Bacteria moving particles

Behkam and Sitti (2007)

Title: "Bacterial flagella-based propulsion and on/off motion control of microscale objects"

- ***Serratia marcescens***
- **10 µm polystyrene (PS) beads** (PS beads G1000, Duke Scientific, Palo Alto, CA)

Motility test protocol

9 cm swarm plate

- Luria broth:
  - 0.6% Difco Bacto agar
  - 5 g/ l glucose
- Off center inoculation with $1.8*10^{-6}$ cells (grown in L broth)
- Incubation: 19 h at 30 °C

➢ Result: Ø 7 cm swarm colony

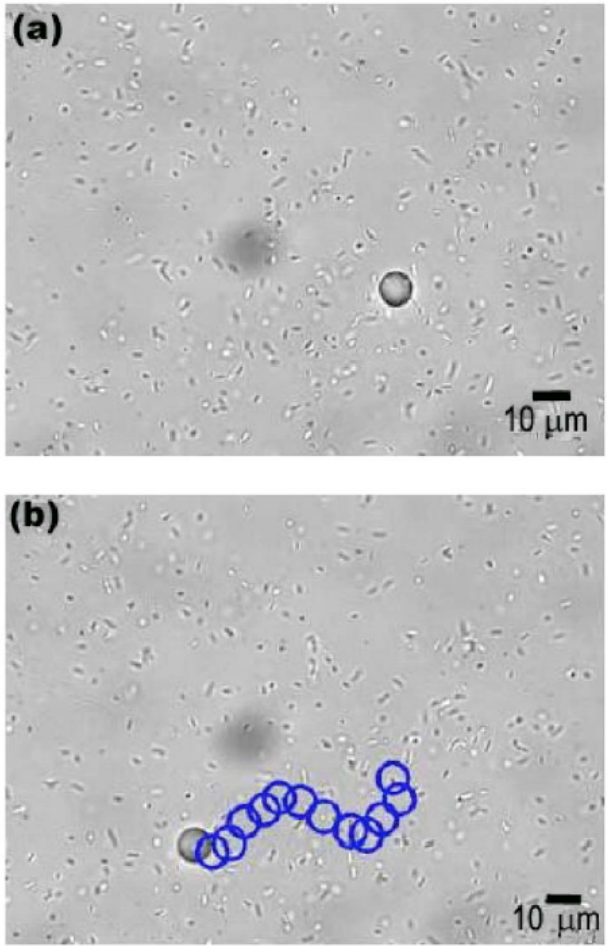
Mobility test

- motility medium:
  - 0.01M potassium phosphate
  - 0.067M sodium chloride
  - 10^{-4}M EDTA
  - 0.01M glucose
  - 0.002% Tween 20
  - pH of 7.0

- Wash beads in motility medium
- Concentrate 5-fold
- 10 µl on leading edge of plate
- 5 min later: pipetting back into 1 ml motility medium
- 10 µl in imaging enclosure (microscope glass slide No. 1 with app 500 µm thick polydimethyl siloxane ring + coverslip)

S. Marcescens move beads

- 60 oil immersion phase objective
- Distance ~90 µm
  - ➢ 100 times bigger than diffusion length
- Ø velocity: 15 µm/s
- Drag force for bead: 1.4 pN
- Drag force of each attached bacterium: 0.45 pN



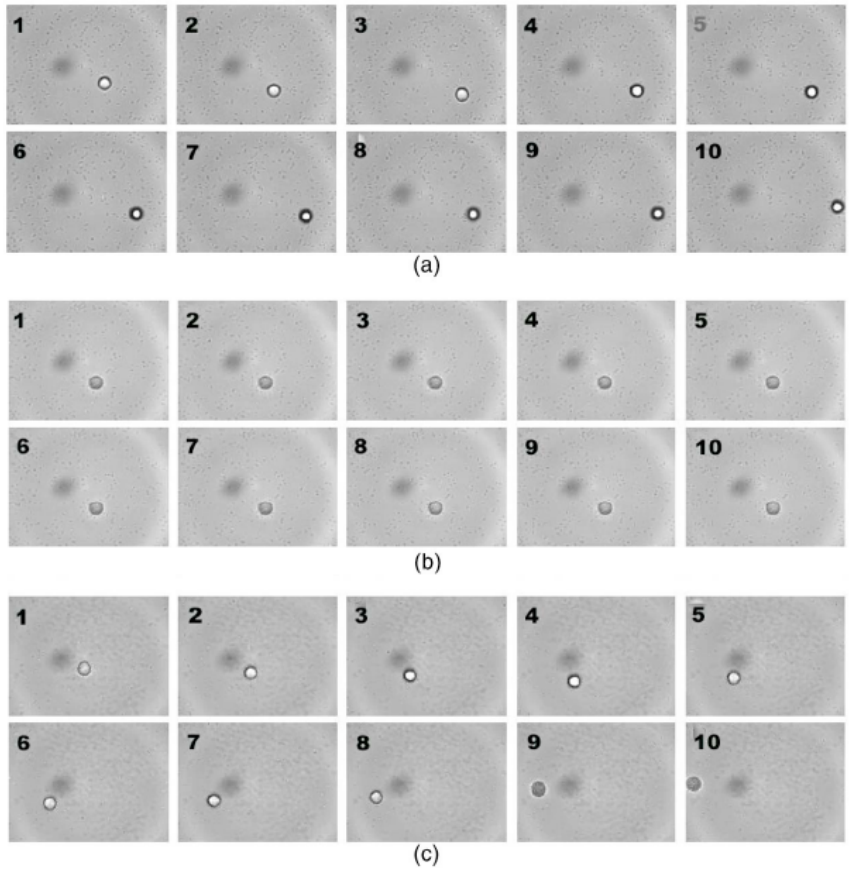
t=0 s

t=6 s

Behkam and Sitti, 2007

On/Off moving

- App. 8 bacteria attached to the bead
- Time intervals of 0.6 s over 6 s
- Repeating 3 times



10 µl bacteria solution in motility medium

↓

5 µl of $5*10^{-3}$M $CuSO_4$
→ no moving

↓

7.5 µl of $5*10^{-3}$M EDTA
→ resumed moving

Behkam and Sitti, 2007

Problems

- Ø: 60% of the beads were mobile
- net displacement and speed of the beads were random and not identical among the experiments (Ø bead displacement over 6 s was 92±35 µm)
- Possible reasons:
  - random run and tumble behavior
  - since the beads were pipetted onto the swarm plate, the quantity, orientation, and spacing of the adhered bacteria were not controlled

Darnton et al. (2004)

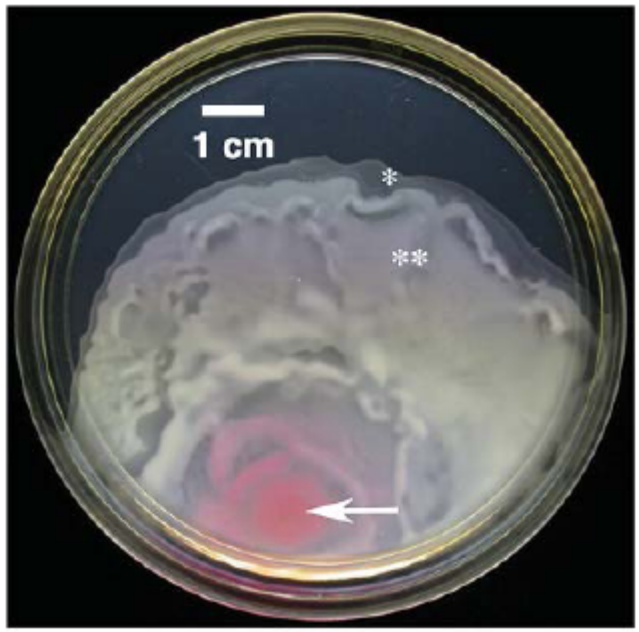
Title: "Moving Fluid with Bacterial Carpets"

- ***S. marcescens***
- Bacteria attached to solid surface
- Polydimethylsiloxane=PDMS (fragments) or polystyrene (beads) in fluid over bacteria
- Visible flow patterns produced by bacteria carpets

15 cm swarm plate

- 33 ml L broth:
  - 0.6% Difco Bacto agar
  - 5 g/ l glucose
- Off center inoculation with $2*10^{-6}$ cells (grown in L broth)
- Incubation: overnight at 30 °C

➢ Result: 10-12 cm swarm colony



Darnton et al., 2004

Bacterial carpet formation

- PDMS-coated coverslip inverted on bacterial swarm on agar plate and removed
- “blotted” bacteria= carpet
- Mobility medium: see Behkam and Sitti (2007)

Fluid-flow assays

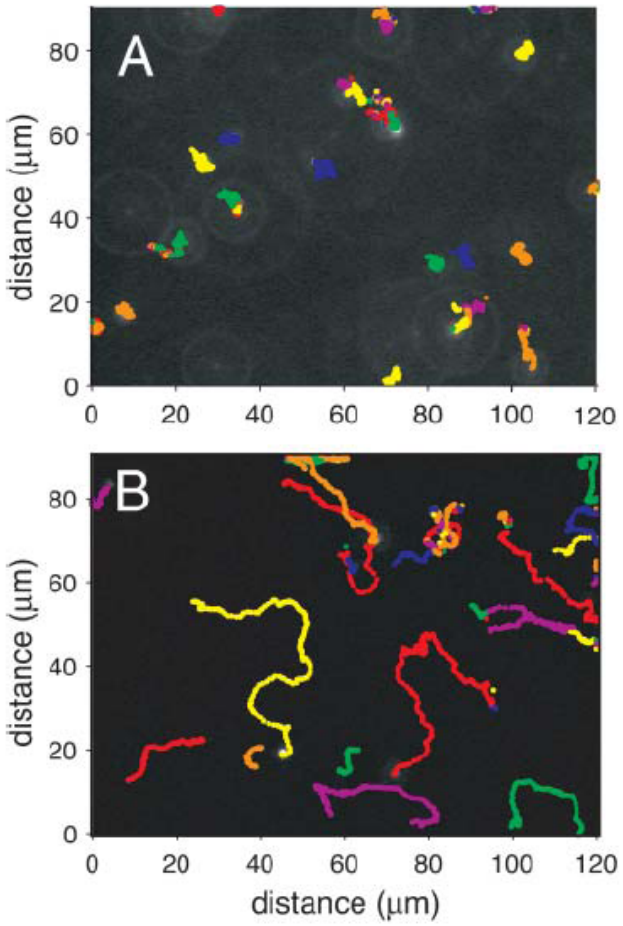
- Viewing area 0.76 cm square by 0.033 cm dept [60x phase objective (Nikon PlanApo DM oil, NA 1.4) and inverted microscope (Nikon Diaphot 200)]
- V(chamber)= 60 µl
- Mobility medium flushed through chamber (10-200 µl/min)
- Ø 1 µm red fluorescing beads (R0100, Duke Scientific, Palo Alto, CA) → 1:50 dilution added
- Imaging of epiflourescence with mercury arc exitation (TRITC fluorescence cube No. 31002, Chroma Technologies, Brattleboro, VT) and a CCD camera (model 1070, Marshall Electronics, Culver City, CA)
- More used hard- and software (e.g. a MATLAB algorithm)

Results

- Highly orientated bacteria domains
- lying flat, parallel to the substrate
- standing on end, normal to the substrate (artificial)
- Longest cells (6.6±1.9 µm) at leading edge on plate
- On slide: applied flow of 10 µm/s
- Flagella orientated on flow direction; rotation rate of the flagella 140±30 Hz (comparable to *E. coli*)
- Swimming speed of *S. marcescens* 47±16 µm/s (at RT)

Tracer bead tracks

- 1 µm beads
- 10 s interval



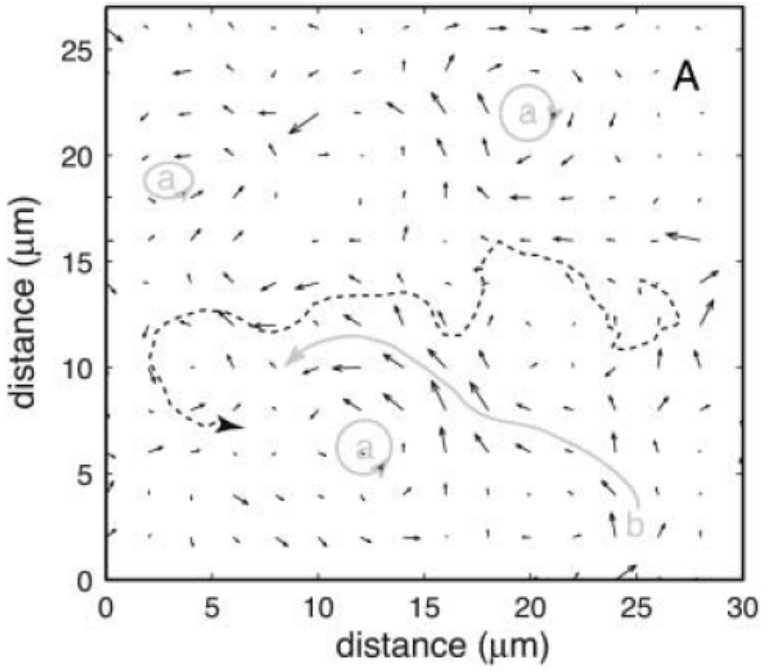
80 µm over the active surface (Brownian motion)

5 µm over the active surface

Darnton et al., 2004

Surface flows

- Whirlpools (a) and rivers (b)
- A few stable for more than 10 min, but normally less



Darnton et al., 2004

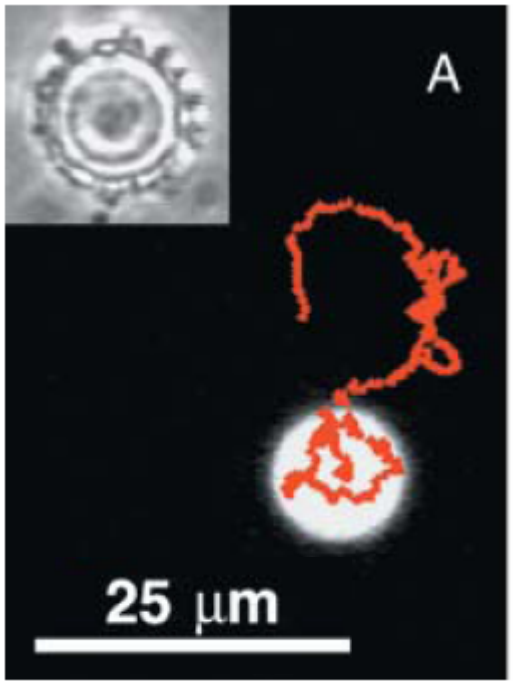
Protocol: Auto-mobile beads and chips

- **Auto-mobile beads:**
  - Red flourescing PS beads (Ø 10 µm F-8834, Molecular Probes, Eugene, OR)
  - Concentrated in motility medium and added to leading edge of swarm on plate
  - Pipetted into 1 ml motility medium on slide with grease ring
  - 40x phase contrast dry objective and fluorescence (Texas Red cube No. 31004, Chroma Technologies)
  - Individual bacteria on the bead's surface were identified by eye
- **Automobile chips:**
  - Slide covered with fractured PDMS-plates
  - Bacteria "blotted" on surface
  - Scraping off the pieces with razorblade
    - Bacterial-carpet-coated PDMS
    - Imaging: 10x phase contrast objective

Auto-mobile bead

- 10 μm diameter fluorescent auto-mobile bead (red= bead center)
- phase-contrast image of the same bead (inset):
  - adhered bacteria are visible as dark spots around the bead's circumference
  - ~50 attached bacteria (~1 bacterium/6 μm^2)
- Root mean-square speed for bead 4.7 μm/s

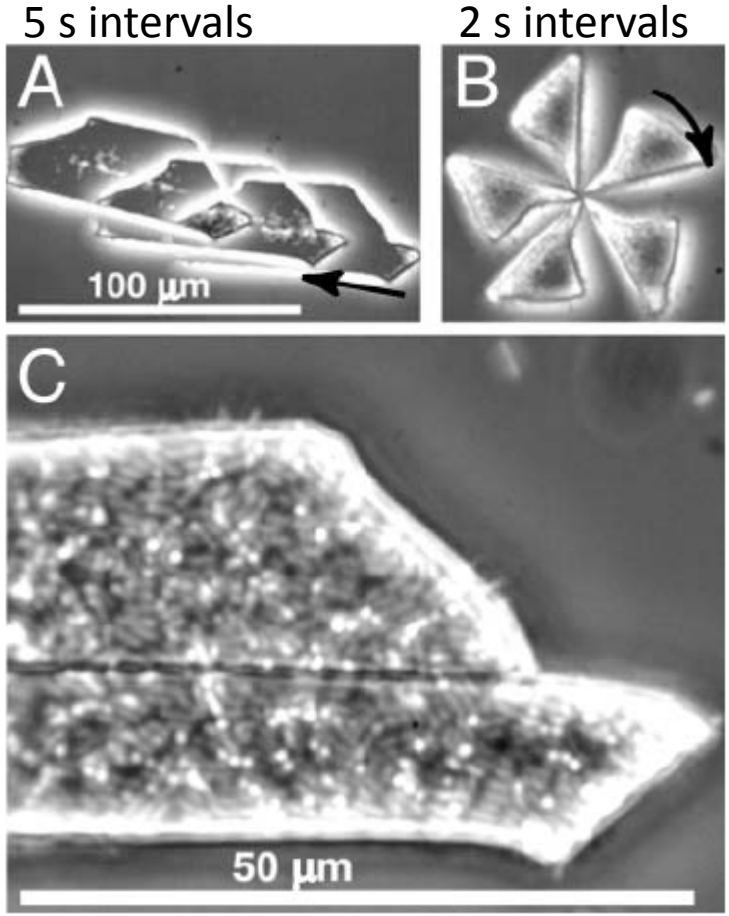
1/10 intervals over 30 s



Darnton et al., 2004

Auto-mobile chips

- Auto mobile chip in A) ~5 µm/s
- In B) rotation at ~6 rpm
- 3 times higher density of bacteria on the surface than on beads



Darnton et al., 2004

Important!!!

- *S. marcescens*: equally bounding to native (hydrophobic) PDMS and to oxygen-plasma-treated (hydrophyllic) PDMS, but not *E. coli*!!!