The Biofilm Hypothesis of Chronic Infection
Prof. Philip
A Webber Training Teleclass

MONTANA STATE UNIVERSITY
Center for Biofilm Engineering

The Biofilm Hypothesis of Chronic Infection

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Hosted by Martin Kiernan
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The Black Death

**Victims…
"ate lunch with their friends and dinner with their ancestors in paradise."**
Boccacio

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Cholera

Drinking from the Ganges River during a Hindu ceremony

"What frightened people about cholera was the speed at which it struck the victim and brought about a painful death."

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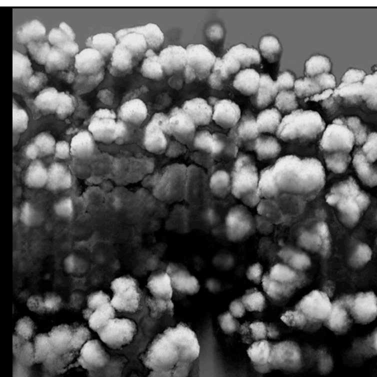

Staphyloccal biofilm imaged by CSLM, Betsey Pitts, 2005, MSU-CBE

Biofilm on pacemaker lead

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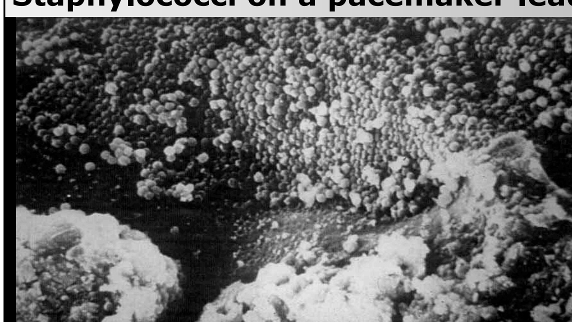
Staphylococci on a pacemaker lead



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Biofilm in CF pneumonia

- Genetic defect in chloride ion channel
- Lung is never cleared of bacteria despite aggressive chemotherapy
- Massive neutrophil invasion contributes to gradual loss of lung function
- Evidence of low oxygen/anaerobic bacterial metabolism

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Biofilm in periodontitis

- Tooth surface poorly defended compared to vascularized tissues
- Tetracycline, antiseptic mouthrinses have little efficacy
- Host responses, bacterial virulence factors lead to progressive bone loss
- Malodor

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Biofilm in chronic wounds

- Biofilm forms on necrotic tissues
- Symptoms wax and wane over weeks to months
- Marginal response to topical antimicrobials, systemic antibiotics
- Normal healing process arrested
- Malodor

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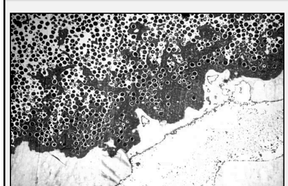
Biofilm in chronic osteomyelitis



- Biofilm forms on dead bone
- Infection persists despite antibiotic therapy
- Involucrum of fibrous tissue decreases vascularization of the infection site

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Features of biofilm infections

- Form preferentially on foreign bodies, dead or damaged tissue
- Slow to develop, but persistent
- Respond poorly or only temporarily to antibiotics, antiseptics
- Collateral damage to neighboring healthy tissue
- Anoxic niches, anaerobic metabolism

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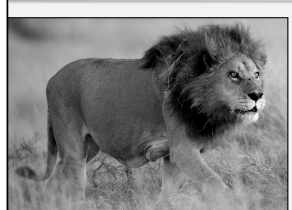
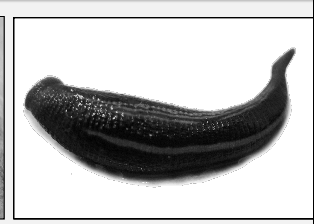
Planktonic vs Biofilm



“Predatory”



“Parasitic”

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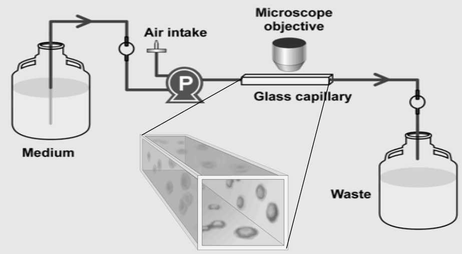
Glass capillary biofilm reactor



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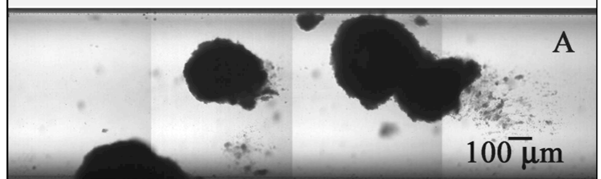
S. epidermidis biofilm



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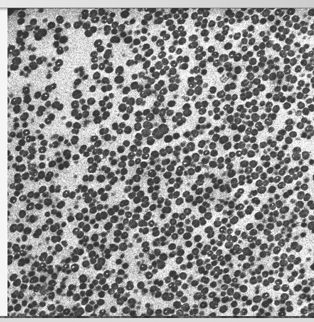
S. epidermidis biofilm - TEM



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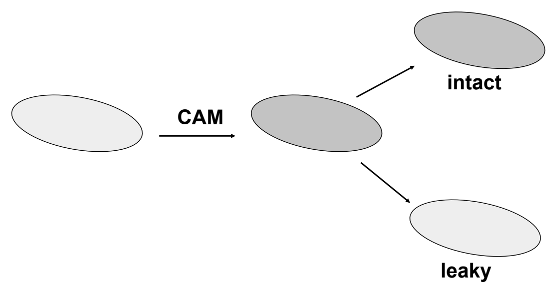
Visualizing antimicrobial action



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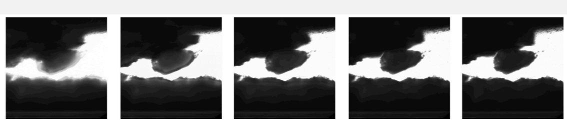
Treatment with 50 mg/l quat



0 min 10 min 20 min 30 min 40 min

Davison et al (2010) *Antimicrob. Agents Chemother.* **54**:2920.

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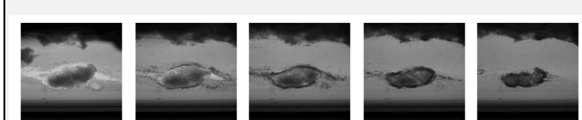
Treatment with 50 mg/l chlorine



0 min 10 min 20 min 30 min 40 min

Davison et al (2010) *Antimicrob. Agents Chemother.* **54**:2920.

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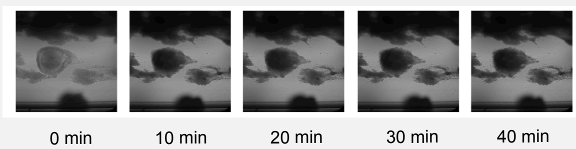
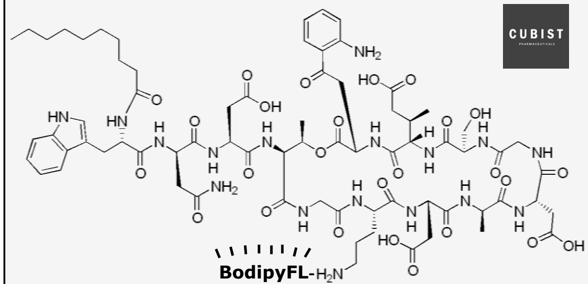
Treatment with 50 mg/l nisin

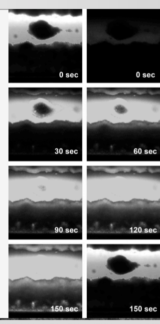


Davison et al (2010) *Antimicrob. Agents Chemother.* **54**:2920.

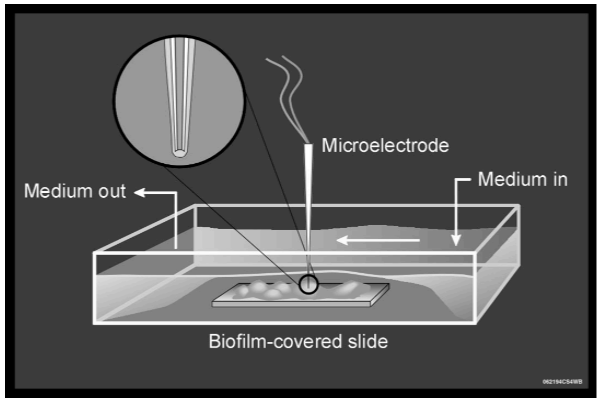
Daptomycin



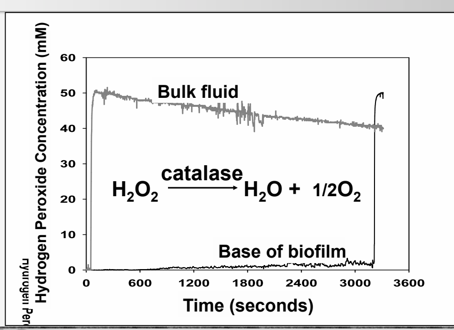
Daptomycin penetration in biofilm



Stewart et al (2009) *Antimicrob. Agents Chemother.* **53**:3505.



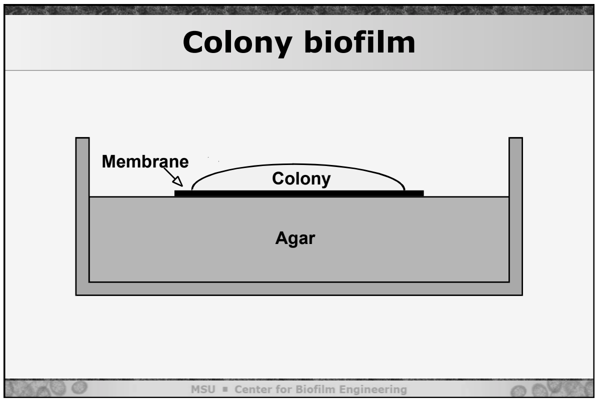
H_2O_2 fails to penetrate biofilm



Stewart et al (2000) *Appl. Environ. Microbiol.* **66**:836.

Antimicrobial penetration

- Antibiotics, peptides penetrate readily
- Reactive oxidants may fail to penetrate


Colony biofilm
Membrane
Colony
Agar
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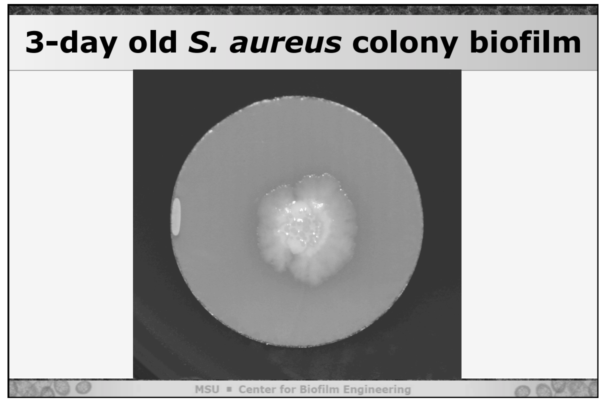
3-day old *S. aureus* colony biofilm
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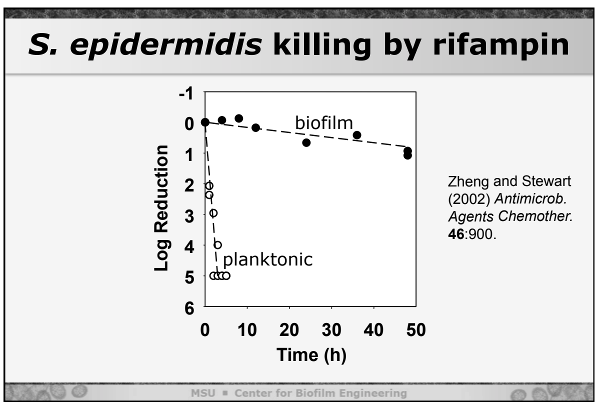
S. epidermidis killing by rifampin
Log Reduction
-1
0
1
2
3
4
5
6
biofilm
planktonic
0 10 20 30 40 50
Time (h)
Zheng and Stewart (2002) *Antimicrob. Agents Chemother.* **46**:900.
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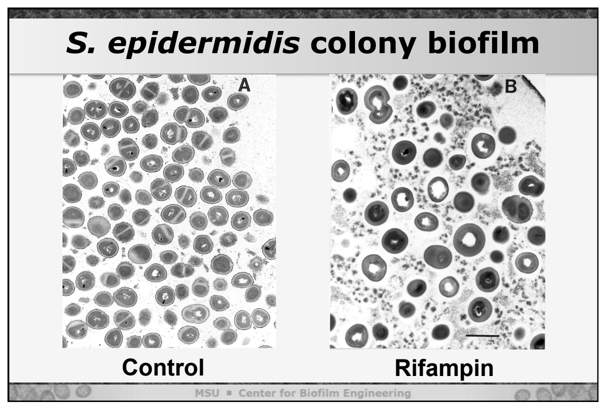
S. epidermidis colony biofilm
A
B
Control
Rifampin
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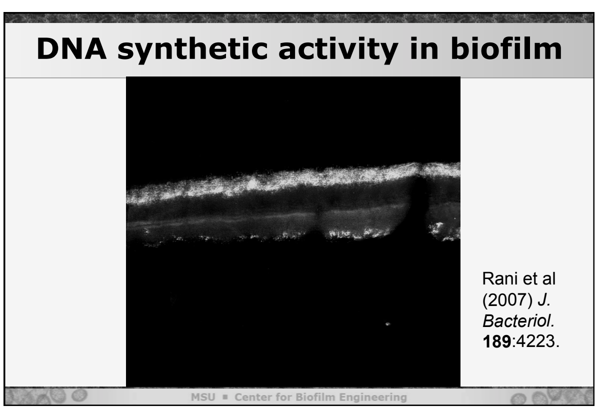
DNA synthetic activity in biofilm
Rani et al (2007) *J. Bacteriol.* **189**:4223.
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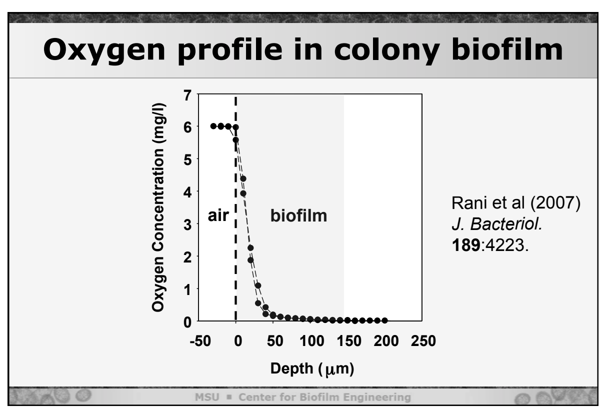
Oxygen profile in colony biofilm
Oxygen Concentration (mg/l)
0 1 2 3 4 5 6 7
air
biofilm
-50 0 50 100 150 200 250
Depth (μm)
Rani et al (2007) *J. Bacteriol.* **189**:4223.
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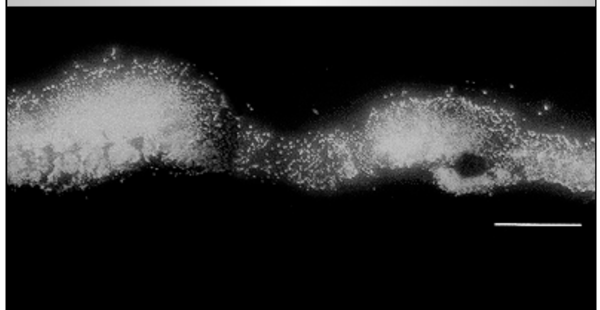
Physiological heterogeneity

- Growing aerobically
- Growing fermentatively
- Dead
- Dormant

K. pneumoniae biofilm - growth



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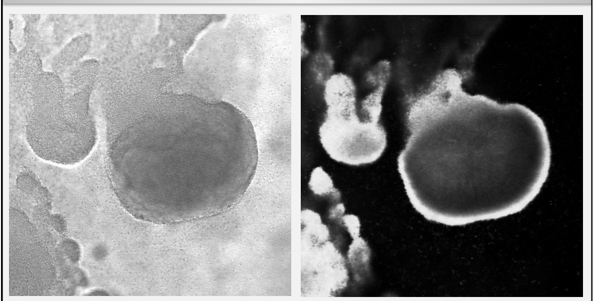
Protein synthetic activity in biofilm



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Pseudomonas aeruginosa biofilms Consensus genes - 10 studies

Transcriptomic	Proteomic
Whiteley 2001	Sauer 2002
Hentzer 2005	Seyer 2005
Waite 2006	Southey-Pillig 2005
Mikkelsen 2009	Mikkelsen 2007
Folsom 2010	Patrauchan 2007

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Top 26 genes in *Pseudomonas aeruginosa* biofilms

PA0139	PA1905	PA4236
PA0263	PA2274	PA4352
PA0515	PA2386	PA4610
PA0588	PA2782	PA5427
PA0713	PA3126	PA5460
PA1555	PA3309	PA5475
PA1556	PA3572	
PA1673	PA4067	
PA1746	PA4211	
PA1904	PA4217	

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Table 3. Activities or functions in *P. aeruginosa* biofilms as indicated by overlap with independently derived gene lists.

Pa biofilm consensus genes

Activity	P value
Oxygen downshift	~0
Oxygen limitation	$4x10^{-11}$
Stationary phase	$8x10^{-8}$
Phenazine biosynthesis	$2x10^{-6}$
Peroxide stress	0.02
------------------------------	---------
Iron limitation	0.25
HSL quorum sensing	0.29
Mg limitation	0.76
Osmotic stress	0.77
Efflux pumps	0.83
c-di-GMP	0.84
Nitrosative stress	0.88

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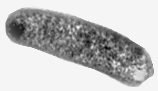
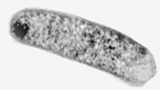
Table 3. Activities or functions in *P. aeruginosa* biofilms as indicated by overlap with independently derived gene lists.

Adaptive stress response



Antimicrobial exposure

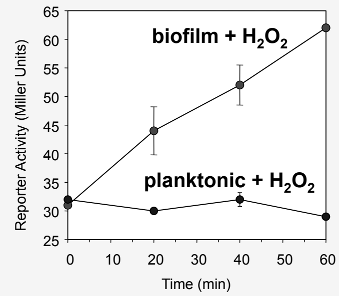


Unstressed, susceptible

Stress response induced, protected

Table 3. Activities or functions in *P. aeruginosa* biofilms as indicated by overlap with independently derived gene lists.

Catalase induction in Pa biofilm

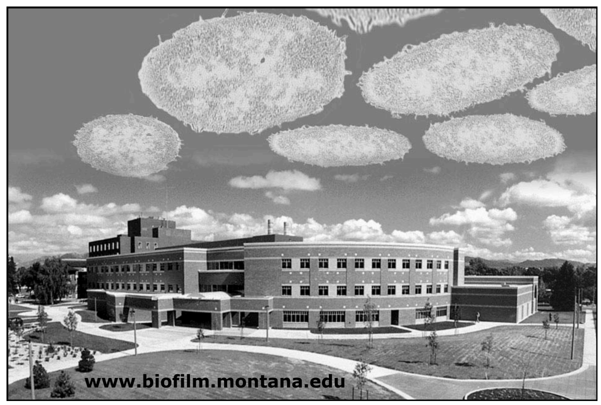


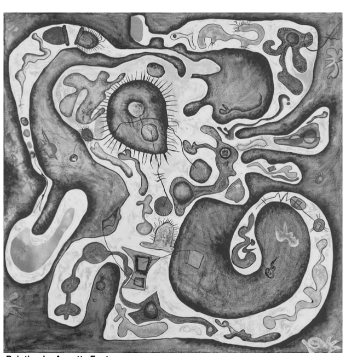
***katB*-lacZ**

Elkins et al (2000) *Appl. Environ. Microbiol.* **65**:4594

Summary

- Biofilms can cause chronic infections
- Bacteria in biofilms evade killing by host antimicrobials and antibiotics
- Multiple mechanisms of protection in biofilms





Painting by Annette Foster

2695_99cs

01 March	**Developing a Sustainable and Effective Approach to Hygiene and Infection Prevention in Home and Everyday Life Settings** Speaker: Dr. Sally Bloomfield, International Scientific Forum on Home Hygiene
07 March	***(FREE … WHO Teleclass - Europe)* Achievements in Improving Injection Safety Worldwide** Speaker: Prof. Chuck Gerba, University of Arizona Sponsor: World Health Organization First Global Patient Safety Challenge
22 March	**Hand Hygiene: New Frontiers in Messaging and Measurement** Speaker: Dr. Katherine Ellingson, Centers for Disease Control Sponsor: Diversey Inc.
29 March	**Water and Infection Control** Speaker: Andrew Streifel, University of Minnesota

www.webbertraining.com/schedulep1.php