

Bacteria moving particles

# Behkam and Sitti (2007)

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

- *Serratia marcescens*
- **10  $\mu\text{m}$  polystyrene (PS) beads** (PS beads G1000, Duke Scientific, Palo Alto, CA)

# Motility test protocol

## 9 cm swarm plate

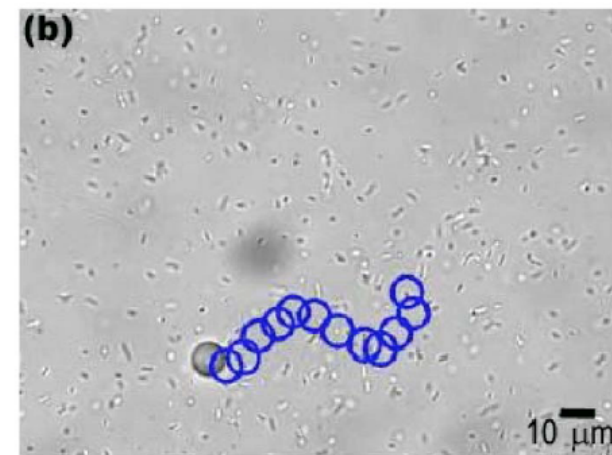
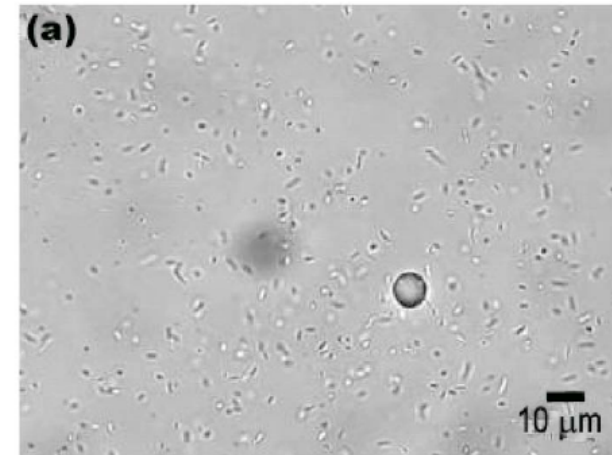
- Luria broth:
  - 0.6% Difco Bacto agar
  - 5 g/l glucose
- Off center inoculation with  $1.8 \times 10^{-6}$  cells (grown in L broth)
- Incubation: 19 h at 30 °C
- Result:  $\varnothing$  7 cm swarm colony

## Motility 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  $\mu$ l on leading edge of plate
- 5 min later: pipetting back into 1 ml motility medium
- 10  $\mu$ l in imaging enclosure (microscope glass slide No. 1 with app 500  $\mu$ m thick polydimethyl siloxane ring + coverslip)

# *S. Marcescens* move beads

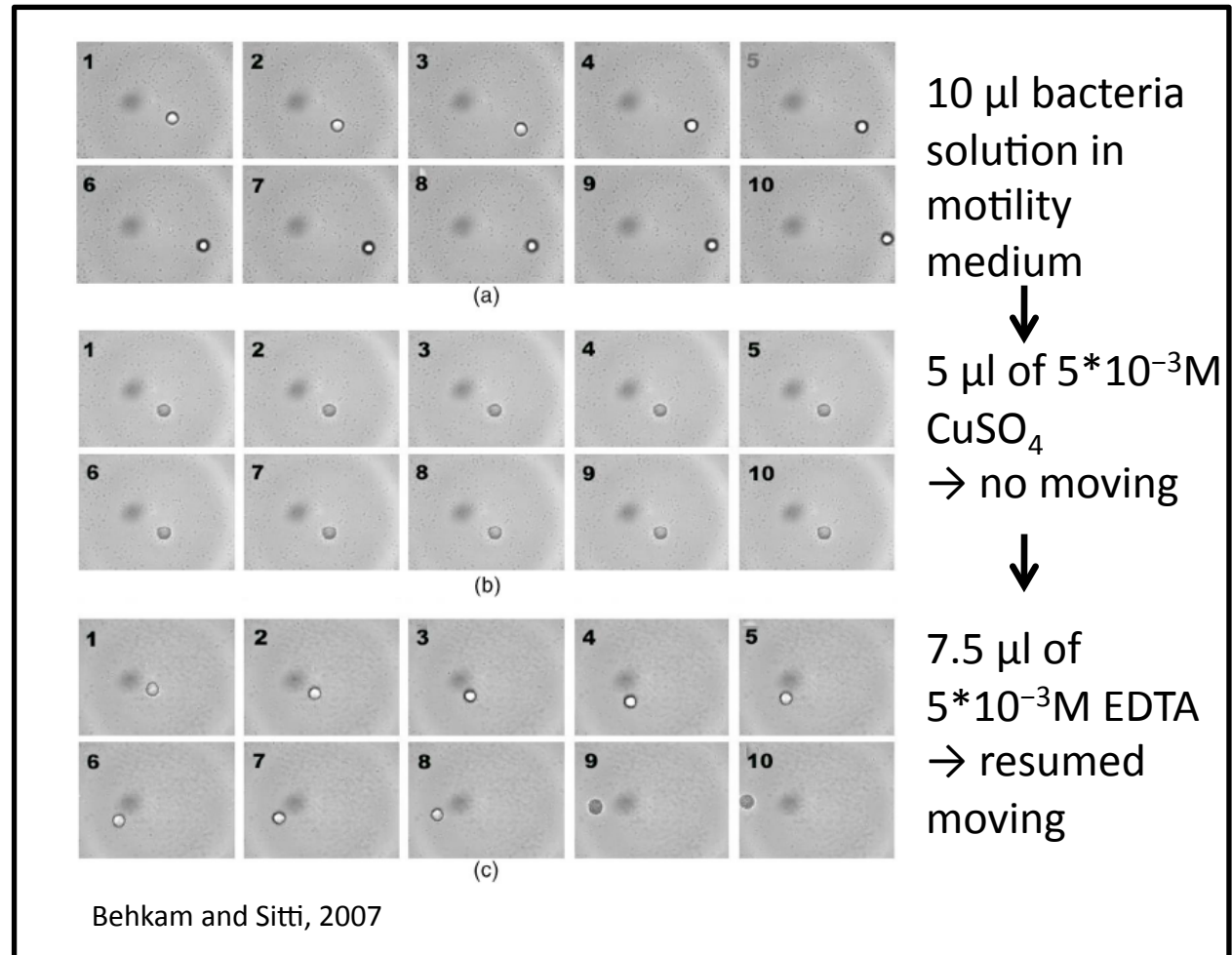
- 60 oil immersion phase objective
- Distance  $\sim 90 \mu\text{m}$ 
  - 100 times bigger than diffusion length
- $\emptyset$  velocity:  $15 \mu\text{m/s}$
- Drag force for bead:  $1.4 \text{ pN}$
- Drag force of each attached bacterium:  $0.45 \text{ pN}$



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



# Problems

- $\emptyset$ : 60% of the beads were mobile
- net displacement and speed of the beads were random and not identical among the experiments ( $\emptyset$  bead displacement over 6 s was  $92 \pm 35 \mu\text{m}$ )
- 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

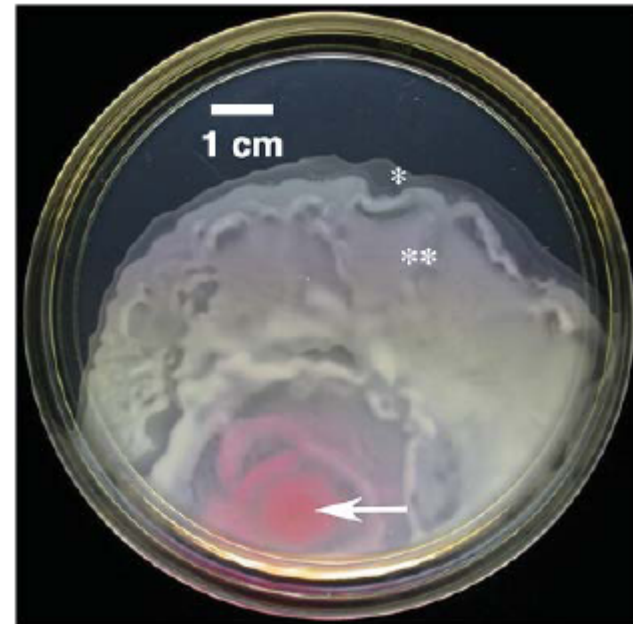
# Darnton et al. (2004)

## **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  $2 \times 10^6$  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

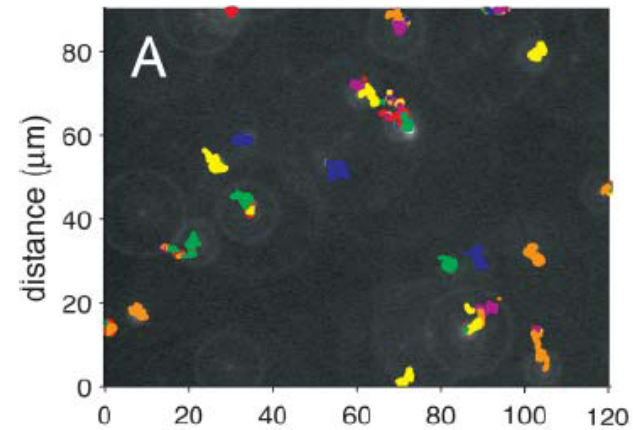
- Viewing area 0.76 cm square by 0.033 cm dept [60x phase objective (Nikon PlanApo DM oil, NA 1.4) and inverted microscope (Nikon Diaphot 200)]
- $V(\text{chamber}) = 60 \mu\text{l}$
- Mobility medium flushed through chamber (10-200  $\mu\text{l}/\text{min}$ )
- $\emptyset$  1  $\mu\text{m}$  red fluorescing beads (R0100, Duke Scientific, Palo Alto, CA)  $\rightarrow$  1:50 dilution added
- Imaging of epifluorescence with mercury arc excitation (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

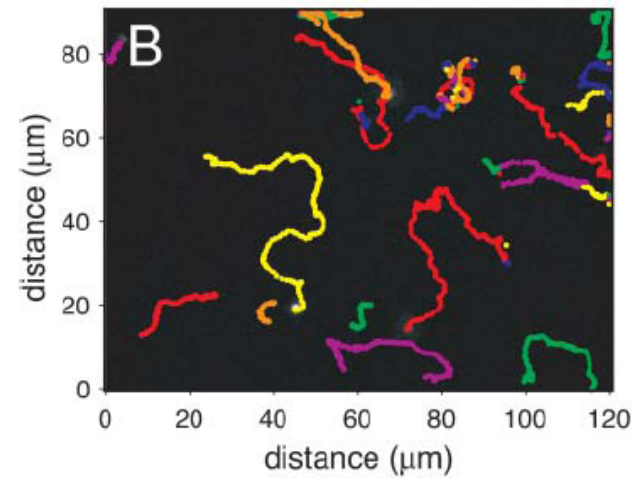
- Highly orientated bacteria domains
- lying flat, parallel to the substrate
- standing on end, normal to the substrate (artificial)
- Longest cells ( $6.6 \pm 1.9 \mu\text{m}$ ) at leading edge on plate
- On slide: applied flow of  $10 \mu\text{m/s}$
- Flagella orientated on flow direction; rotation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

# Tracer bead tracks

- 1  $\mu\text{m}$  beads
- 10 s interval



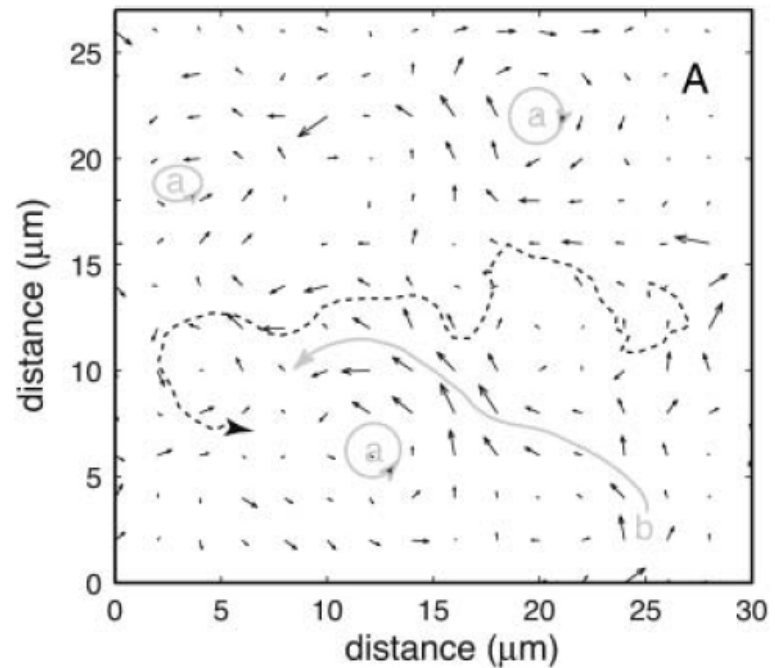
80  $\mu\text{m}$  over  
the active  
surface  
(Brownian  
motion)



5  $\mu\text{m}$  over  
the active  
surface

# Surface flows

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



# Protocol: Auto-mobile beads and chips

- **Auto-mobile beads:**

- Red fluorescing PS beads ( $\varnothing$  10  $\mu\text{m}$  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

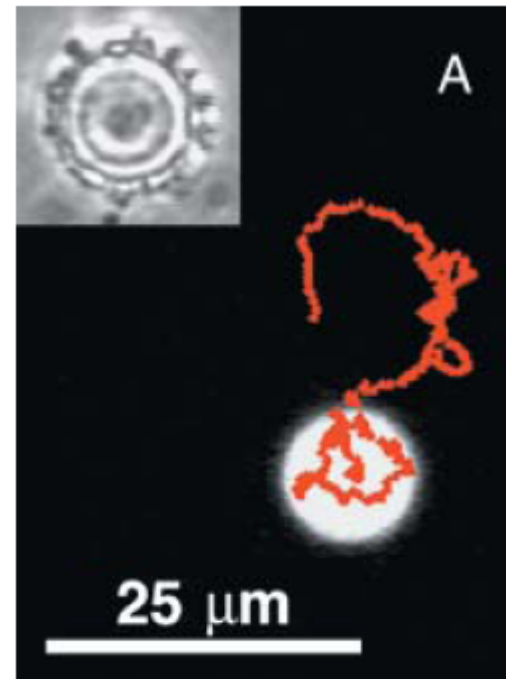
- **Automobile chips:**

- 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  $\mu\text{m}$  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
  - $\sim 50$  attached bacteria ( $\sim 1$  bacterium/ $6 \mu\text{m}^2$ )
- Root mean-square speed for bead  $4.7 \mu\text{m/s}$

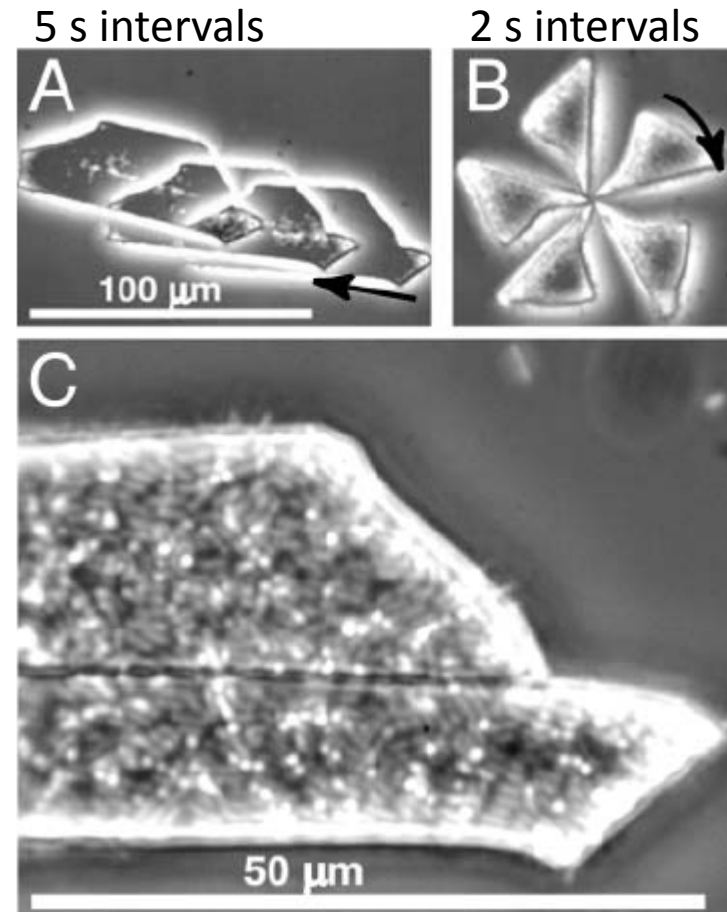
1/10 intervals over 30 s



Darnton et al., 2004

# Auto-mobile chips

- Auto mobile chip in A)  
~5  $\mu\text{m}/\text{s}$
- In B) rotation at ~6 rpm
- 3 times higher density of bacteria on the surface than on beads





# Important!!!

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