A bacterial extracellular DNA inhibits settling of motile progeny cells within a biofilm

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Biofilm

- An aggregate of microorganisms
- Attachment on a surface
Impact of biofilm
Biofilm resistance

- Degradation enzyme
- Resistance gene
- Antibiotics
- Resistance mechanism
Biofilm formation

- Biofilms: extracellular matrix (great resistance)
- Detachment
- Dispersal: deterioration of habitat
  - planktonic phase
Extracellular DNA

- eDNA stabilized biofilm

Listeria monocytogenes

Tetz et al, DNA AND CELL BIOLOGY (2010)

Caulobacter crescentus

• An innocuous oligotrophic $\alpha$-proteobacterium
• colonizes streams, rivers, and water pipes
• strongest adhesion force of any known natural material

--- *PNAS* (2006) 103: 5764-5768

• biodegradable surgical adhesive
Motile swarmer cell and stalked cell

Electron micrograph of Caulobacter crescentus
Rationale

• The role of eDNA within C. cresentus biofilm
• The balance between biofilm and dispersal
In this paper....
Structure

What role does eDNA play in *C. crescentus*?

Where does the eDNA come from?

How does the eDNA work?
DNase I recovered the inhibition of biofilm formation.

→ eDNA inhibited biofilm formation.
The eDNA in the spent medium

Lower molecular weight DNA fragments inhibited biofilm formation.

Addition of digested gDNA had the same inhibition as spent medium.
Structure

What role does eDNA play in *C. crescentus*?

Where does the eDNA come from?

How does the eDNA work?
Correlation between cell death, eDNA release and biofilm inhibition

eDNA was released during cell death.
Structure

What role does eDNA play in *C. crescentus*?

Where does the eDNA come from?

How does the eDNA work?
The inhibition in pili and flagellum mutant

The biofilm inhibition was not due to interaction between eDNA and pili or eDNA and flagellum.

70% decrease

Blank: no gDNA/eDNA
Black: digested gDNA (wt)
Stripe: digested gDNA (ΔpulA)
Dot: digested gDNA (ΔflgE)
Later attachment with eDNA

Holdfast-deficient

eDNA prevented the attachment of swimming cells by holdfast.
Colocalization of eDNA and holdfast

eDNA inhibited biofilm formation by binding to holdfast.
Summary
Discussion (I)

Previous studies:
eDNA stabilized biofilm formation

- *Pseudomonas aeruginosa*

- *Staphylococcus epidermidis*

The addition of DNase I inhibited biofilm formation and dissolved established biofilm.
The addition of Dnase I

Biofilm formation within DNase I (red)

Effect of DNase I (red) on established biofilms.

Dnase I did not inhibit biofilm formation and dissolve established biofilms. Therefore, eDNA did not stabilize biofilm of C. crescentus.
Discussion (II)

- eDNA stabilized biofilm in previous studies: 20~30Kb
eDNA inhibited biofilm formation in this paper: 500bp

Grande et al, Applied Microbiology (2010)

• high-molecular-weight DNA (20~30Kb): stability
low-molecular-weight DNA (500bp): inhibition

*Listeria monocytogenes.*

The size of eDNA is probably a key factor of decision between stability and inhibition.

Caulobacter crescentus

eDNA

![Image of biofilms and environmental impacts](Image)

![Image of eDNA and DNA samples](Image)

![Graphs showing biofilm formation](Image)

![Immunofluorescence images](Image)
Thanks for your attention!

Special thanks for Professor Ching-Tsan Huang