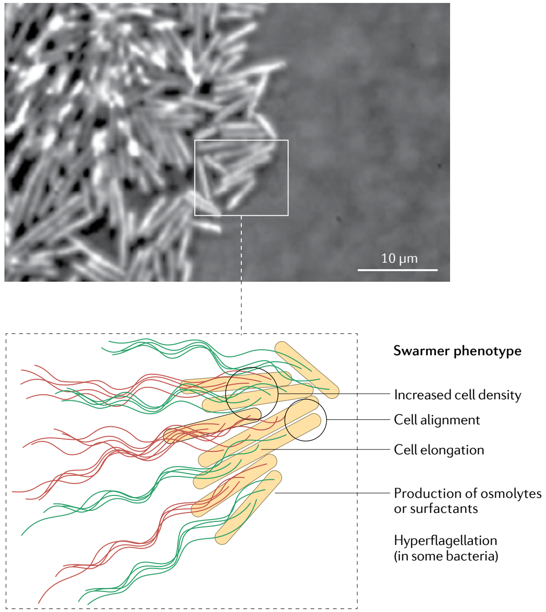
Slowed 2.5x

Serratia marcescens

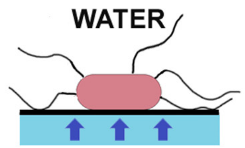
Salmonella enterica

Partridge, JD. and Harshey RM. *J. Bacteriology* 195: 909-918. (2013)

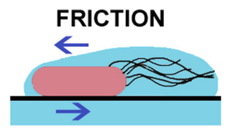
Swarming state: *Physical constraints*



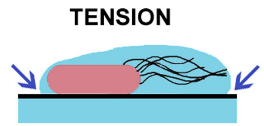
1. Maintaining a hydrated, fluid medium
   - Osmotic regulation of fluid uptake



2. Reducing and opposing frictional forces
   - Substrate interactions
   - Propulsion by flagella
   - Cell length and fluid drag



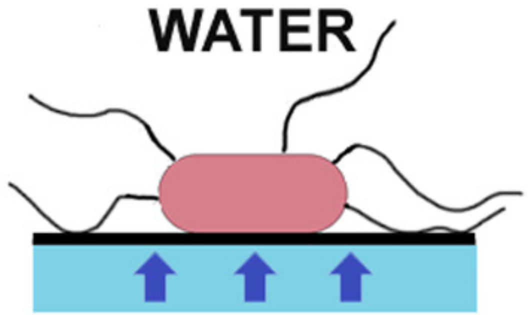
3. Reducing Surface tension: wettability
   - Surfactants and other wetting agents



Partridge, JD. and Harshey RM. *J. Bacteriology* 195: 909-918. (2013)

Transition to swarming state: fluidization

- Secretion of osmolytes by bacteria give rise to water osmotic flow from agar to the bacteria media
- This decreases the viscosity of the bacteria swarm and favors motility



Gram negative bacteria secrete osmolytes, polysaccharides to draw water from agar into the swarm

- In *Proteus mirabilis, there is* secretion of extracellular matrix (ECM) composed of polysaccharides, the osmolyte glycine betaine, etc.
- Acidic polysaccharide Cmf (colony migration factor) is essential for swarming and supposed to increase colony hydration.

- Some swarmers (*P. Aeruginosa*) up regulate the osmolytes glutamate and proline.
- Evidence of altered metabolome in bacteria at the surface of agar. This induces synthesis and modification of lipopolysaccharides (LPS)

Partridge, JD. and Harshey RM. *J. Bacteriology* 195: 909-918. (2013)

Lahaye E, Aubry T, Fleury V, Sire O. Does water activity rule P. mirabilis periodic swarming? II. Viscoelasticity and water balance during swarming. *Biomacromolecules* **8**:1228 –1235. (2007)

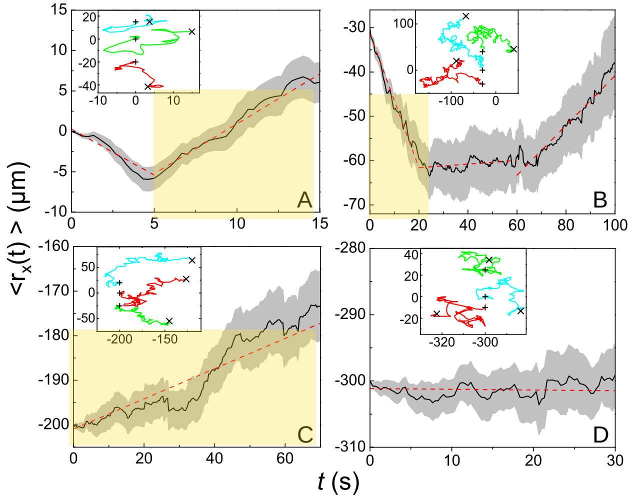
Transition to swarming state: fluidization

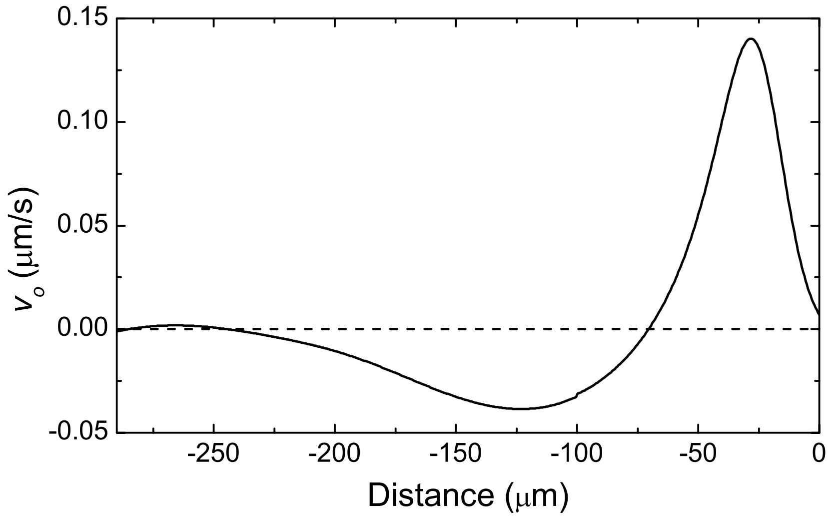
Observation of fluid flows at the edge and inside a swarm of *E. coli*
Prediction of osmotic fluid flows from the agar into the swarm

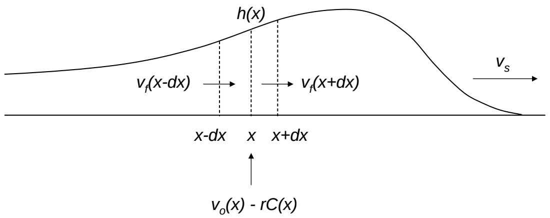
Microbubbles fluid flow profiles as a function of distance from the edge of the swarm edge

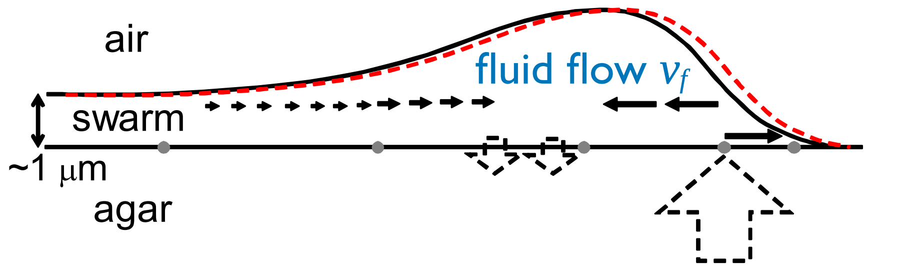
Cell growth and metabolic activity is required for fluid flow

typical bubble tracks in the laboratory frame measured in μm, beginning at + and ending at x









osmotic flow speed: $v_o(x) = rC(x) - v_s \frac{dH(x)}{dx} + \frac{d[v_f(x)H(x)]}{dx}$.

growth rate x cell volume

swarm speed

flow speed

Wu, Y., and H. C. Berg. *Proc. Natl. Acad. Sci. USA*. 109:4128–4133. (2012)

Transition to swarming state: fluidization

Osmolarity is patterned at the swarm edge consistent with predicted osmotic fluid flow

Wu, Y., and H. C. Berg. *Proc. Natl. Acad. Sci. USA.* 109:4128–4133. (2012)

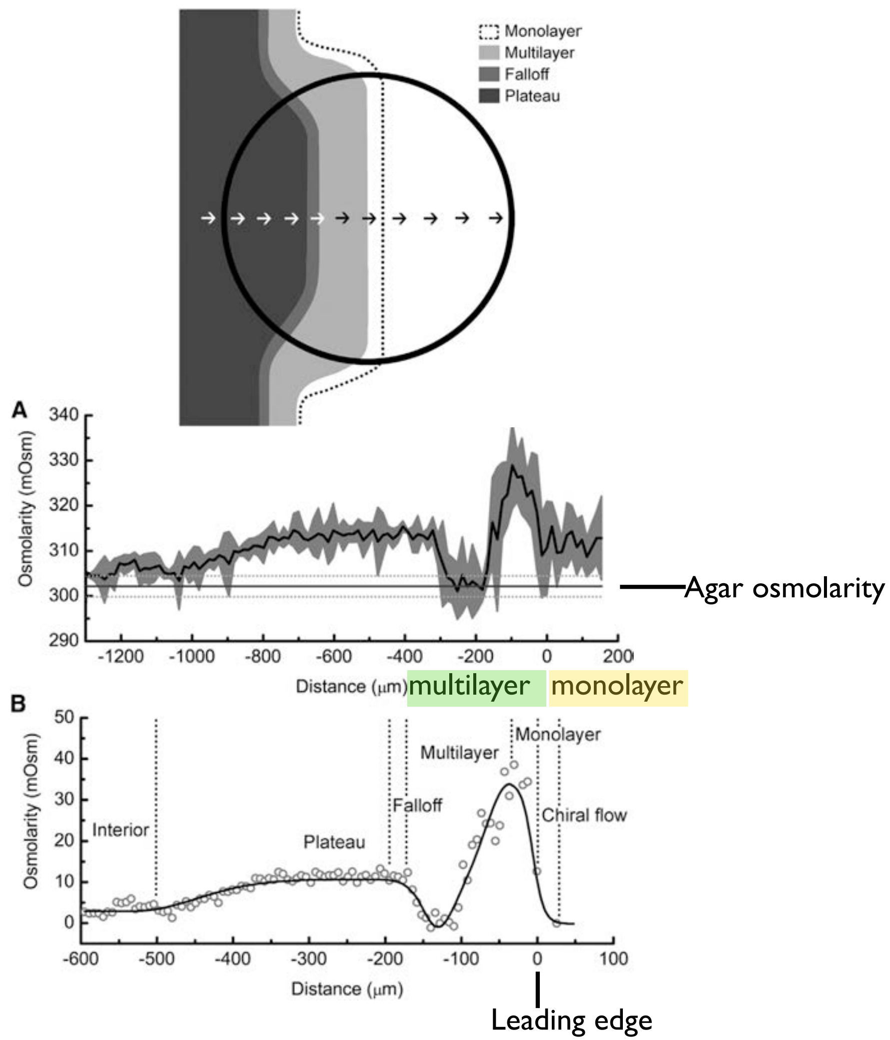
Secretion of osmolytes by bacteria gives rise to water osmotic flow from agar to the bacteria media

Liposomes are prepared with 2 fluorescent dyes: one that self-quenches when liposomes shrink (G, green dye calcein) and one that does not (R, red dye sulforhodamine-101).

The G/R ratio reflects the osmolarity of the surrounding fluid.

Measure osmolarity as swarm of *E. coli* cells spread across large liposome (450µm)

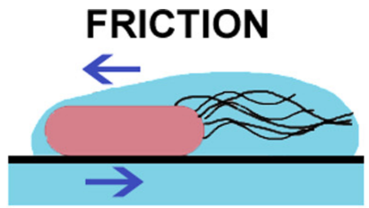
Two bands: high osmolarity at the edge (+25mOsm) and lower osmolarity behind, isotonic to agar



Ping et al. and HC. Berg. *Biophysical Journal* 107 :871–878 (2014)

Transition to swarming state: Overcoming friction

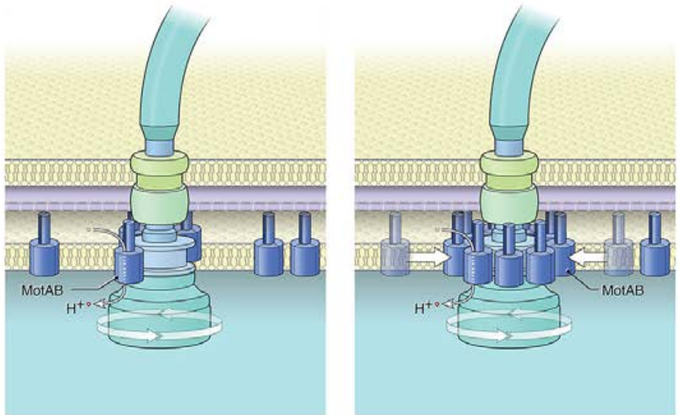
– Reducing and opposing frictional forces
- Substrate interactions
- Propulsion by flagella
- Cell length and fluid drag



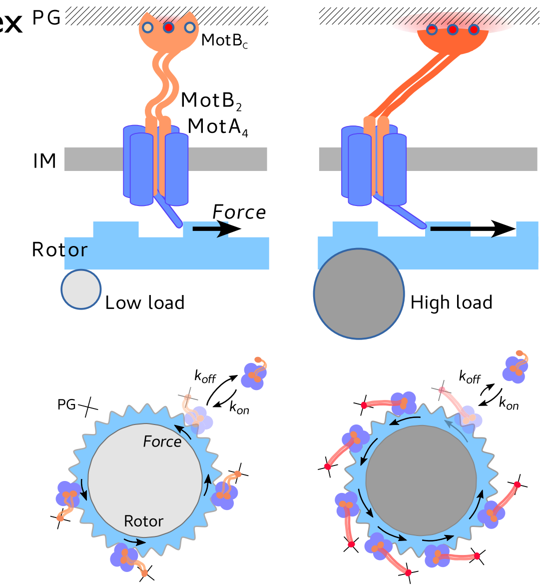
- Substrate interaction:
  — role of charges (could be regulated by LPS)
  — biosurfactants are lubricants due to amphipathic (hydrophilic and hydrophobic) structure that could reduce friction. LPS (gram- bacteria), rhamnolipids (*Pseudomonas)* and lipopeptides have surfactant properties.

- Propulsion by flagella:
  — Hyperflagellation associated with increase in body length. The increased propulsion forces counteract friction
  — Specific stator units that increase active torque on rotor/flagella complex

P. aeruginosa has five stator proteins (MotAB, MotCD, and MotY), which likely associate into two sets of stators, whereas only two such proteins (MotAB) drive swimming motility in *E. coli* and *Salmonella*.



A. Baker and G. O'Toole. *J. Bacteriol.* doi:10.1128/JB.00088-17 (2017)

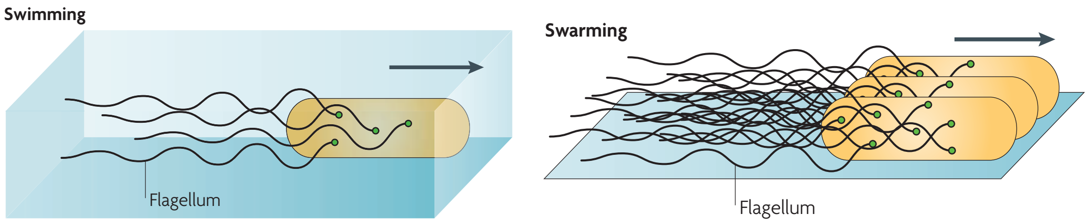


N. Wadhwa, R. Phillips and HC. Berg. *PNAS.* 116:11764-11769 (2019)

Transition to swarming state: Overcoming friction

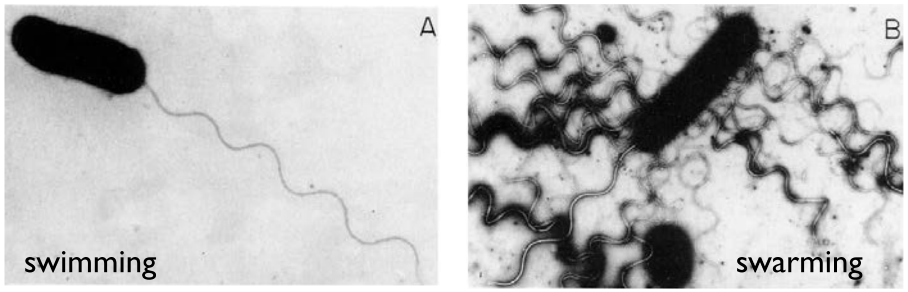
- Bacteria contact with a soft surface is associated with induction of hyper flagellation

—Induction of flagellar genes and **increase in number of flagella per cell**
Flagella cover the entire cell (peritrichous flagella), eg. From 5 to 50 flagella.



—synthesis of new flagella (lateral flagella) in *Vibrio parahaemolyticus*

Sheathed polar flagellum (Fla genes)



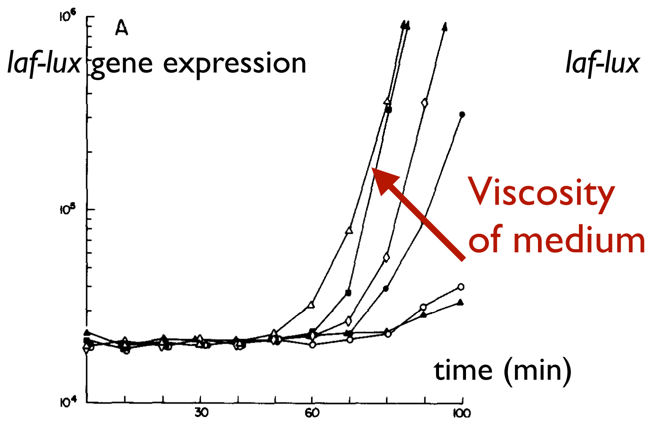
Unsheathed lateral flagella (Laf genes)

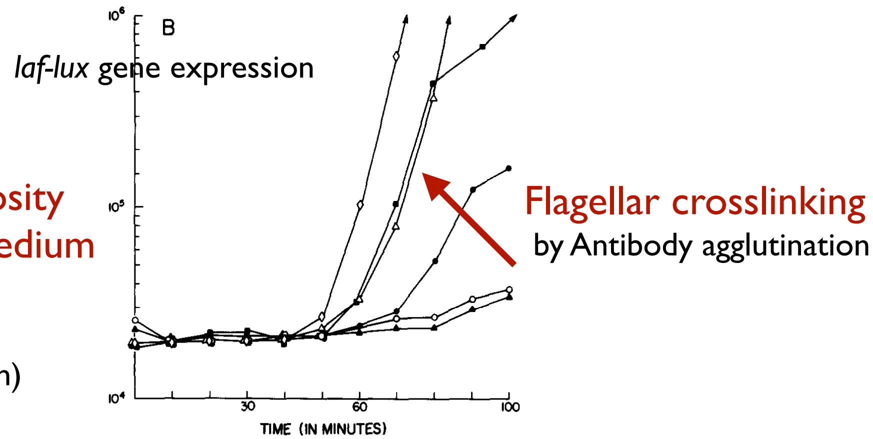
L. McCarter et al. *Cell* 54: 345-351 (1988)

Transition to swarming state: hyperflagellation

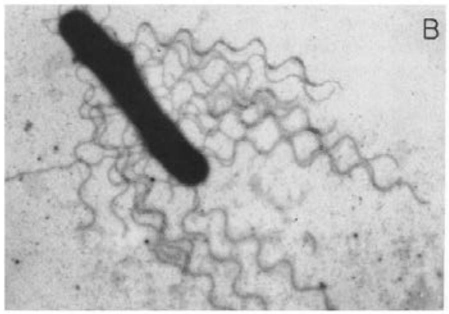
— When bacteria sense a more viscous environment at a surface they differenciate
— Cells detect physical properties of the surface
— The induction of lateral flagella is regulated mechanically
— The polar flagella works as a dynamometer/viscometer

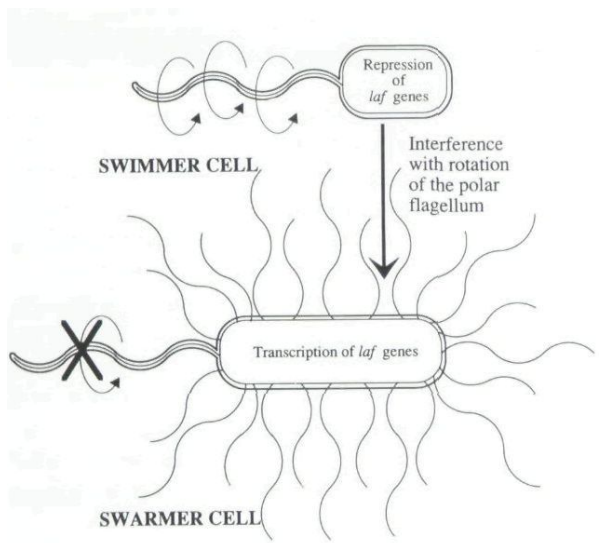
- Reporter of *lab* gene expression with light
- Increasing the viscosity of the medium induces Laf expression
- Blocking flagellar rotation with an antibody also induces Laf





- Laf is constitutively induced in bacteria in solution when the flagellum is defective (*flagellin C* or *mot* mutant)



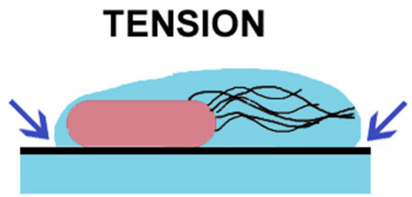


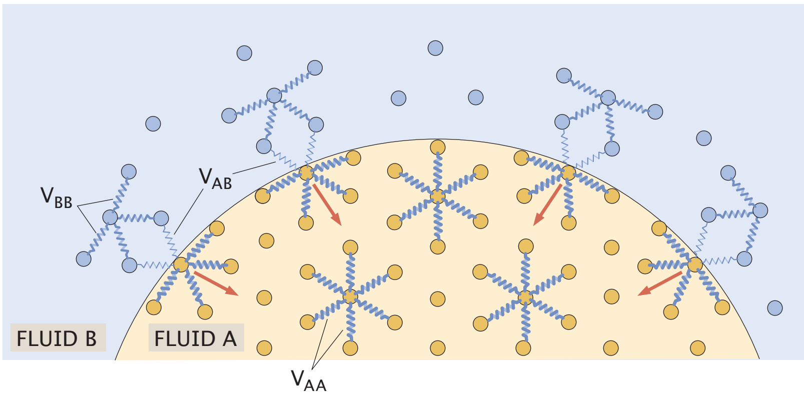
L. McCarter and M. Silverman *Cell* 54: 345-351 (1988)
L. McCarter and M. Silverman *Mol. Microb.* 4(7), 1057-1062 (1990)

Transition to swarming state: surfactants and wetting

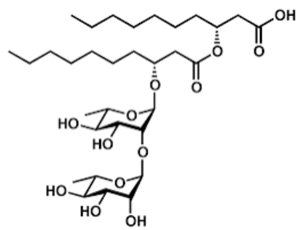
Reducing Surface tension: wettability

- Surfactants and other wetting agents
- Hypothesis: Gradient in wetting agents generates Marangoni stresses and capillary flows that drive swarm expansion.



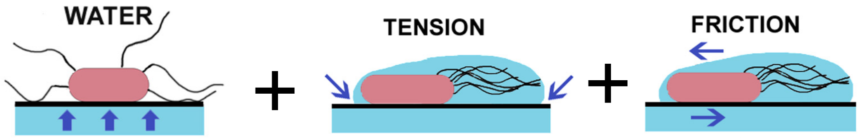


— The agar substrates is partially hydrophobic.
— Need of wetting agents, eg. amphipathic molecules.
—Lipopeptides and rhamnolipids produced by *Serratia, Bacillus, Pseudomonas* etc
— Cyclic lipopeptides: Surfactin produced by *B. subtilis* and serrawetin produced by *S. marcescens*.
— Surfactant deficient bacteria loose swarming behavior. This can be rescued by adding exogenous surfactant.



rhamnolipid

A theoretical model of swarming



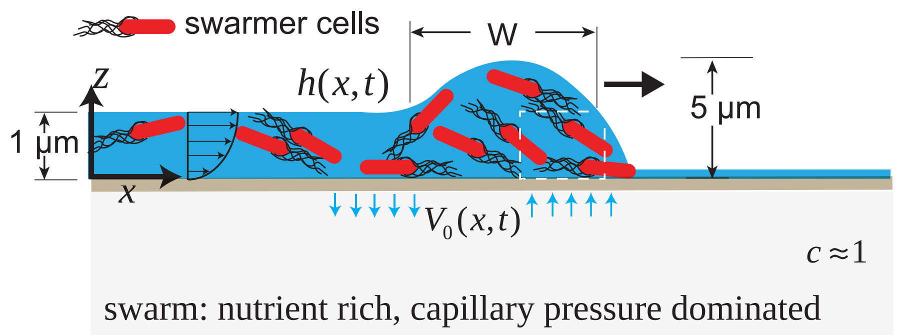
Osmotic flow + Marangoni flow + Friction and viscous resistance

Governing equations based on mass and momentum conservation:

$$((h\phi))_t + (Q_1(x))_x = g_1(h,\phi,c),$$

$$(h(1-\phi))_t + (Q_2(x))_x = (1-\phi)V_0(x),$$

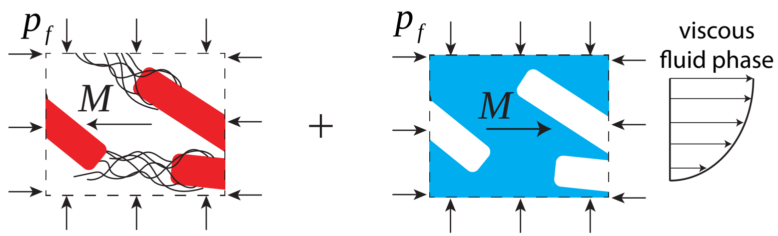
$$c_t - Dc_{xx} = g_2(h,\phi,c).$$



$\phi_1 = \phi(x,t)$ is the active (cell) phase

$Q_1(x)$ is the horizontal flux in the active phase, $Q_2(x)$ is the horizontal flux in the fluid phase and $V_0(x)$ is the osmotically-driven net vertical fluid influx per unit length across the permeable substrate

$g_1(h;c;\phi)$ is the depth integrated active phase growth rate within the bacterial colony, and $g_2(h;c;\phi)$ is the depth integrated nutrient uptake rate

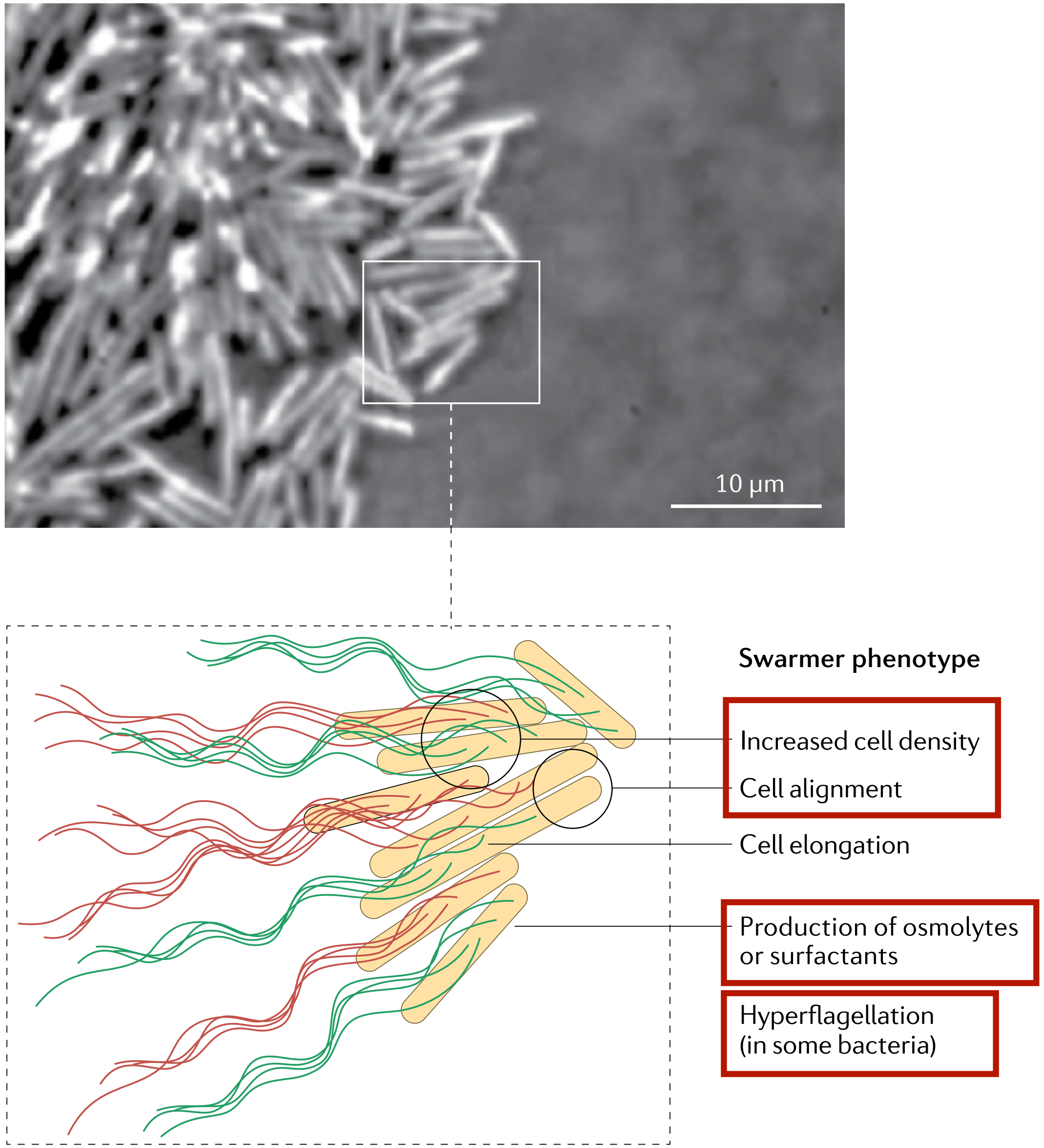


$\phi(x,t)$ active phase

$1-\phi(x,t)$ passive phase

Srinivasan et al. and L. Mahadevan *eLife* 8:e42697. DOI: https://doi.org/10.7554/eLife.42697 (2019)

Motility properties of swarming cells

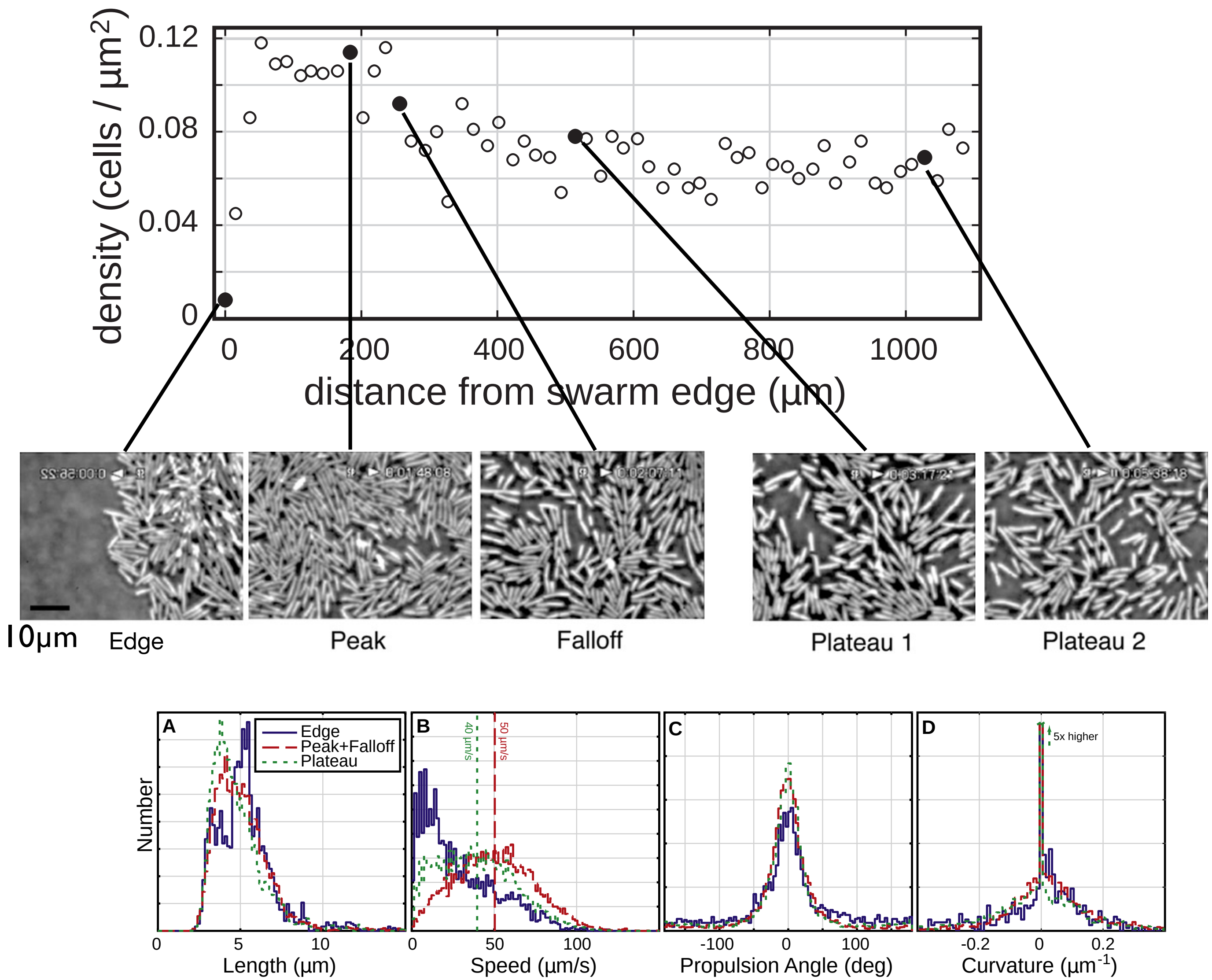


Motility properties of swarming cells

—The swarm monolayer acts like a 2D gas of self- propelled, polar particles.

- The density profile of *E. coli* cells in the swarm varies with distance from the edge.

  Highest density is just behind the edge
- Cells at highest density region have higher motility and motility is broadly distributed (compared also to swimming bacteria)
- Speed is the same as in swimming bacteria
- Cell movement is mostly linear (low curvature) with a narrow propulsion angle compared to swimming bacteria that undergo tumbling and nearly random reorientation

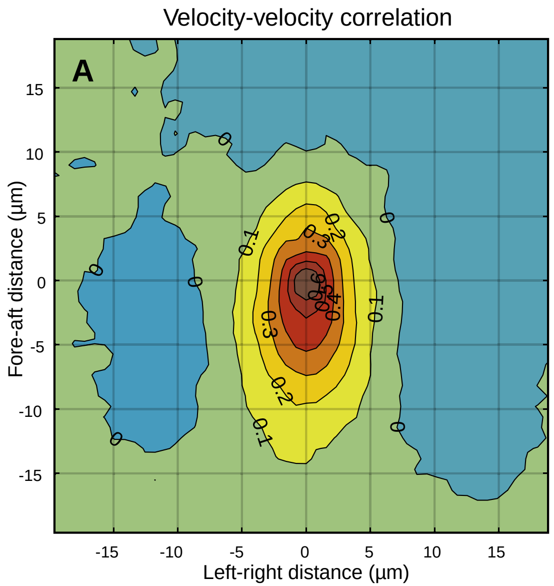


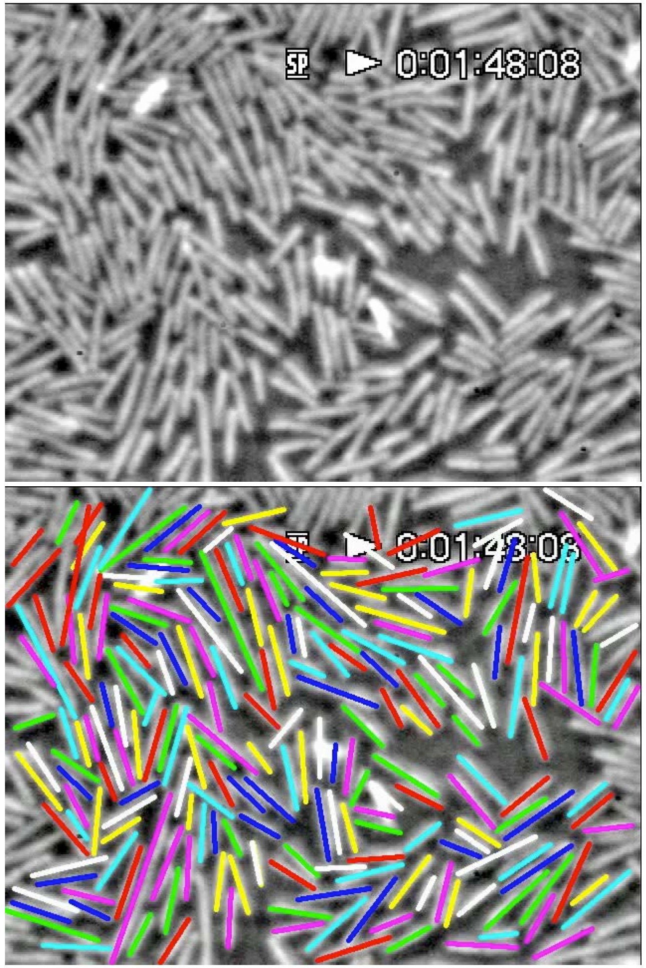
N. Darnton et al and H. Berg. *Biophysical Journal* 98(10) 2082–2090 (2010)

Motility properties of swarming cells

—The swarm monolayer acts like a 2D gas of self- propelled, polar particles.

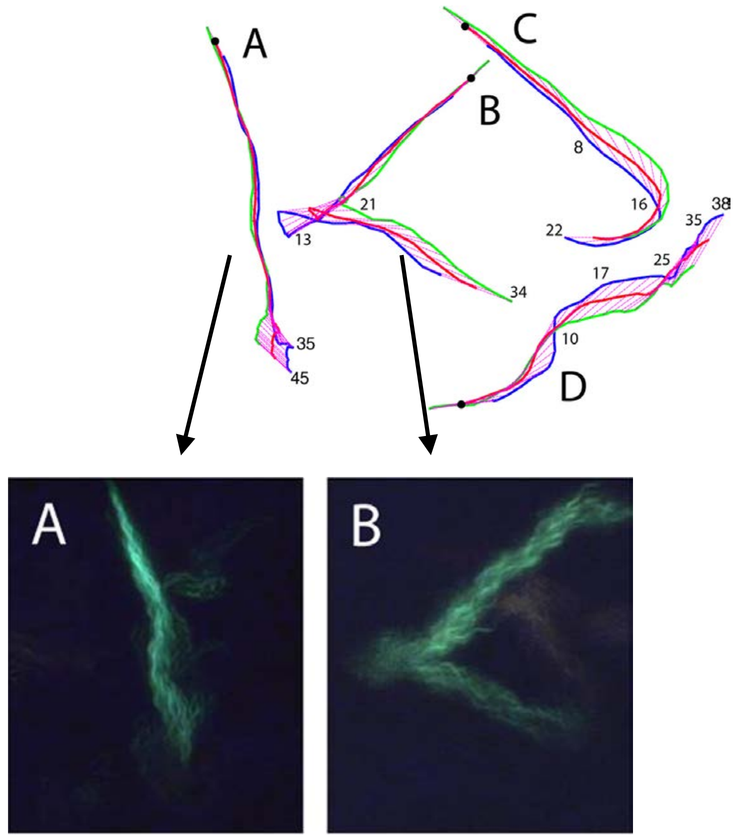
- Microscopic properties of the « bacterial atoms of this 2D gas », such as speed distributions and correlation functions.
- Cells move in coordinated packs
- Cells tend to align laterally but less so at front/back





N. Darnton et al and H. Berg. *Biophysical Journal* 98(10) 2082–2090 (2010)

Motility of swarming cells

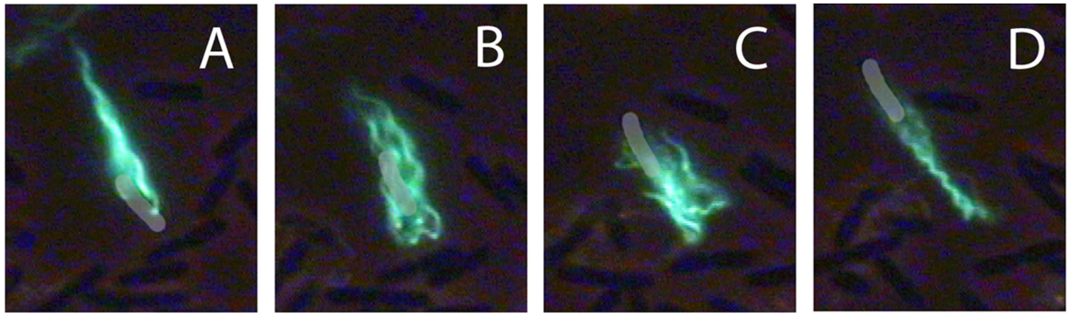


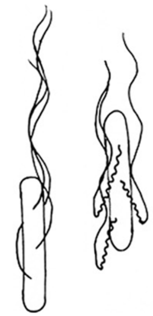
Cell tracking and time projections of cell positions
Cell body: head (blue), tail (green), middle (red)

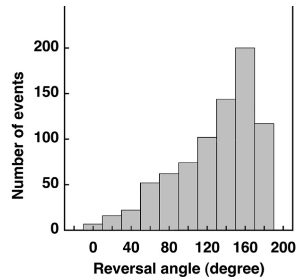
Flagella (light blue)

- Forward motion
  - Bundles of flagella are aligned with long axis of the cell and propel it forward at the same speed as free swimmers in 3D (few 10 µm/s)

- Reversals
  - Cells reorganize the flagella as they stop, and rebundle them in the opposite orientation so change in direction is mostly a reversal



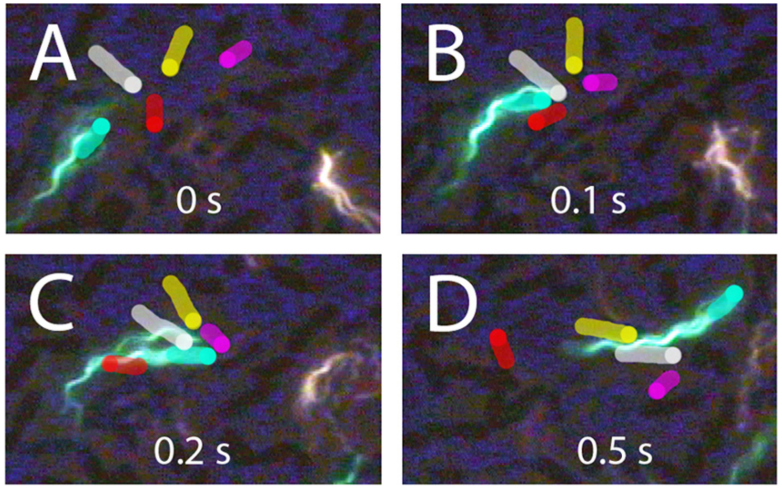




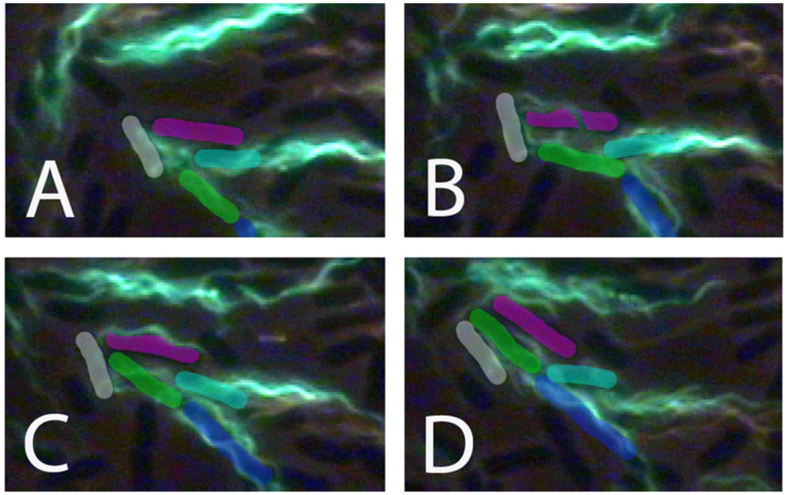
L. Turner et al and H. Berg. *Journal of Bacteriology*, 192: 3259–3267 (2010)

Motility of swarming cells

- Lateral motion and Collisions
  - Cell collisions are responsible for lateral motion of cells and for the wide distribution of velocities



- Cell alignement
  - Cells tend to align, most likely via entrainement due to motion, interactions among flagella, and the cell elongation (long aspect ratio of cells).
  - Not due to co-bundling of flagella



L. Turner et al and H. Berg. *Journal of Bacteriology*, 192: 3259–3267 (2010)

Motility of swarming cells

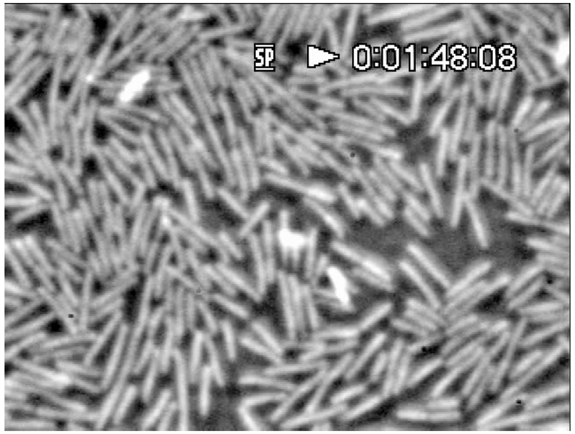
- Comparison of swimmers and swarmers:

  Similarities

  - Cells run, stall and re-run in both cases and this is due to a transition from bundled to « curly » flagella presumably due to a transition from CCW to CW rotation
  - In bot cases, run are powered by bundled flagella.

  Differences

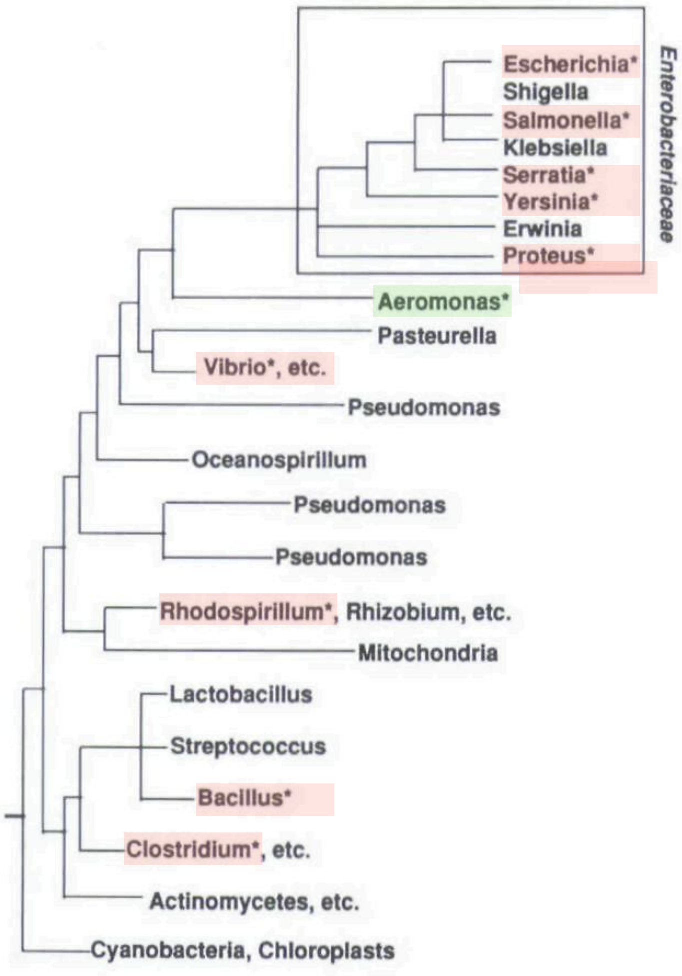
  - Swimmers have a random walk in 3D
  - Swarmers have a constrained movement in 2D.
  - Tumbling is frequent in swimmers but rare in swarmers.
  - Contrary to swimmers, swarmer cells are not free to change direction at random after they stall/stumble and undergo a new run.
  - Rather cells tend to align and to reverse in the opposite direction. This is due to the high density of cells.



- Chemotaxis is essential for swimmers but dispensable for swarmers.

L. Turner et al and H. Berg. *Journal of Bacteriology*, 192: 3259–3267 (2010)

Other modalities of swarming…



Harshey RM. *Molecular Microbiology* 13: 389–394. (1994)

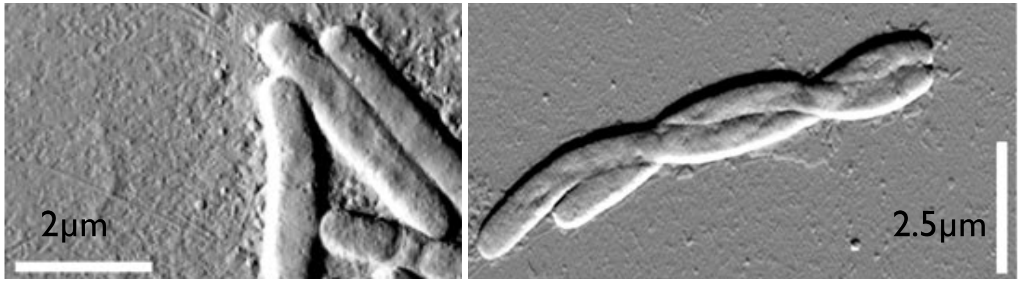
TABLE 1 Main features of various types of surface motility

Types of motility	Motive organelles	Cell differentiation	Colony expansion rates (μm/s)	Function	Bacterial genera[a]
Swarming	Flagella	Yes	2–10	Surface colonization	*Aeromonas, Azospirillum, Bacillus, Clostridium, Escherichia, Proteus, Pseudomonas, Rhodospirillum, Salmonella, Serratia, Vibrio, Yersinia*
Twitching/ social gliding/ retractile motility	Type IV pili	No	0.06–0.3	Surface colonization, biofilm formation, fruiting body development, [b]phage infection, transformation, conjugation	*Aeromonas, Acinetobacter, Azoarcus, Bacteroides, Branhamella, Comomonas, Dichelobacter, Eikenella, Kingella, Legionella, Moraxella, Myxococcus, Neisseria, Pasteurella, Pseudomonas, Ralstonia, Shewanella, Streptococcus, Suttonella, Synechocystis, Vibrio, Wolinella*

R. Harshey *Annu. Rev. Microbiol.*57:249-273 (2003)

Solitary motility of un-flagellated gliding bacteria

Bacteria: *Myxococcus xanthus*: l = 5μm

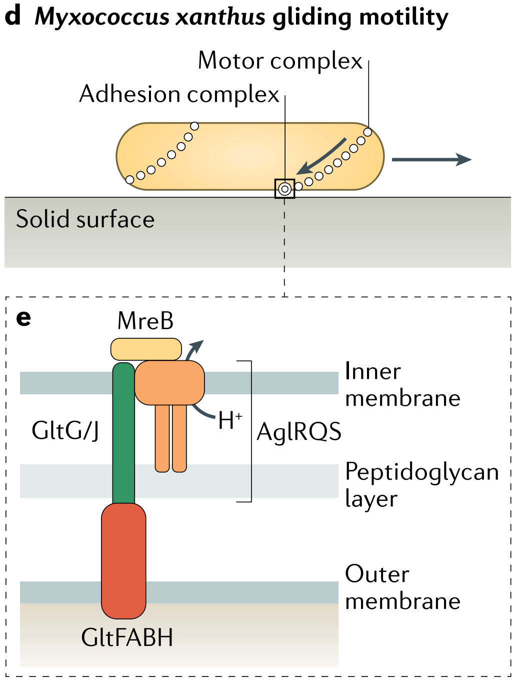


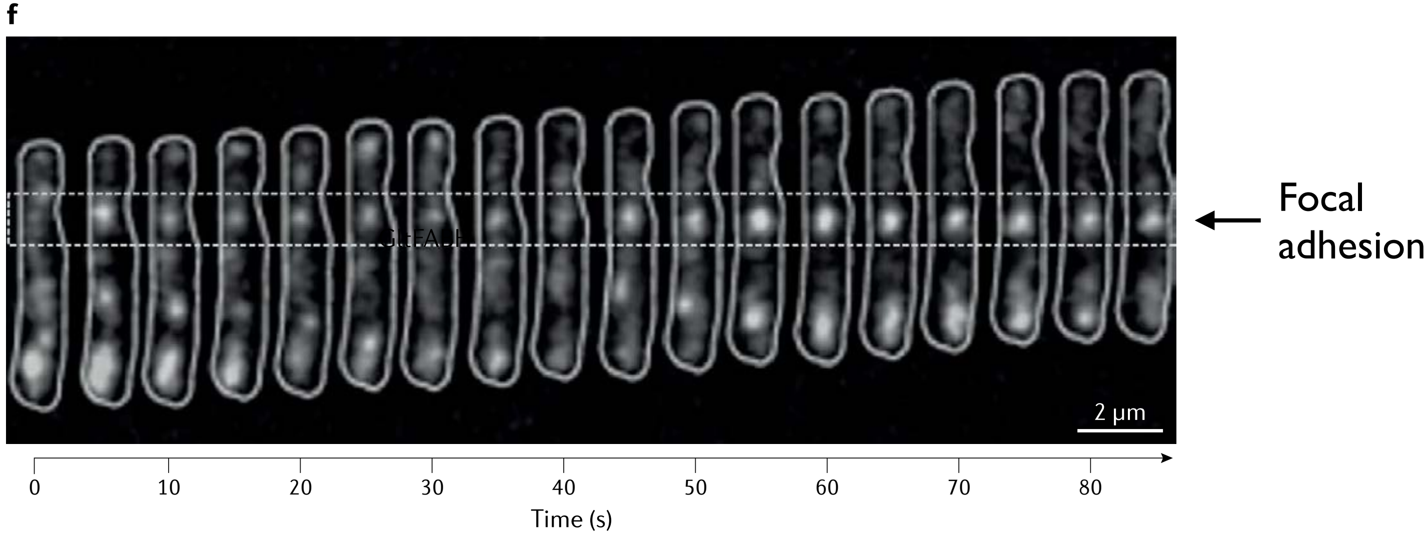
A. Pelling et al. PNAS, 102: 6484–6489 (2005)

speed: 2-4 μm/min

Gliding motility: helicoidal rotation and adhesion

Myxococcus xanthus





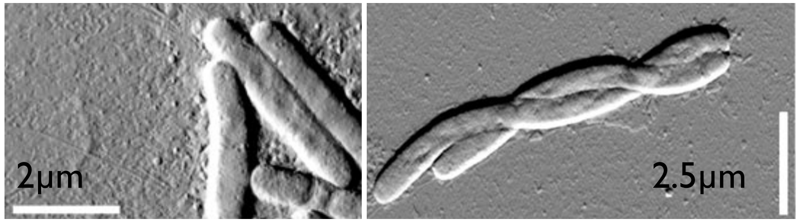
L. Faure et al, and T. Mignot *Nature*, 539:530-535 (2016)

N Wadhwa and HC. Berg. *Nature Rev. Mol. Cell Biol.* 20: 161-173 (2022)

Collective motility of un-flagellated bacteria

Myxococcus xanthus:

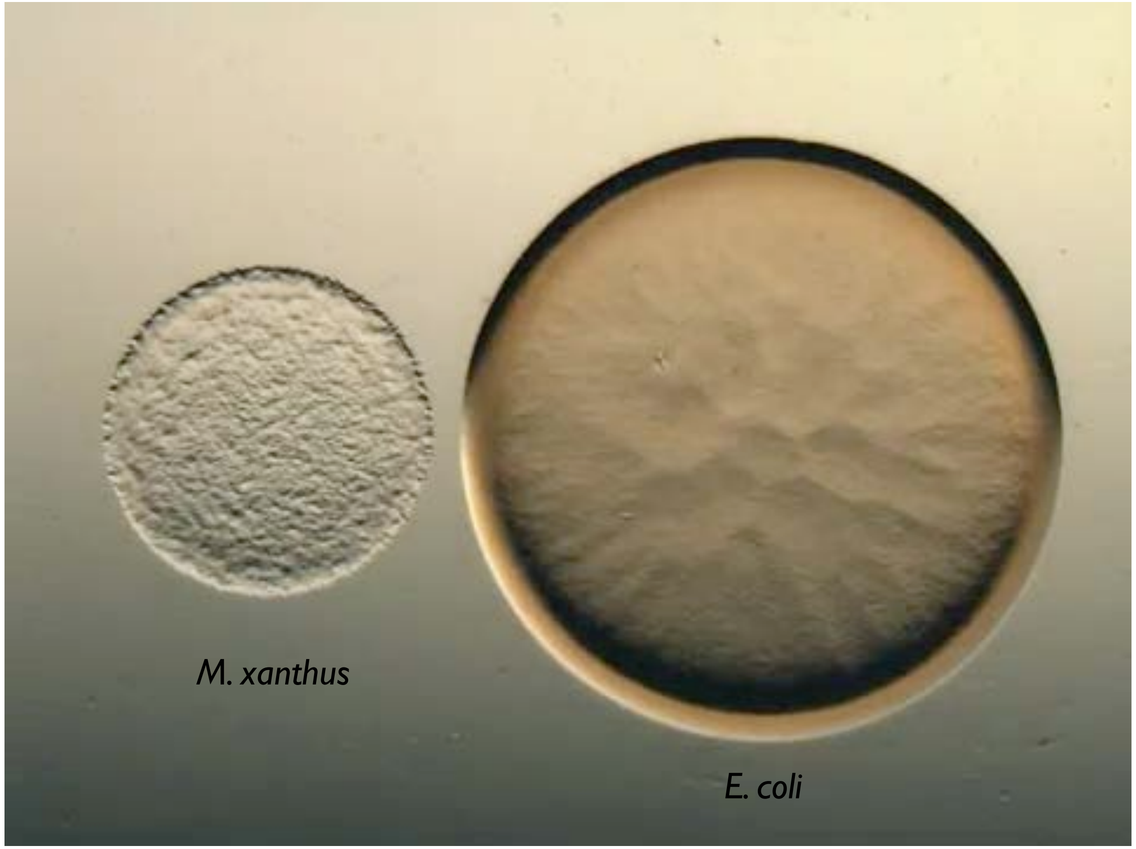
adventurous (solitary) motility: exploration



A. Pelling et al. PNAS, 102: 6484–6489 (2005)

speed: 2-4 µm/min

social motility (predation)

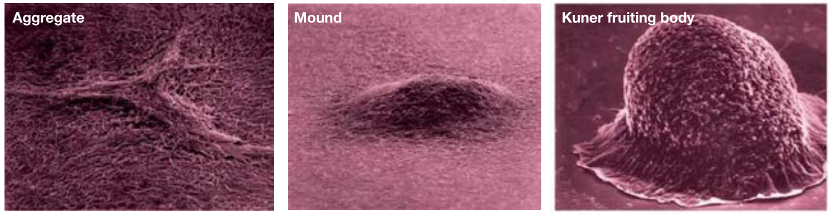


www.youtube.com/watch?v=tstc6doiNCU

Treuner-Lange, MPI Marburg

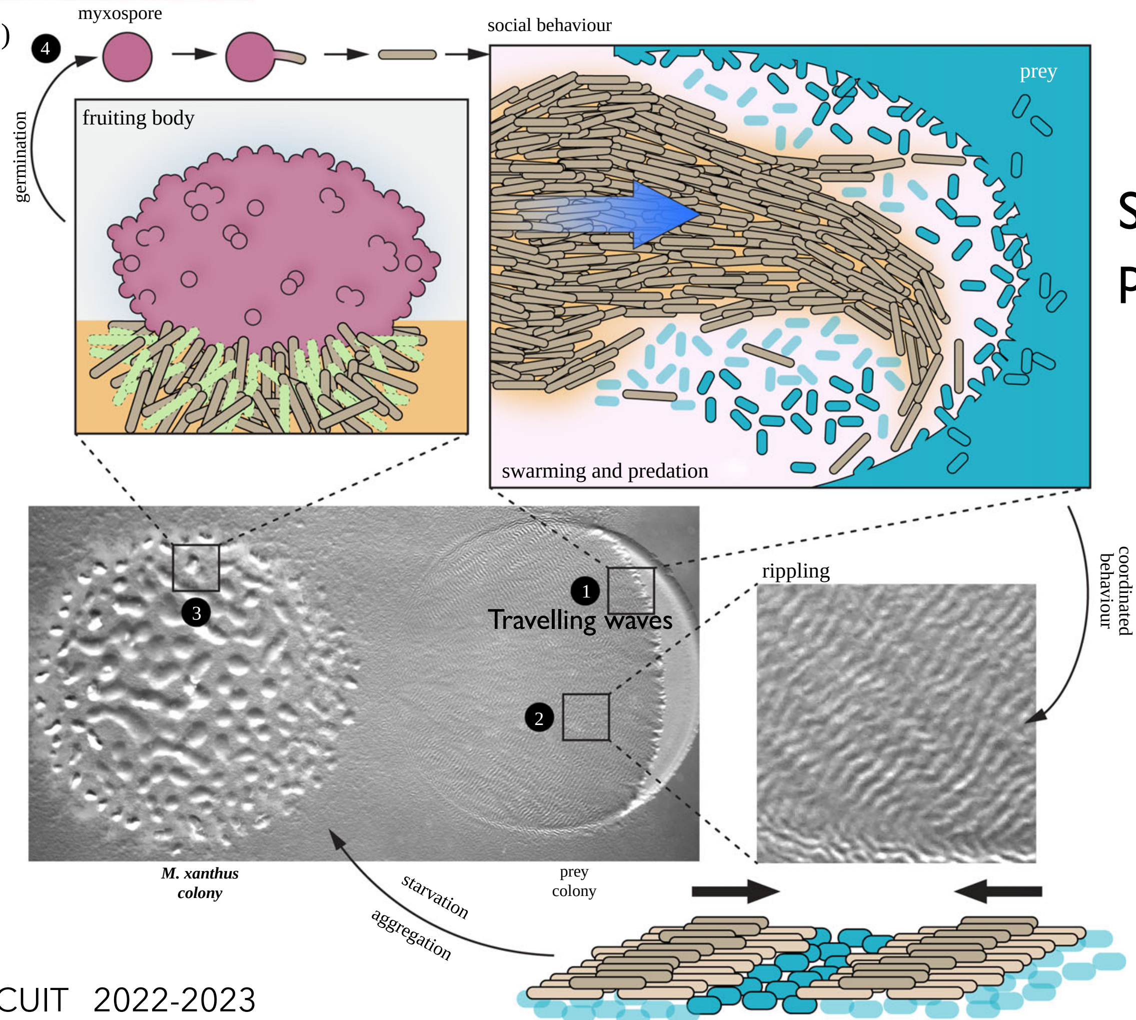
Collective motility of un-flagellated bacteria

Myxococcus xanthus, *Pseudomonas aeruginosa*. And many more



D. Kaiser. *Nature Rev. Microbiol.* 1: 45-53 (2003)

Aggregation and starvation

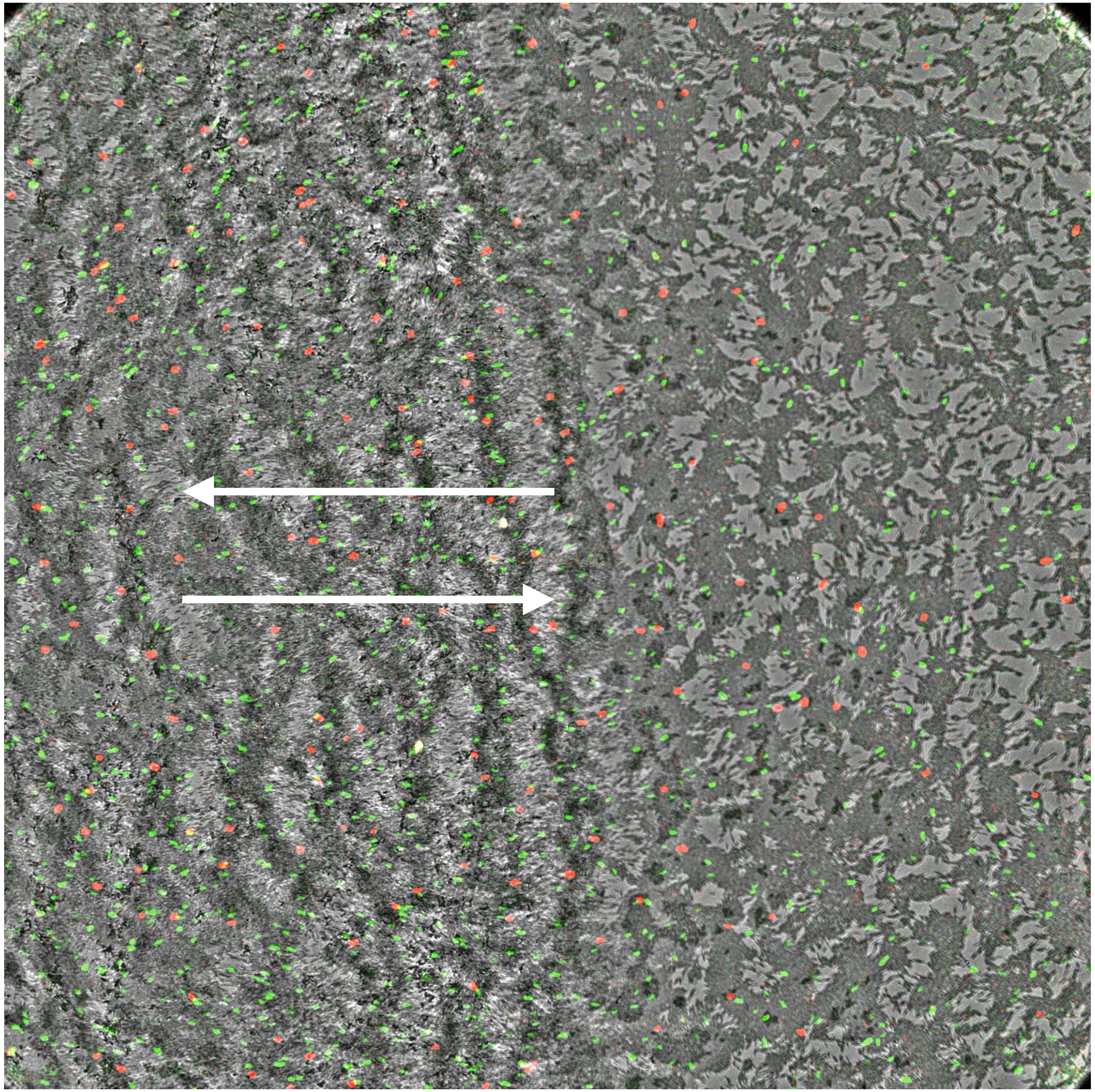


Swarming and predation

Dinet C, Michelot A, Herrou J, Mignot T. *Phil. Trans. R. Soc. B* 376: 20190755. (2021)

Collective motility of un-flagellated bacteria

Rippling waves in *Myxococcus* swarms



- Rippling waves are made of multilayered stacks of bacteria.
- Waves propagate, collide and reflect, in spite of their apparent persistent motion
- Individual bacteria do not follow the apparent dynamics of waves: they exhibit oscillatory motion on the substrate

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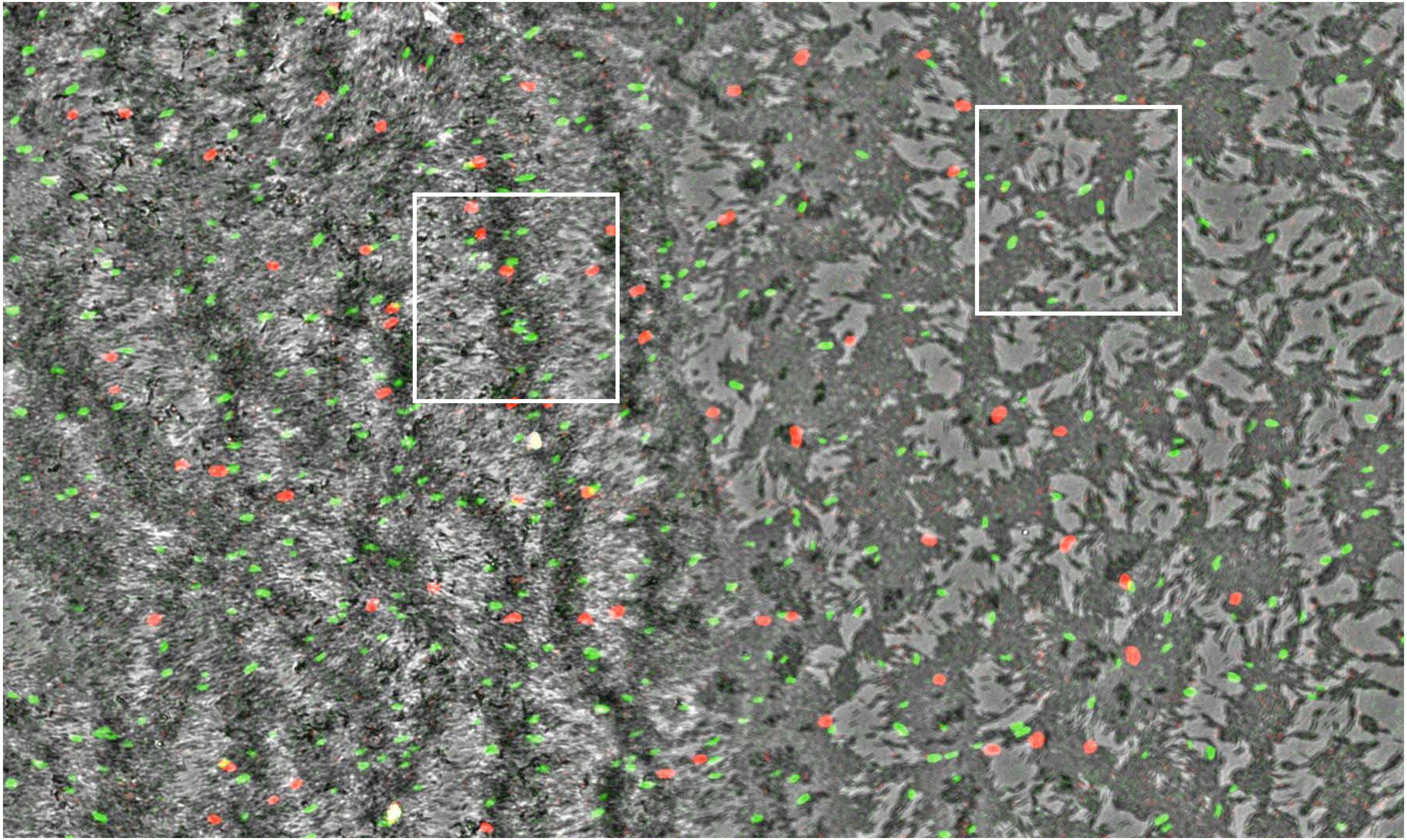
Oscillatory dynamics of un-flagellated swarming bacteria



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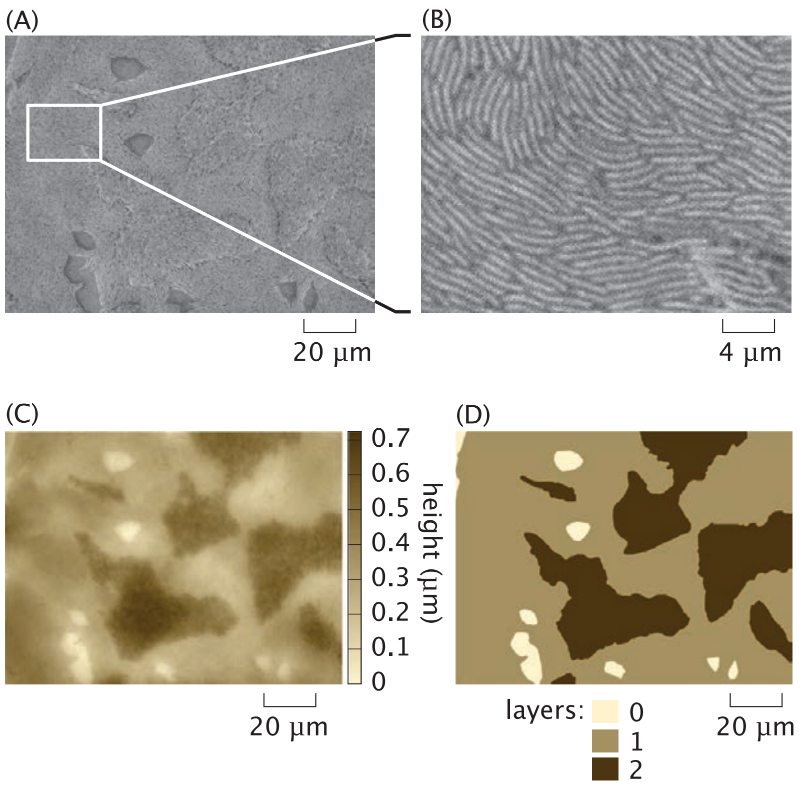
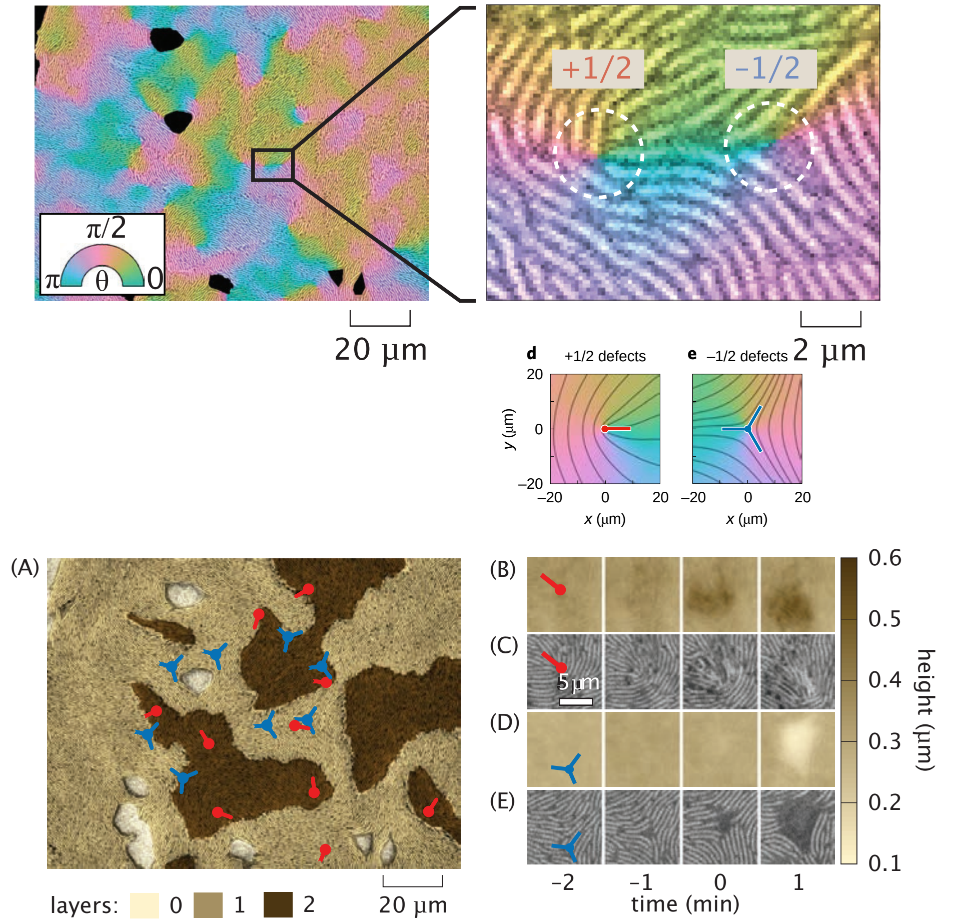
Layer formation in bacteria colonies: Topological defects

Asymmetric cell flows around topological defects
explain the formation of new layers and holes.

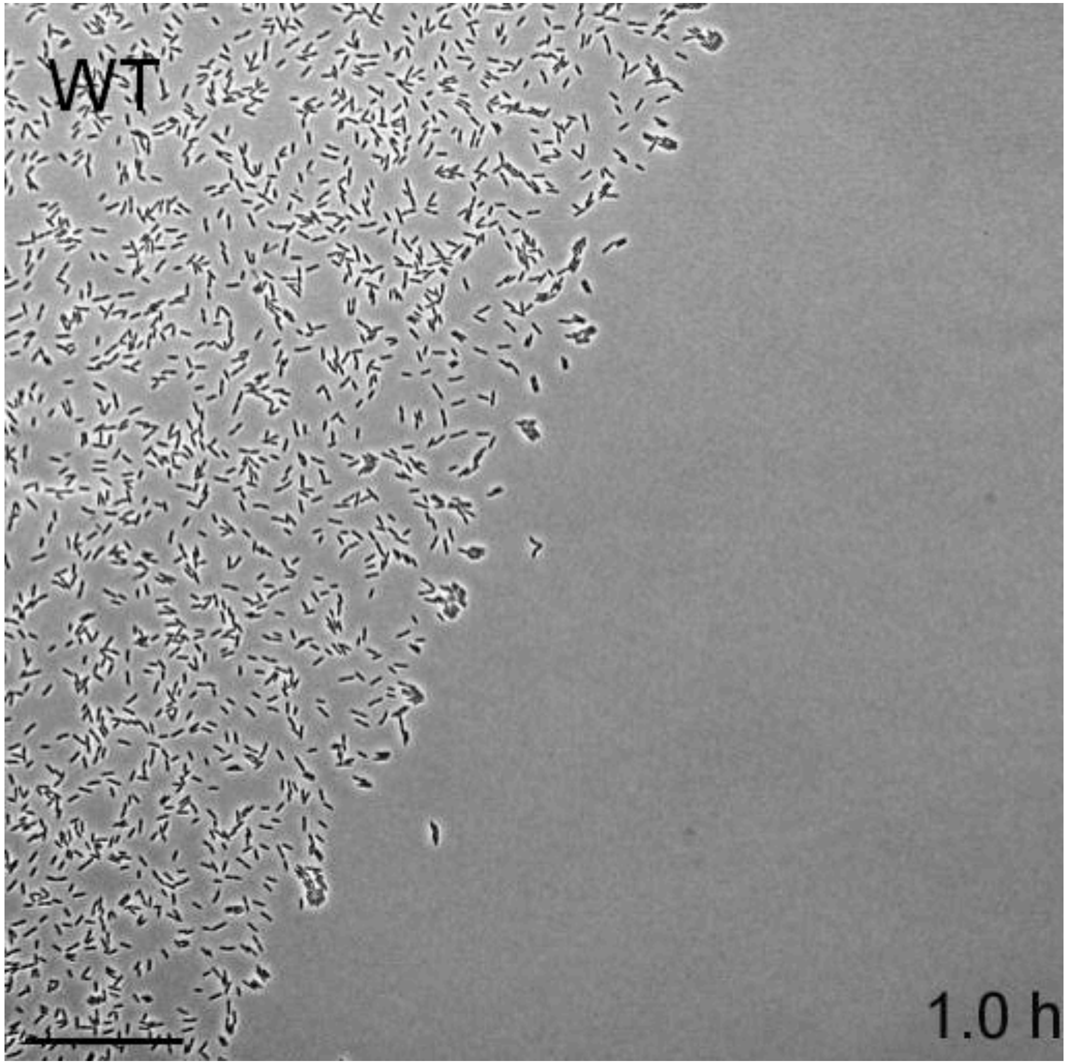




Copenhagen, K, Alert, R, Wingreen, NS and Shaevitz, JW *Nature Phys.*, 17, 211-215. (2021)

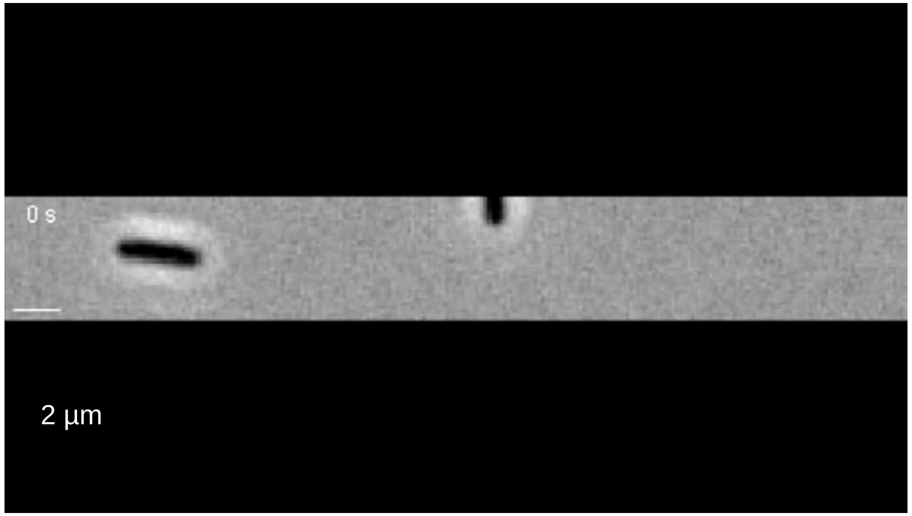
Un-flagellated bacteria swarm via twitching motility

Pseudomonas aeruginosa move forward and reverse by twitching



50 µm

- Bidirectional (oscillatory) motion in the bulk
- More directional motion at the edge

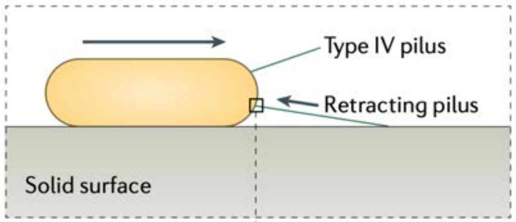


2 µm

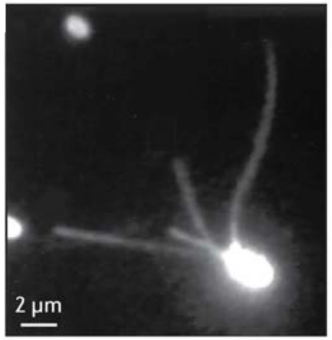
Kühn, M. J. et al. and A. Persat. *PNAS* 118, e2101759118 (2021).

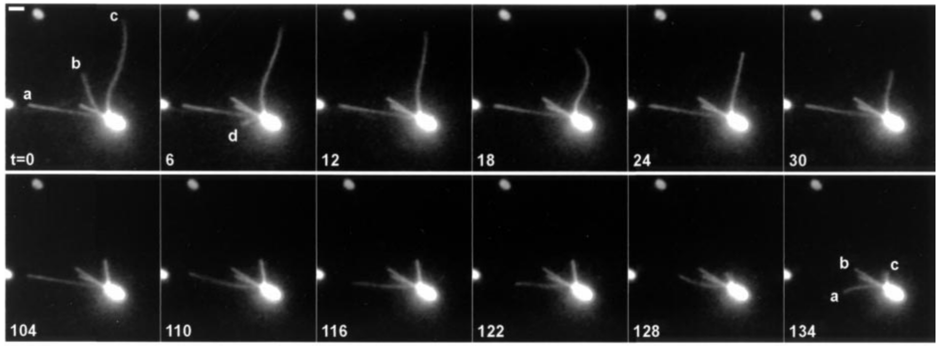
Mechanisms of twitching motility: *mechanics*

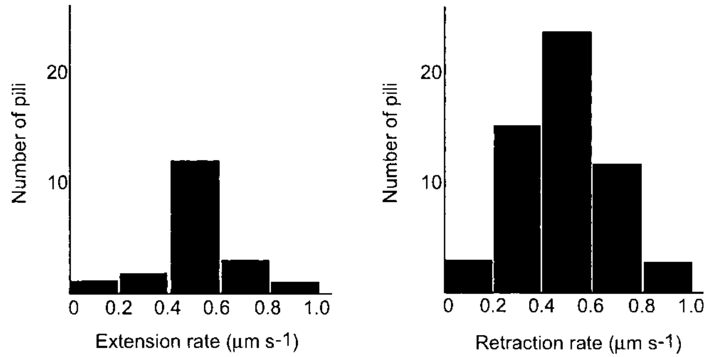
Cycles of pili extension and retraction, adhesion to a self-secreted matrix, drive forward and reverse movement



The pilus functions as a grappling hook







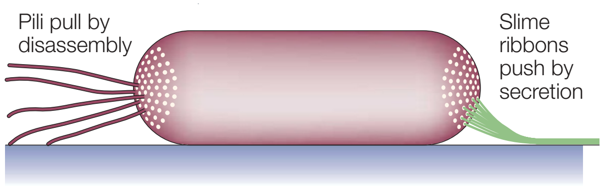
- *Pseudomonas aeruginosa* presents multiples thin filaments called pili at one pole of the bacterium.
- Type IV pili undergo cycles of rapid extension and retraction at rates of ~0.5-1 µm s^{-1}

JM. Skerker and HC. Berg. *PNAS* 98: 6901–6904 (2001)

N Wadhwa and HC. Berg. *Nature Rev. Mol. Cell Biol.* 20: 161-173 (2022)

Mechanisms of twitching motility: *mechanics*

Cycles of pili extension and retraction, adhesion to a self-secreted matrix, drive forward and reverse movement



D. Kaiser. *Nature Rev. Microbiol.* 1: 45-53 (2003)

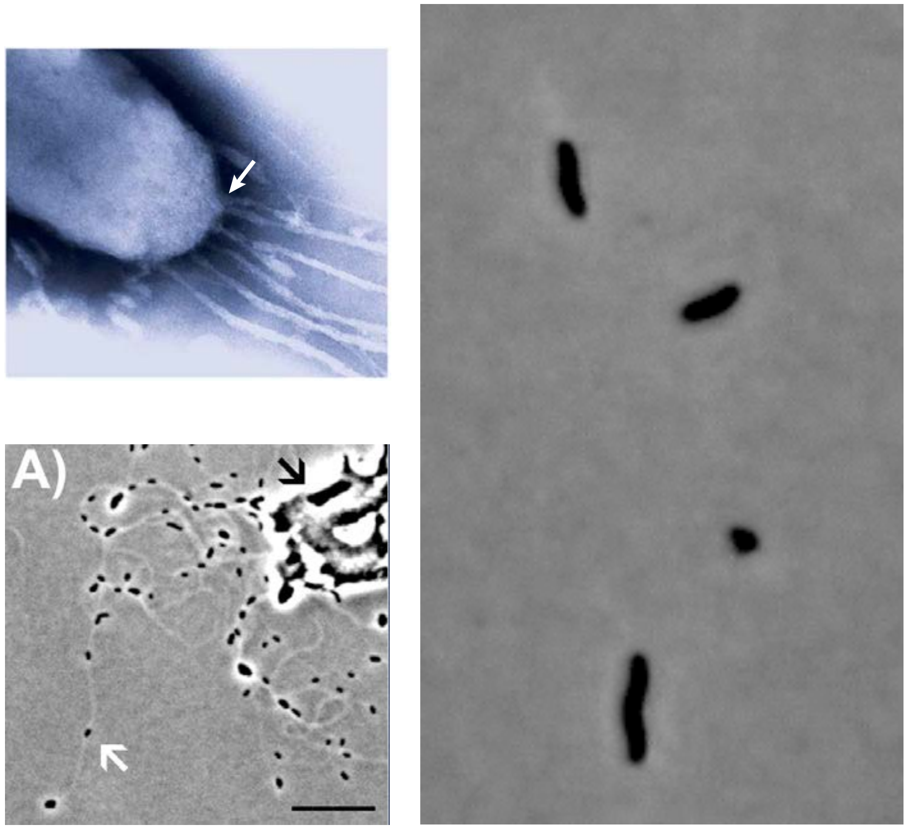
- Cells secrete exopolysaccharides that enhance adhesion to the substrate (agar)
- Reinforcement by feedback of cell motility on tracks used by other bacteria.

- The environment thereby guides the bacteria: concept of stigmergy
  (PP. Grassé, *Insectes sociaux* 1959)

LA RECONSTRUCTION DU NID
ET LES COORDINATIONS INTERINDIVIDUELLES
CHEZ *BELLICOSITERMES NATALENSIS*
ET *CUBITERMES SP.*
LA THÉORIE DE LA STIGMERGIE :
ESSAI D'INTERPRÉTATION
DU COMPORTEMENT DES TERMITES CONSTRUCTEURS.

par Pierre-P. GRASSÉ

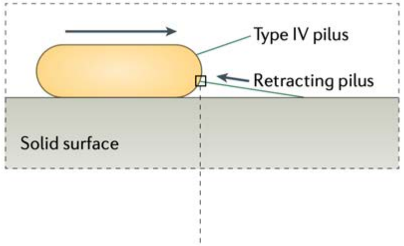
La coordination des tâches, la régulation des constructions ne dépendent pas directement des ouvriers, mais des constructions elles-mêmes. *L'ouvrier ne dirige pas son travail, il est guidé par lui.* C'est à cette stimulation d'un type particulier que nous donnons le nom de STIGMERGIE (*stigma*, piqûre ; *ergon*, travail, œuvre=œuvre stimulante).

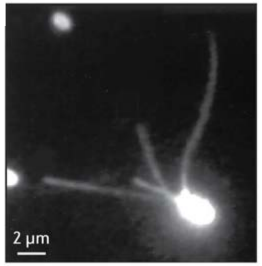


S.T. Islam, T. Mignot / *Seminars in Cell & Developmental Biology* 46 (2015) 143–154

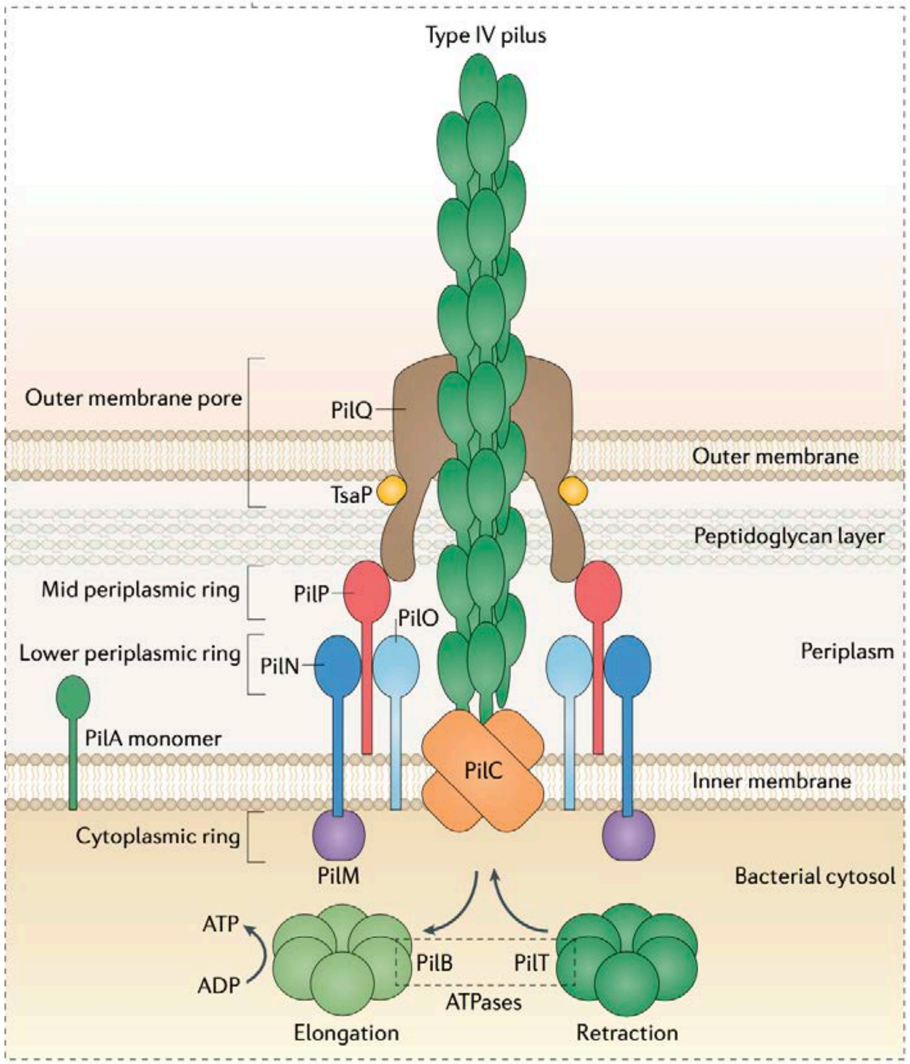
Mechanisms of twitching motility: *mechanics*

Pili extension and retraction





- Type IV pilus machinery is closely related to the type II secretion system of Gram-negative bacteria and the archaeal flagellar machinery
- Type IV pili are ubiquitous in bacteria and archaea and mediate adhesion, DNA uptake, predation, virulence, phototaxis, chemotaxis and surface sensing.
- Single pili are polymers of PilA, or Pilin monomers assembled from the cytoplasmic side.
- PilT and PilB at hexameric motors that induce assembly and disassembly of PilA, and thereby elongation and retraction of the pili.
- The is an energy consuming (ATP dependent) active process.



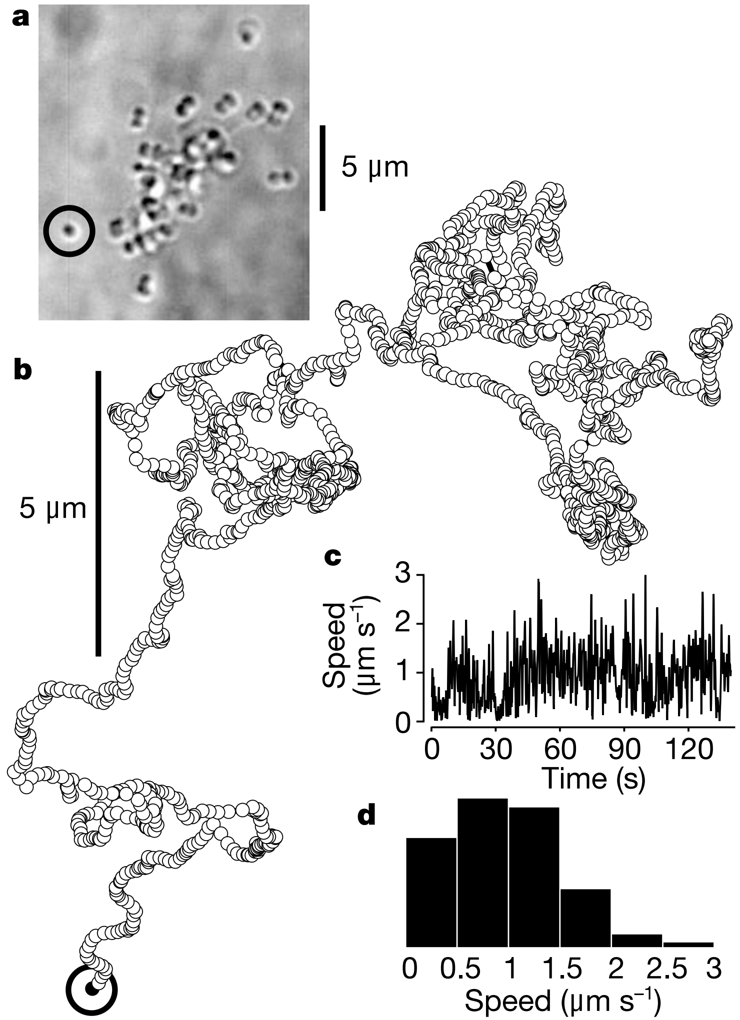
Motor	Exemplar bacterial species	Force-generating proteins	Type of motor	Associated secretion system	Energy source
Flagellar motor	*Escherichia coli*	MotA–MotB	Rotary	T3SS	Proton or ion motive force
Type IV pilus	*Myxococcus xanthus*	PilB and PilT	Rotary	T2SS	ATP

N Wadhwa and HC. Berg. *Nature Rev. Mol. Cell Biol.* 20: 161-173 (2022)

Mechanisms of twitching motility: *mechanics*

Pili retraction powers bacterial twitching motility: *evidence*

- *Neisseria gonorrhoeae* are spherical ~1µm long bacteria that undergo twitching motility via pili.
- Cells form small swarms
- Cells move at a speed of ~1µm/s.

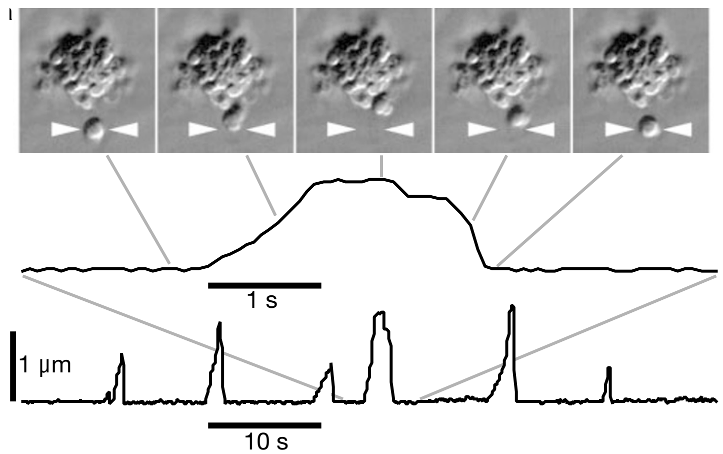


A. Merz, M. So and M. Sheetz. *Nature* 407:98-102 (2000)

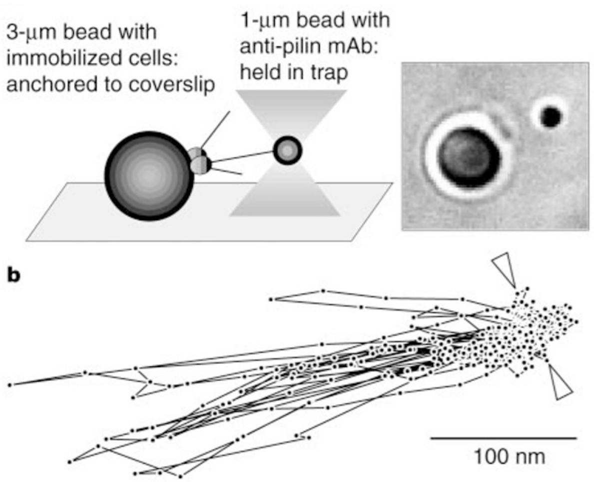
Mechanisms of twitching motility: *mechanics*

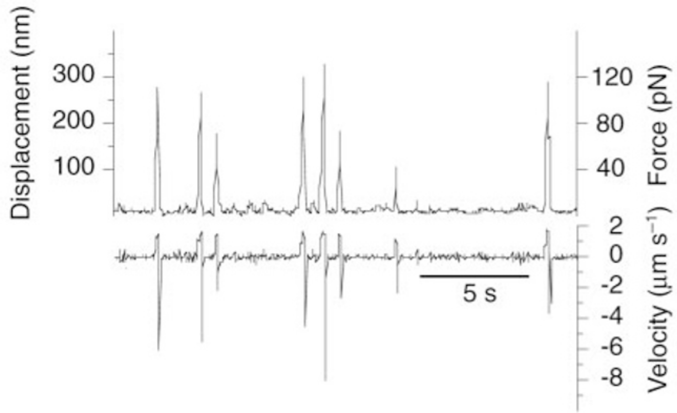
Pili retraction powers bacterial twitching motility: *evidence*

- Use optical tweezer to hold a cell at the edge of a small colony
- The cell moves towards the colony and escapes the trap revealing retractile forces between cells.
  Cells usually move back towards the trap revealing the transient nature of retractile force



- Cells immobilized on a bead anchored to the substrate:
- An optical tweezer was used to hold a cell a few microns away and measured the retractile forces
- Retractile forces are in the range of 100pN



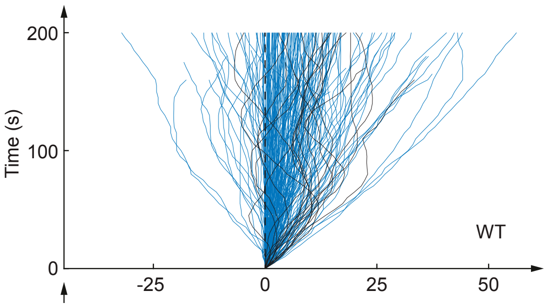


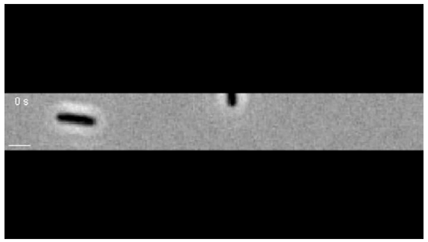
A. Merz, M. So and M. Sheetz. *Nature* 407:98-102 (2000)

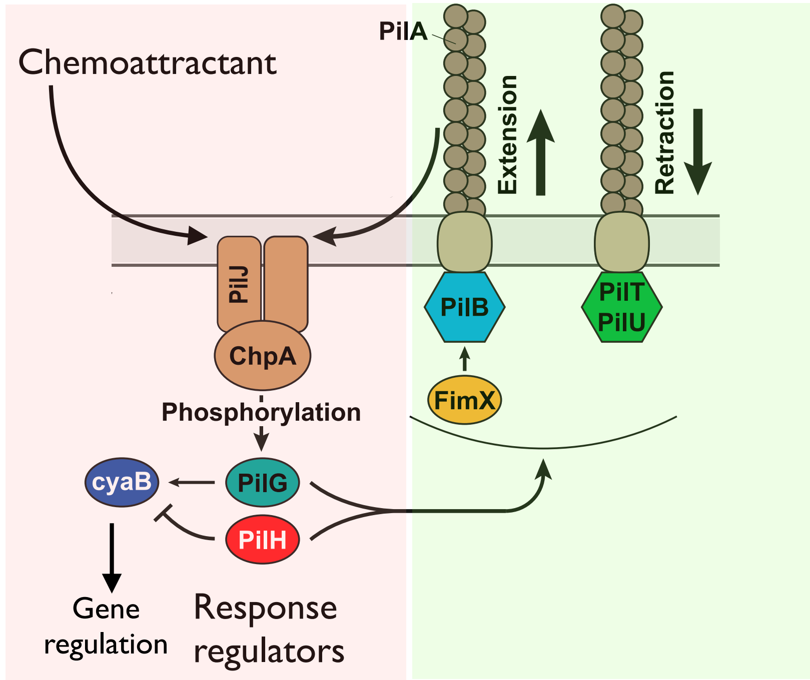
Mechanisms of twitching motility: *regulation*

• Oscillatory dynamics of twitching cells

- *P. aeruginosa* shows oscillatory dynamics but exhibit biased direction.
- This involves in part chemotaxis.
- The ChpA (Chemosensory protein A) sensory system is similar to the CheA/Y system (in *E. coli*) and is likewise involved in chemotaxis

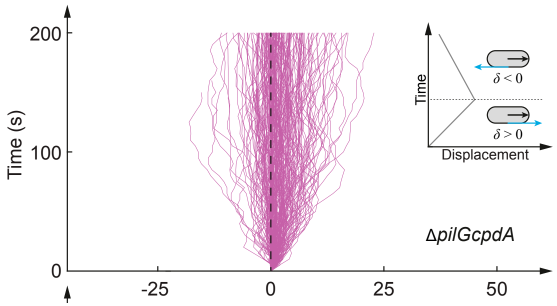






Regulation ⟶ Mechanics

PilG mutant

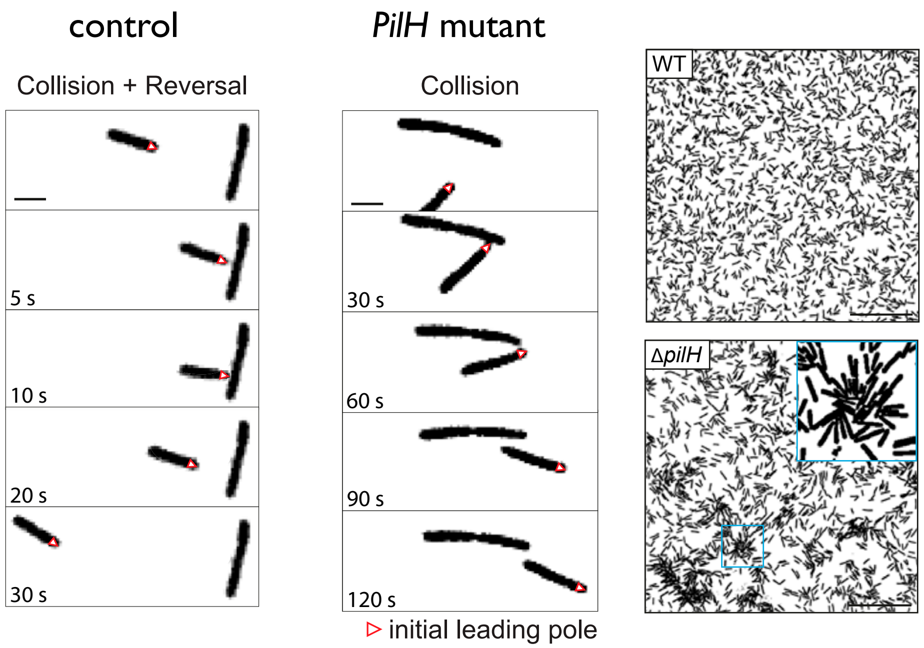


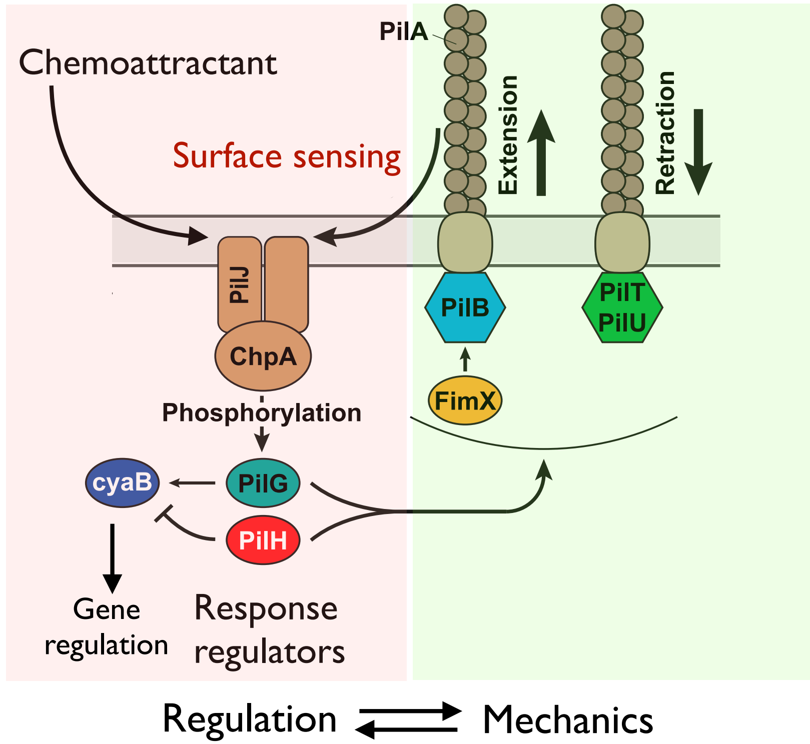
Kühn, M. J. et al. and A. Persat. *PNAS* 118, e2101759118 (2021).

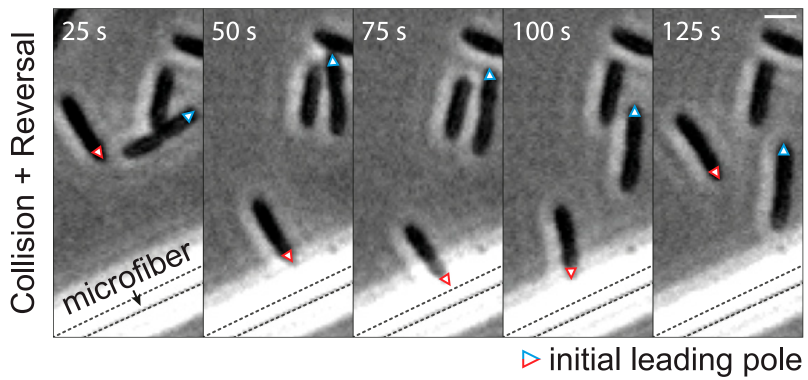
Mechanisms of twitching motility: *regulation*

- Twitching cells detect collisions and reverse motility

- The ChpA/PilG/PilH sensory system is required for the detection of collision
- Detection of collision allows uniform spreading and density of bacteria in swarms.
- Cells detect a synthetic fiber: consistent with mechanotaxis instead of chemotaxis





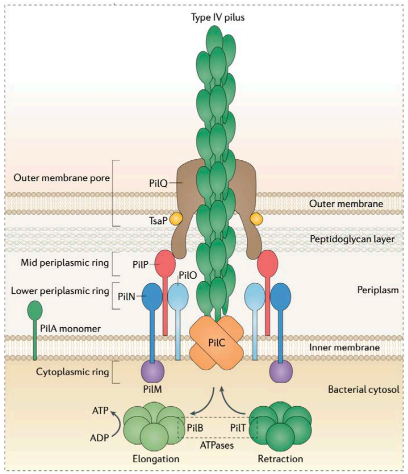


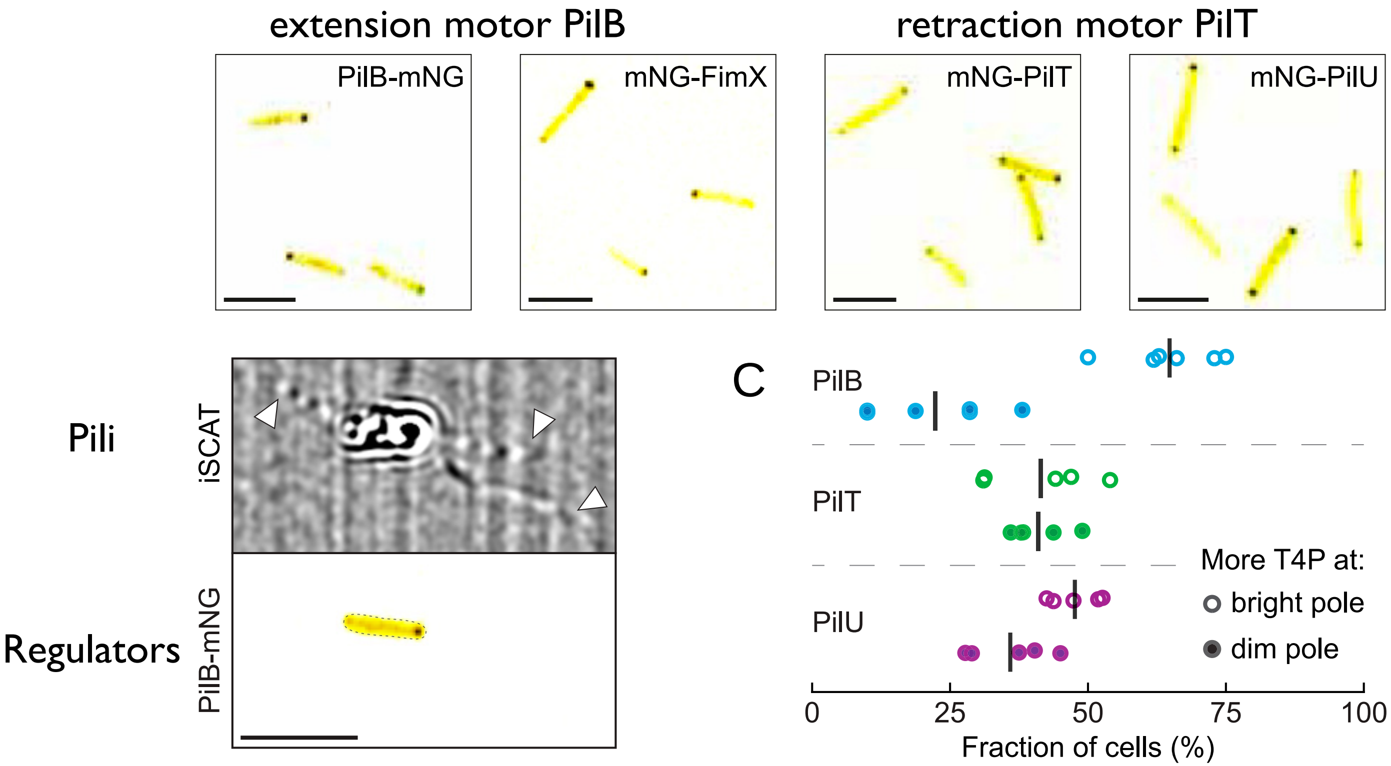
Kühn, M. J. et al. and A. Persat. *PNAS* 118, e2101759118 (2021).

Mechanisms of twitching motility: *regulation*

- PilB and Pili tend to concentrate at the same cell pole

PiliB presents a polarized distribution at the poles of *P. aeruginosa*.
PilB is concentrated at the pole where Type 4 Pili are also concentrated





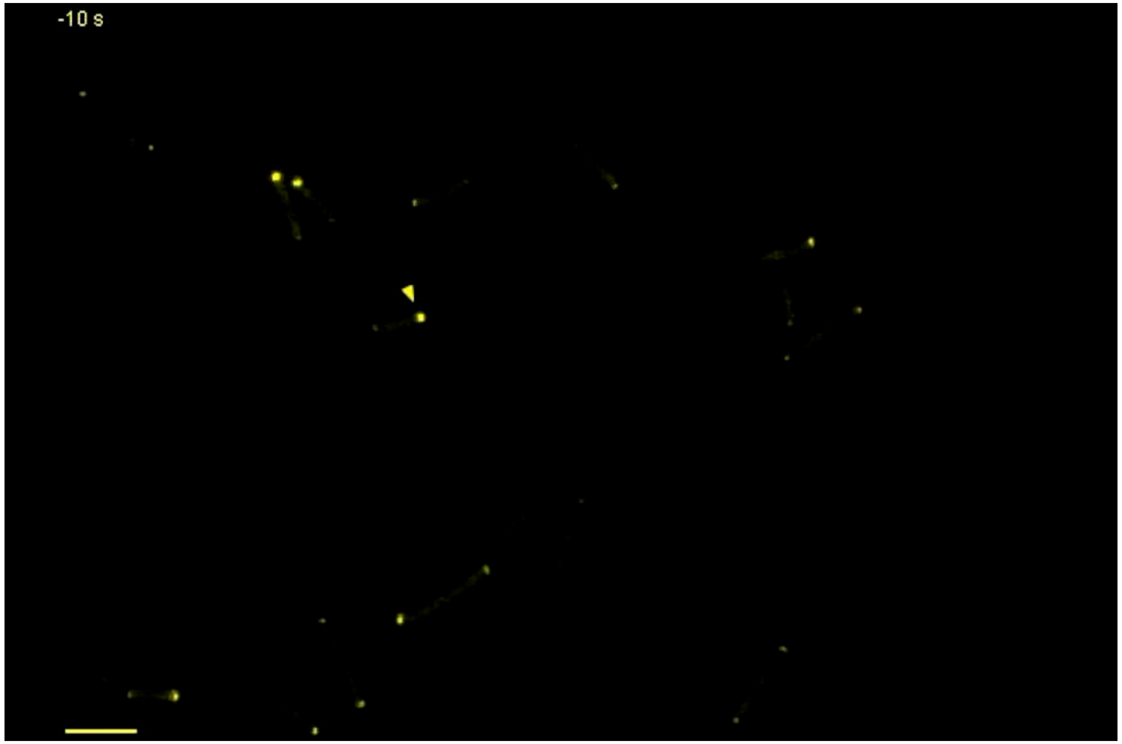
Pili: T4P

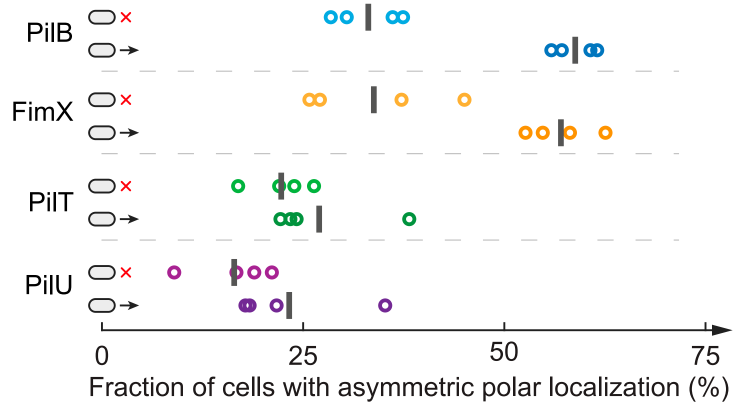
Kühn, M. J. et al. and A. Persat. *PNAS* 118, e2101759118 (2021).

Mechanisms of twitching motility: *regulation*

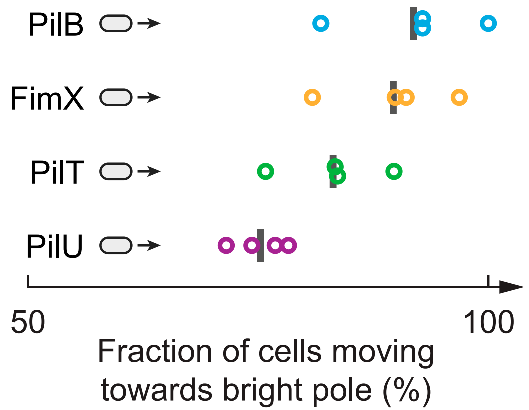
- PilB and Pili concentrate at the leading, forward moving cellular pole

The localization of the extension motor PilB sets the direction of twitching and the polarization of Pili activity
The retraction motor PilT has no biased distribution





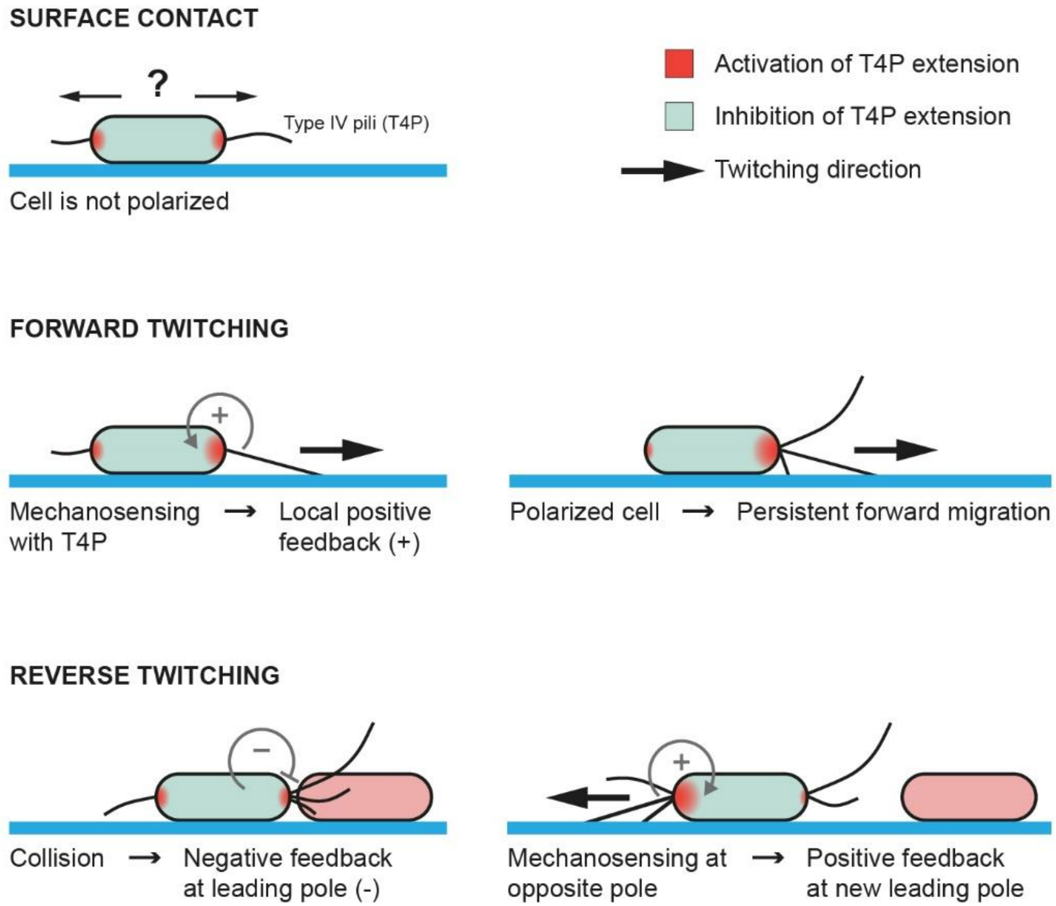
90% of cells move towards PilB pole



Kühn, M. J. et al. and A. Persat. *PNAS* 118, e2101759118 (2021).

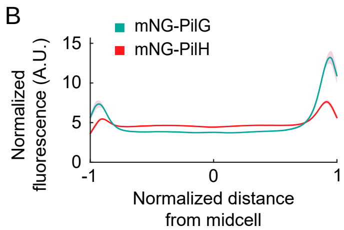
Mechanisms of twitching motility: *regulation*

- Model of mechanotaxis in *P. aeruginosa*



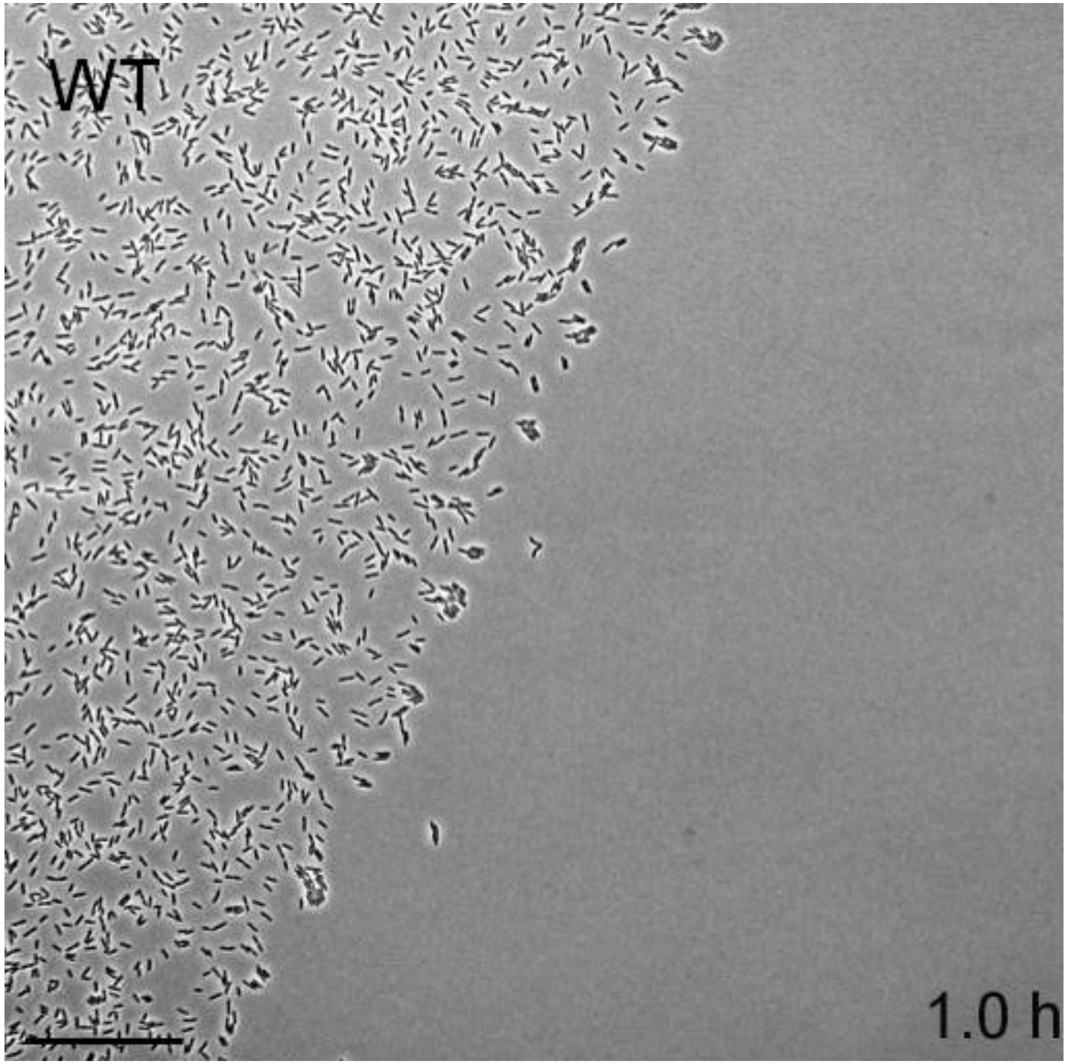
- Chp mechanosensing induces a positive feedback on T4P motors to favor extension at the same pole leading to polarization of the cell and persistent forward motion

- After collision (or loss of T4P attachment/retraction) at the leading pole, a negative feedback down regulates T4P activity. T4P at the opposite pole can then attach and generate a positive feedback that reverses cell polarization and lead to persistent reverse twitching.



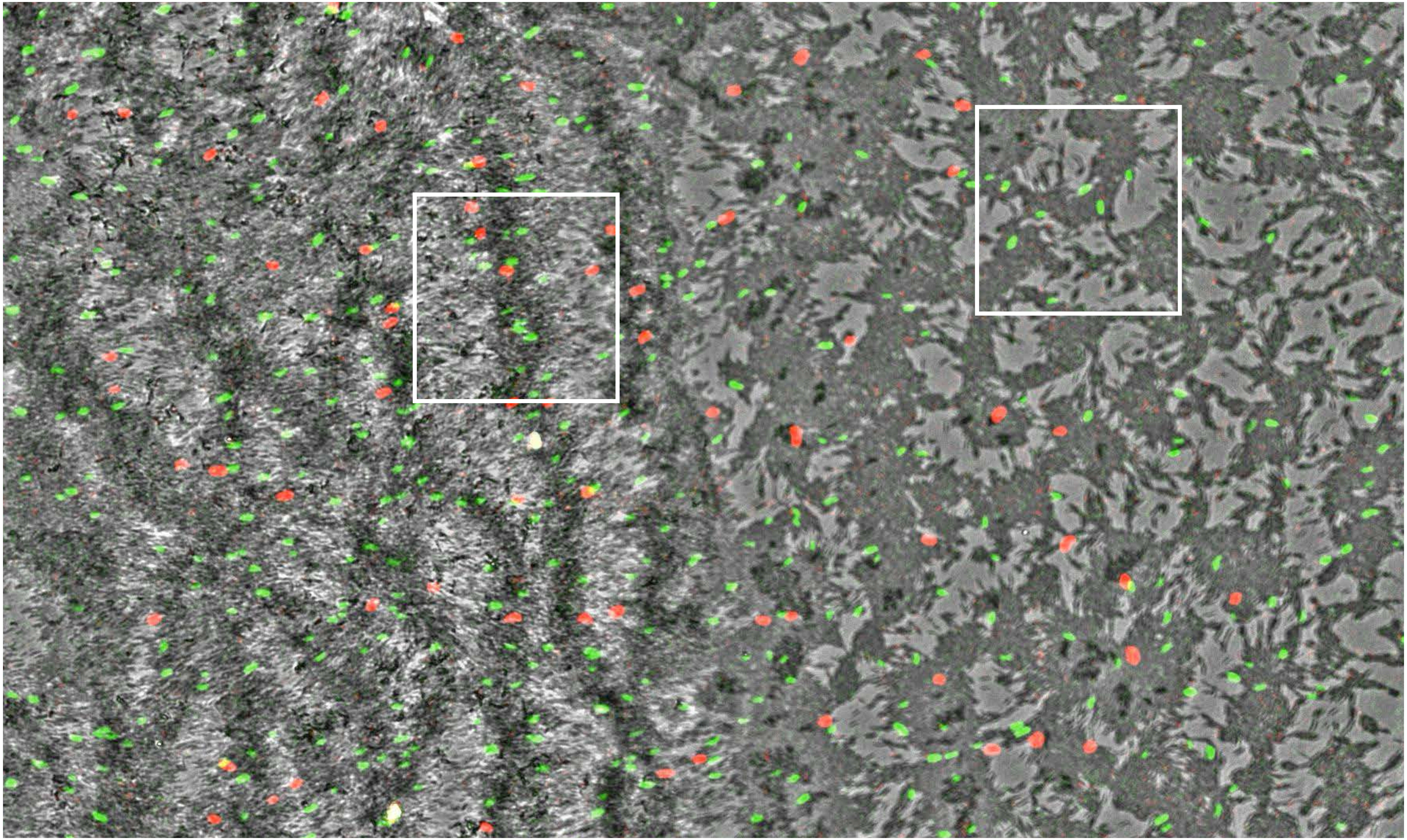
Kühn, M. J. et al. and A. Persat. *PNAS* 118, e2101759118 (2021).

Implications for swarming dynamics in *P. aeruginosa*



- Hypothesis:
- Chemotaxis:
  - The swarm expands towards a chemoattractant that lowers the frequency of reversal. Persistant random walk in 1D/2D.
- Mechanotaxis:
  - The density of collisions is expected to be lower at the edge of the swarm and favors persistent twitching down the density gradient
  - In the bulk of the swarm, reversals are more frequent and collision detection causes a more uniform density distribution.

Oscillatory dynamics of *Myxococcus*



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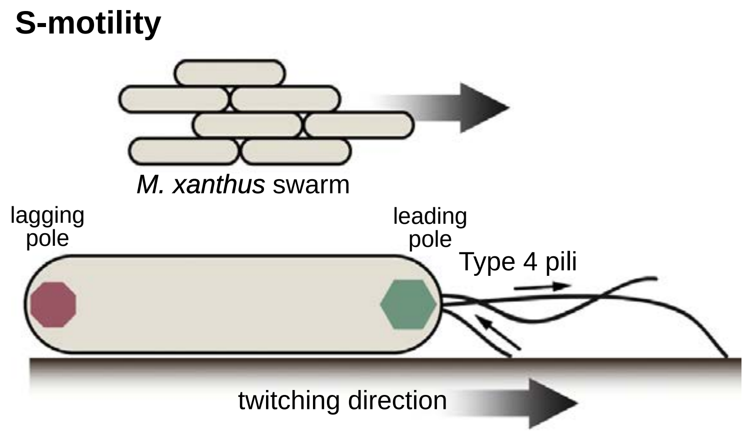
Oscillatory dynamics of twitching *Myxococcus*

- A module of cell polarization driving motility

- Cell polarity:

MglA, a Ras family GTPase, is localized in the leading pole and MglB in the lagging pole.



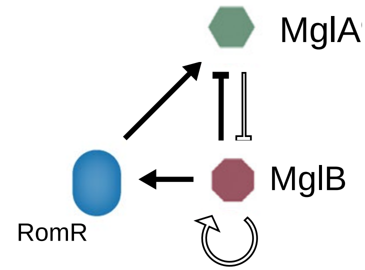
- Control:

Accumulation of MglA at the leading pole is controlled by:
  - The RomRX complex which works as a GEF, that produces MglA-GTP and allows its polar recruitment
  - and by MglB which works as a GAP and inhibits MglA
- MglA, RomRX and MglB define a polarity module that controls the direction of movement.
- During reversal, MglA relocalizes to the opposite pole, switching the polarity axis
- This allows the cells to move in the opposite direction.

Guzzo M, et al. and M. Howard, T. Mignot *Nat Microbiol* 3:948-959 (2018)

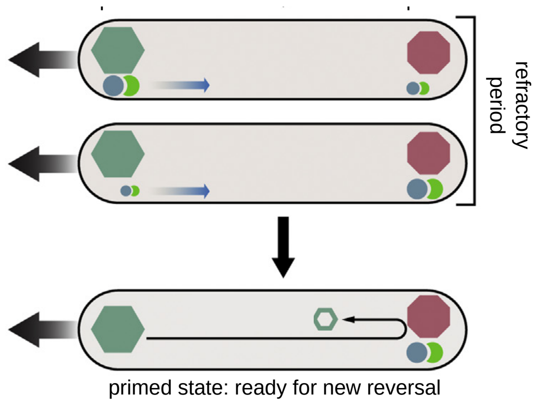
Oscillatory dynamics of twitching *Myxococcus*

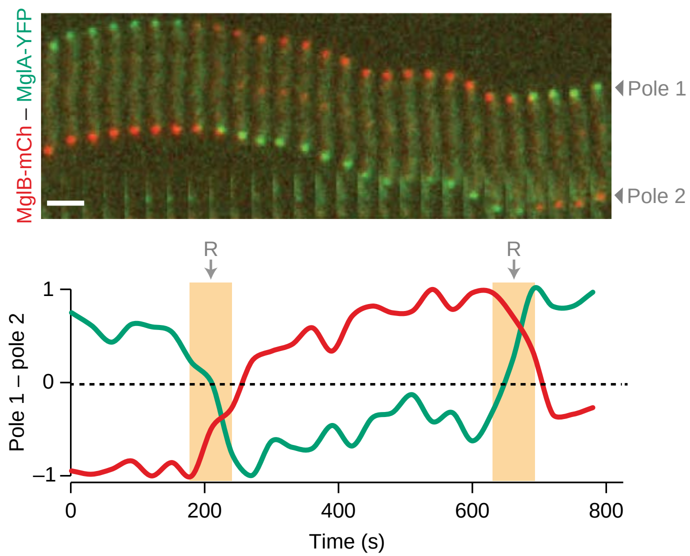
- An oscillatory module underlies reversal

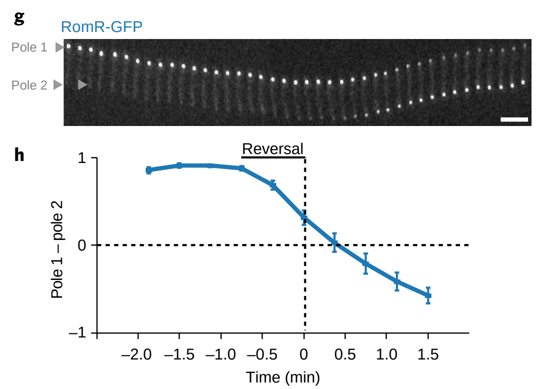


- MglA, and MglB are relocalized at opposite poles during reversal.
- This depends on RomRX oscillations:
  - RomRX recruits MglA to leading pole and subsequently dissociates
  - RomRX relocalises to and primes the lagging pole
- Oscillatory dynamics based on delayed negative feedback.
  The refractory period is set by the time scale of RomRX relocalization

- Yet, RomR:GFP relocalization does not coincide strictly with reversal, suggesting *another signal to trigger reversal.*





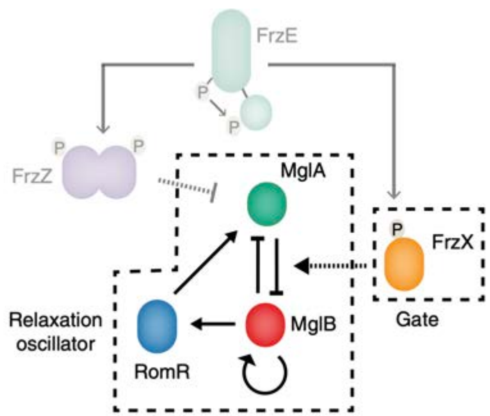


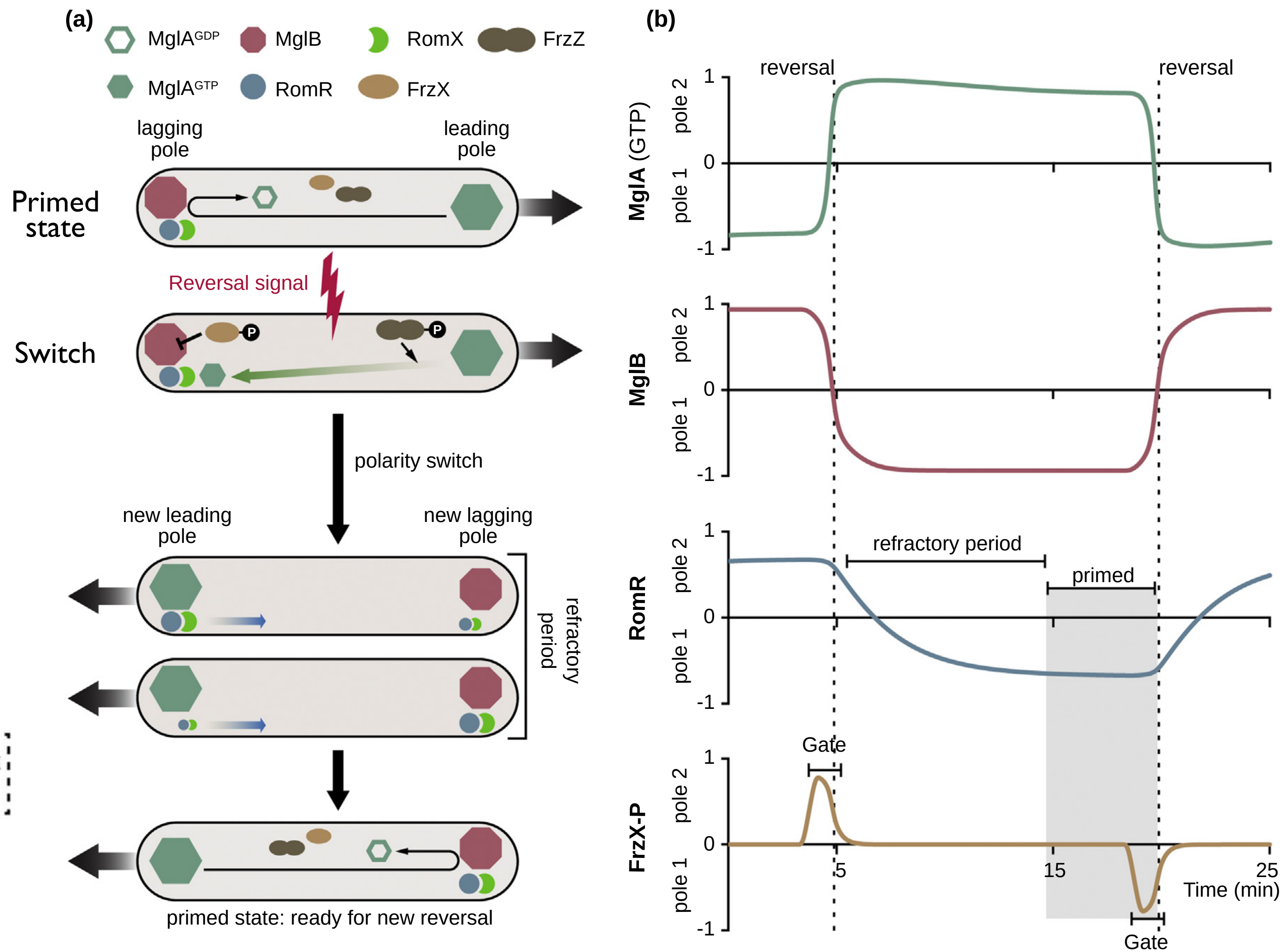
Guzzo M, et al. and M. Howard, T. Mignot *Nat Microbiol* 3:948-959 (2018)

Oscillatory dynamics of twitching *Myxococcus*

- A *gated* relaxation oscillator: a switch and an oscillator

- FrzX inhibits MglB and works as a SWITCH
- Once RomRX is in lagging pole, the lagging pole is primed for activation by FrzX, which inhibits MglB and allows MglA to be recruited, thereby causing reversal
- FrzX reduces the RomR threshold and the effective refractory period for reversal





Current Opinion in Cell Biology

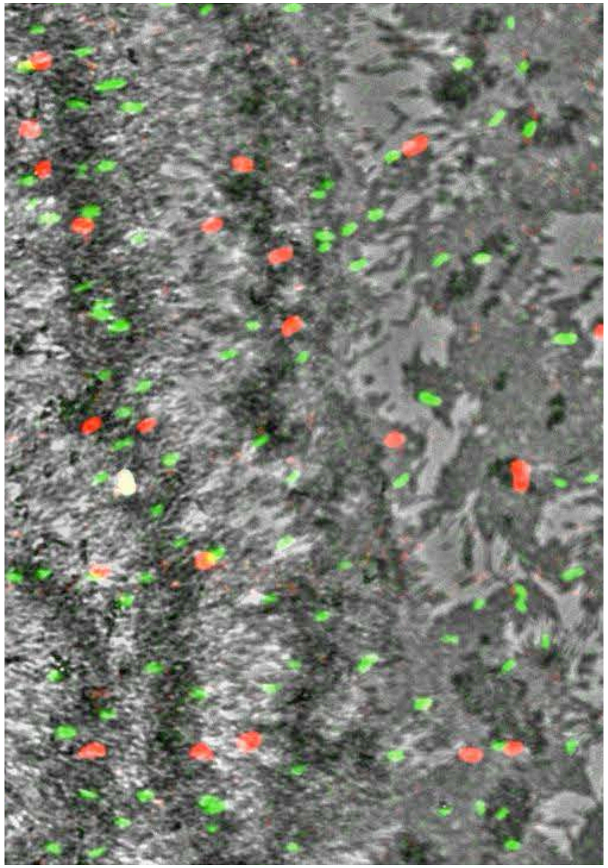
Guzzo M, et al. and M. Howard, T. Mignot *Nat Microbiol* 3:948-959 (2018)

Potential implications for swarming *Myxococcus*

Hypothesis:

Potential implications for swarming dynamics:

- Each cell has an intrinsic oscillator that induces reversals
- This oscillator is gated by a signal (FrzX)
- Presumably collisions induce/activate FrzX and thereby collisions could bias the oscillatory dynamics of twitching cells
- The frequency of collisions would bias the direction of movement.
- Rippling waves are associated with reflection of cells via this mechanism
- On the edge of a swarm where cell density is lower, cells could be biased down the density gradient towards regions with fewer cells, allowing expansion of the swarm (similarities with *Pseudomonas*)



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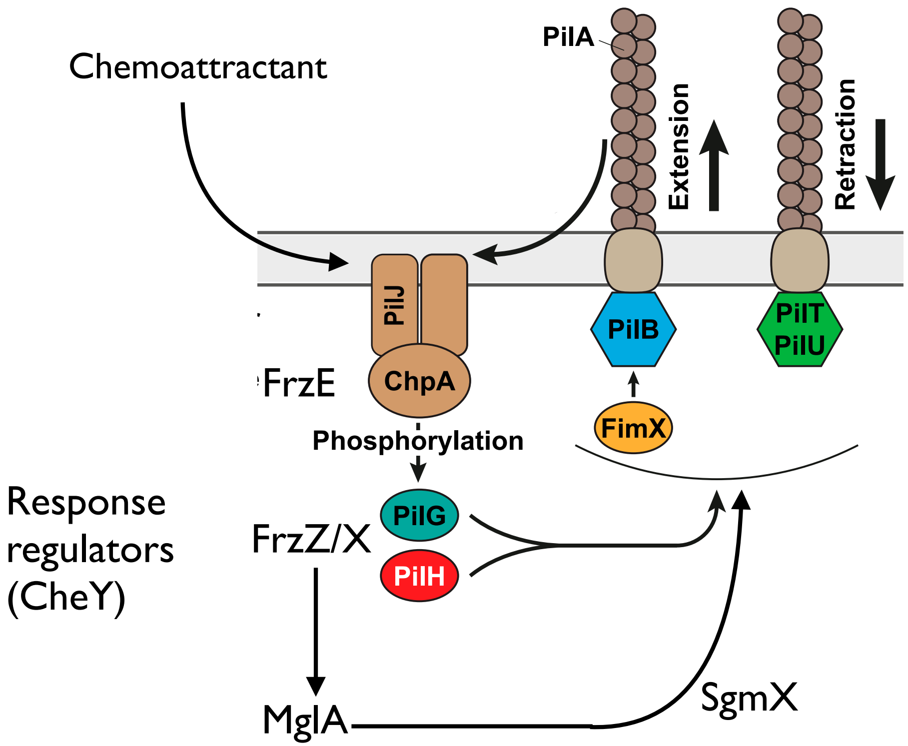
Similarities between *Pseudomonas and Myxococcus*

- Identical Mechanical system
- T4Pili dynamics underlies twitching
- PilB and PilT ATP dependent motors
- Cell reversal induced by collisions.

- Similar regulation
- ChpA is a homologue of FrzE
- They regulate PilG/PilH, response regulators similar to FrzZ/FrzE (CheY type)
- PilG controls the motors (PilB) in *Pseudomonas,* while FrzZ/X control MglA/B in *Myxococcus*
- MglA controls Pili via other proteins



Mercier R, et al. & Mignot T. PNAS 117(45):28366-28373 (2020)
Bautista S, et al, Mignot T, Mercier R. *EMBO J.* 8:e111661 (2022)

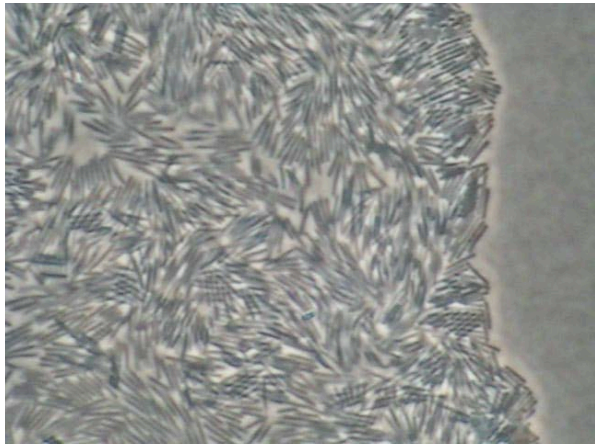
Regulation ⇄ Mechanics

Emergence of collective dynamics in swarms of confluent cells

- Jamming ⇄ - Collective dynamics

www.youtube.com/watch?v=1ZupwFOhjl4

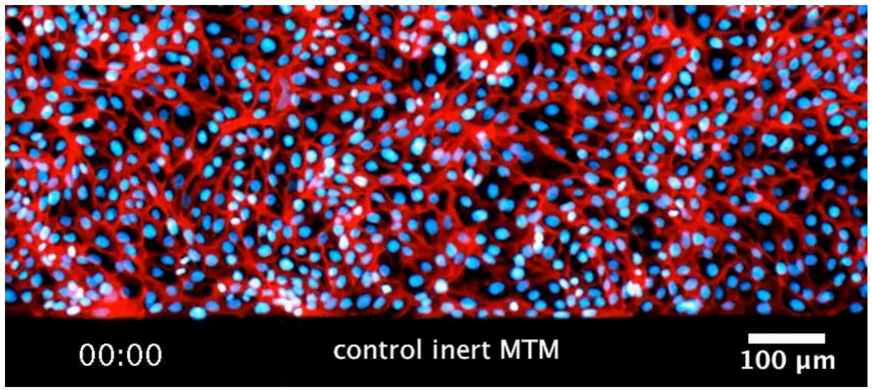
Hi Chi Minh city - Vietnam



Slowed 2.5x

Bacillus subtilis swarm

Partridge, JD. and Harshey RM. *J. Bacteriology* 195: 909-918. (2013)



Confluent MDCK cell monolayer

D. Cohen et al *PNAS* 113: 14698–14703 (2016)

Comparisons between eucaryotes & procaryotes

- *Eukaryotes:*
- Following collisions, cells reorganize their polarities to induce local ordering
- high density induces mechano-chemical coupling that allows global symmetry breaking (leaders) or local symmetry breaking (boundary and leader free).
- Positive density-dependent regulation of motility: coordination of velocity (see Toner Tu model course #1)

- *Prokaryotes*:
- Following collisions, cells move in opposite directions
- Density dependent biased random (oscillatory) 1D walk
- Negative density-dependent regulation of motility?
- But collisions and possible lateral adhesion to promote local ordering?
- Lateral alignement based on morphology of rigid body

- Mechanotaxis: stiffness
- Chemotaxis