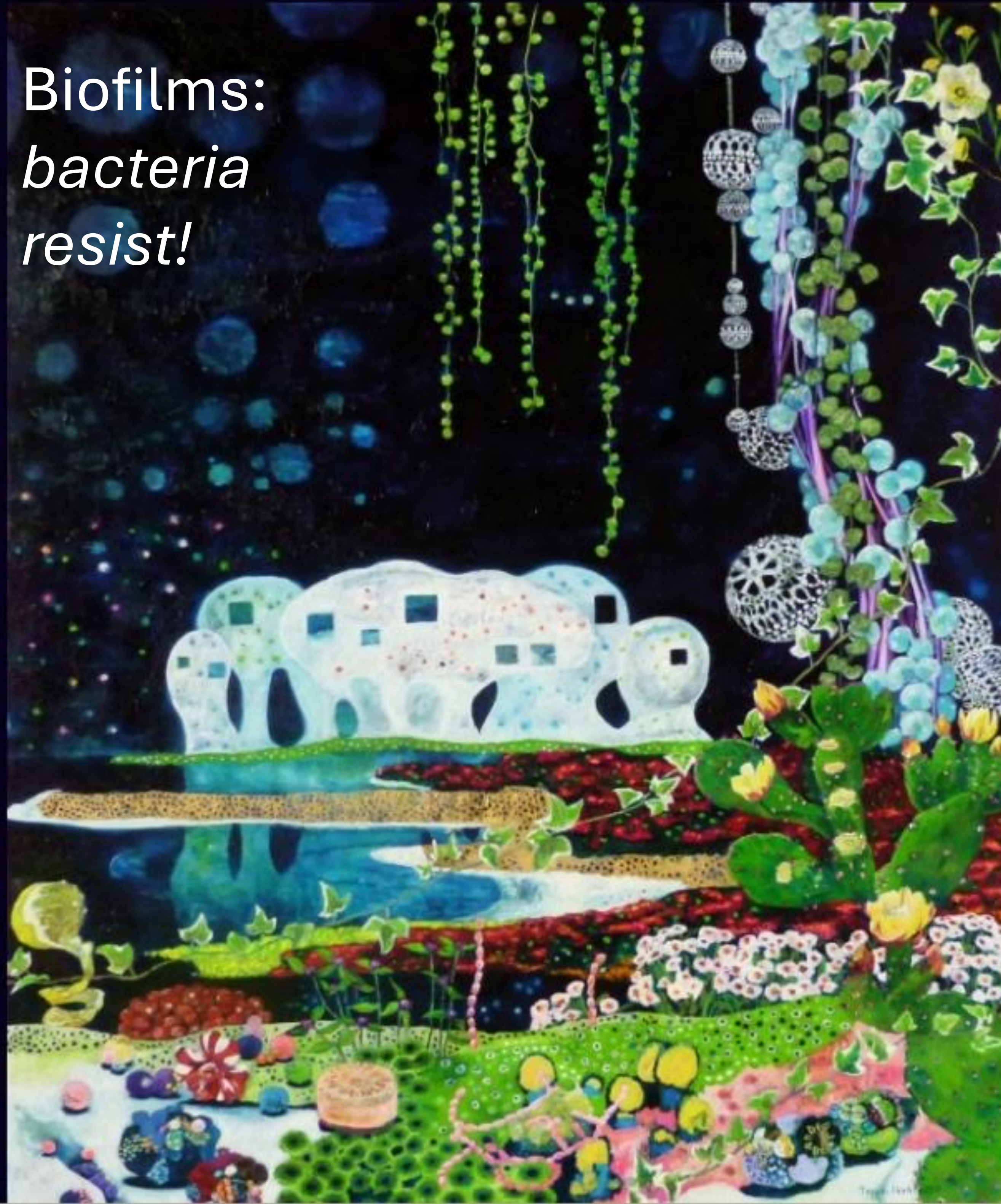
A1 RB-2 : « Tolérance des biofilms : défis et stratégies de contrôle innovantes »

Breaking the biofilm barrier

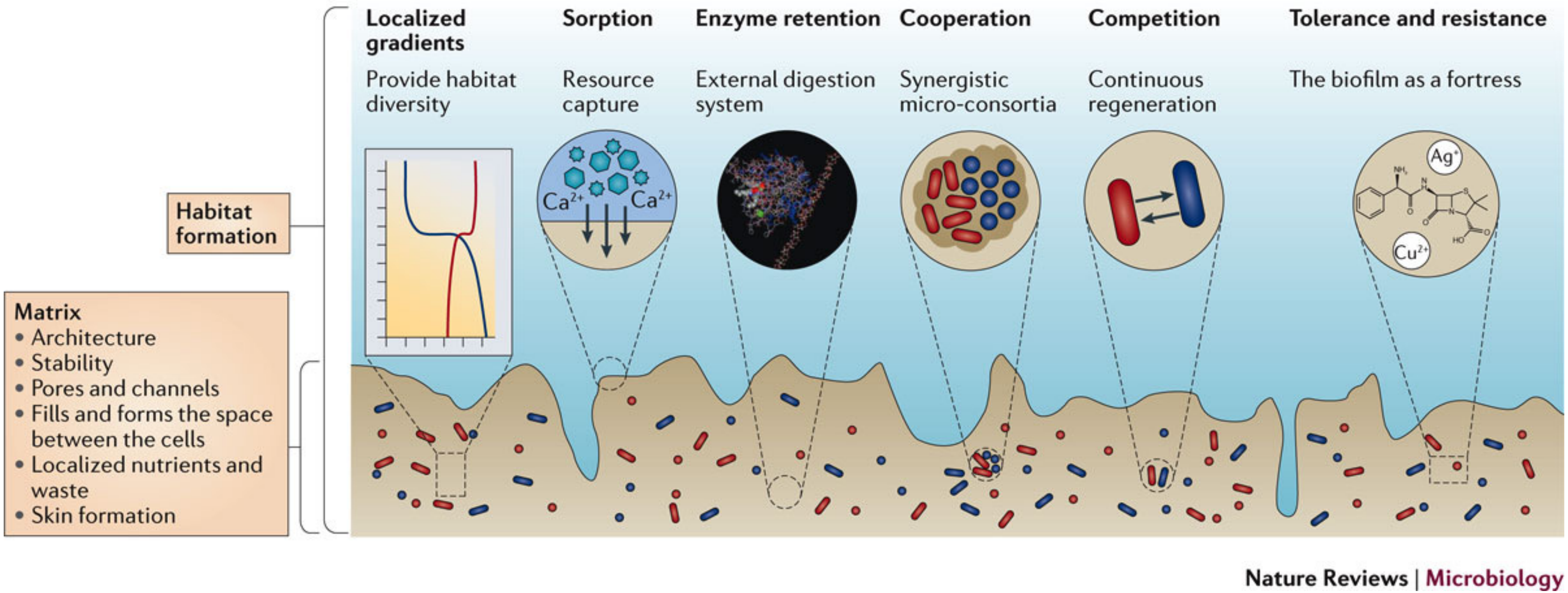
Failures, limits and innovative control approaches

Biofilms:
bacteria resist!

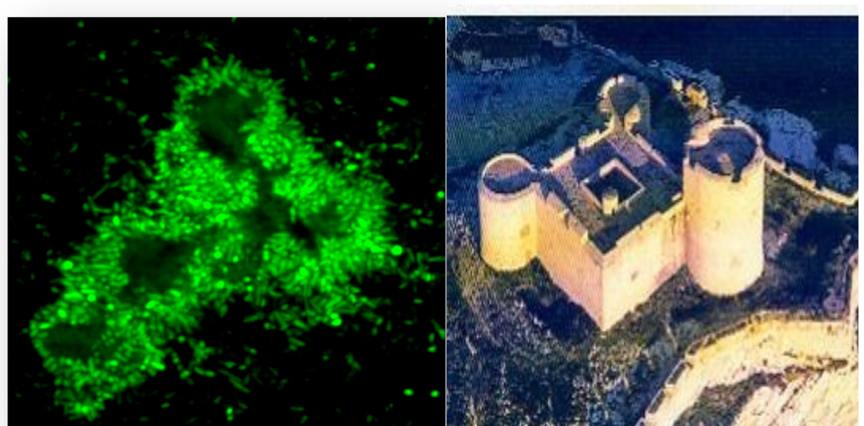


Teppei Ikehila, Tokyo

EPS and emergent functions of biofilms

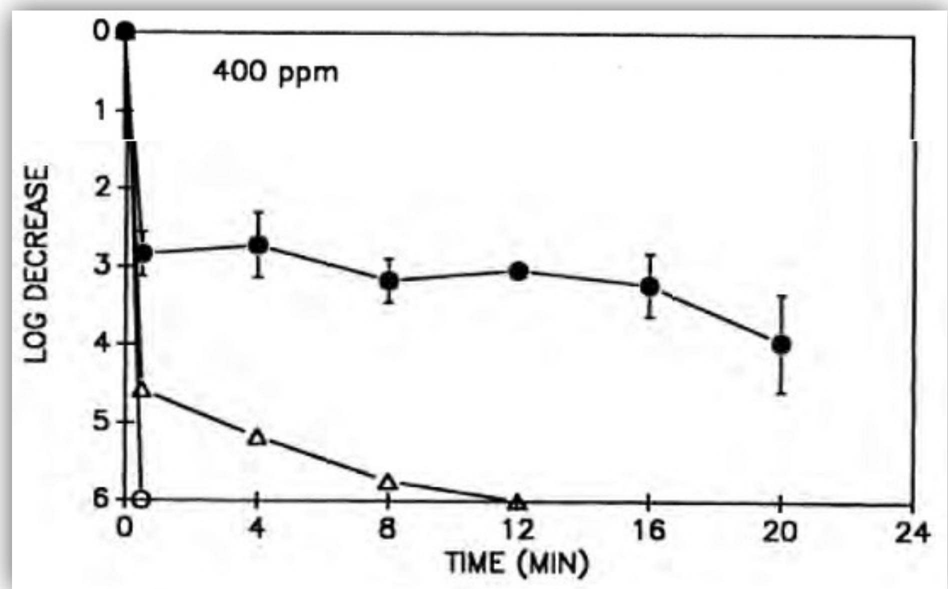


Biofilm as a bacterial fortresses



Disinfectants	Ratio of active concentration between biofilms and free cells
Oxidizing Agents	5 - 600
Quaternary Ammonium	10 - 1000

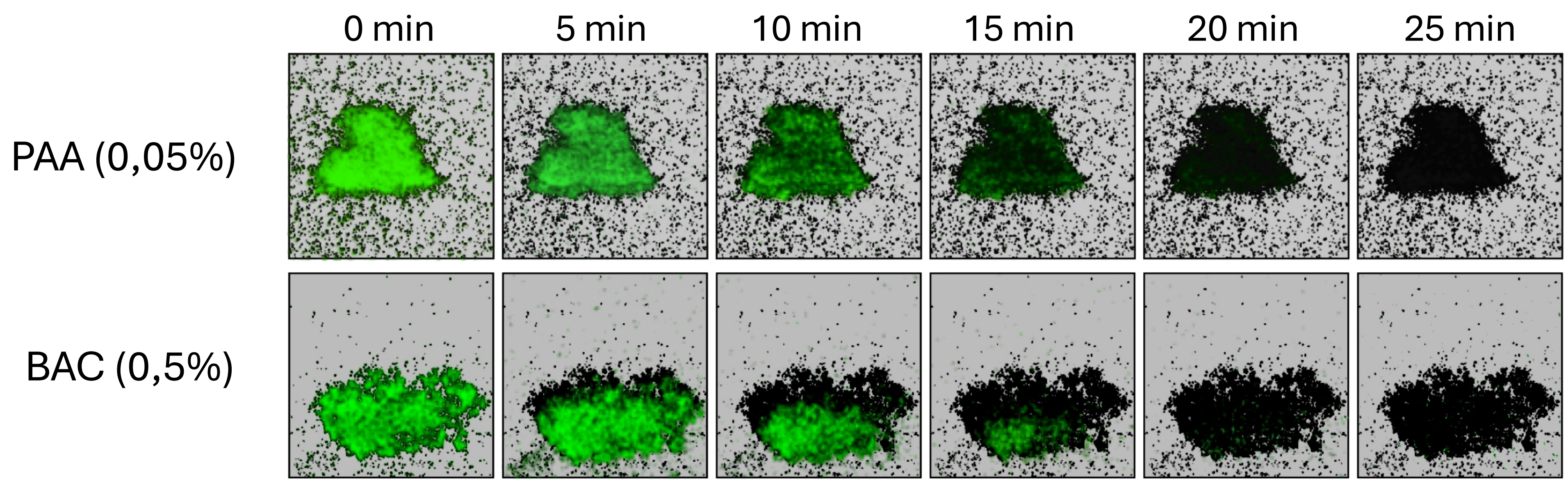
Frank and Koffi ,1990

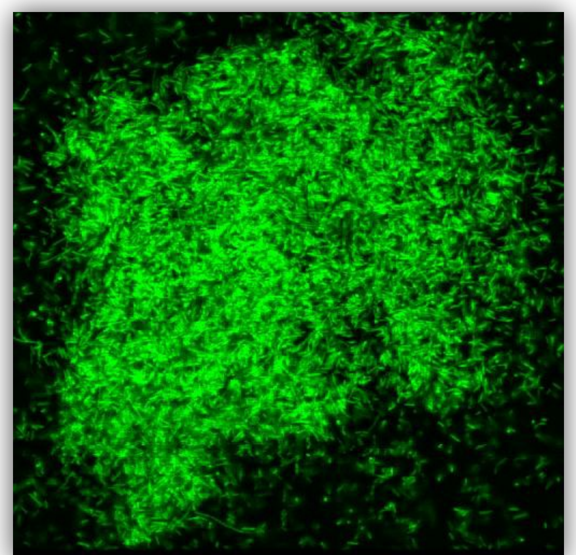


Frank and Koffi ,1990

Activity of BAC on *Listeria monocytogenes*
free cells (O),
adherent cells (∆),
biofilm cells (●)

Direct visualisation of biofilms inactivation

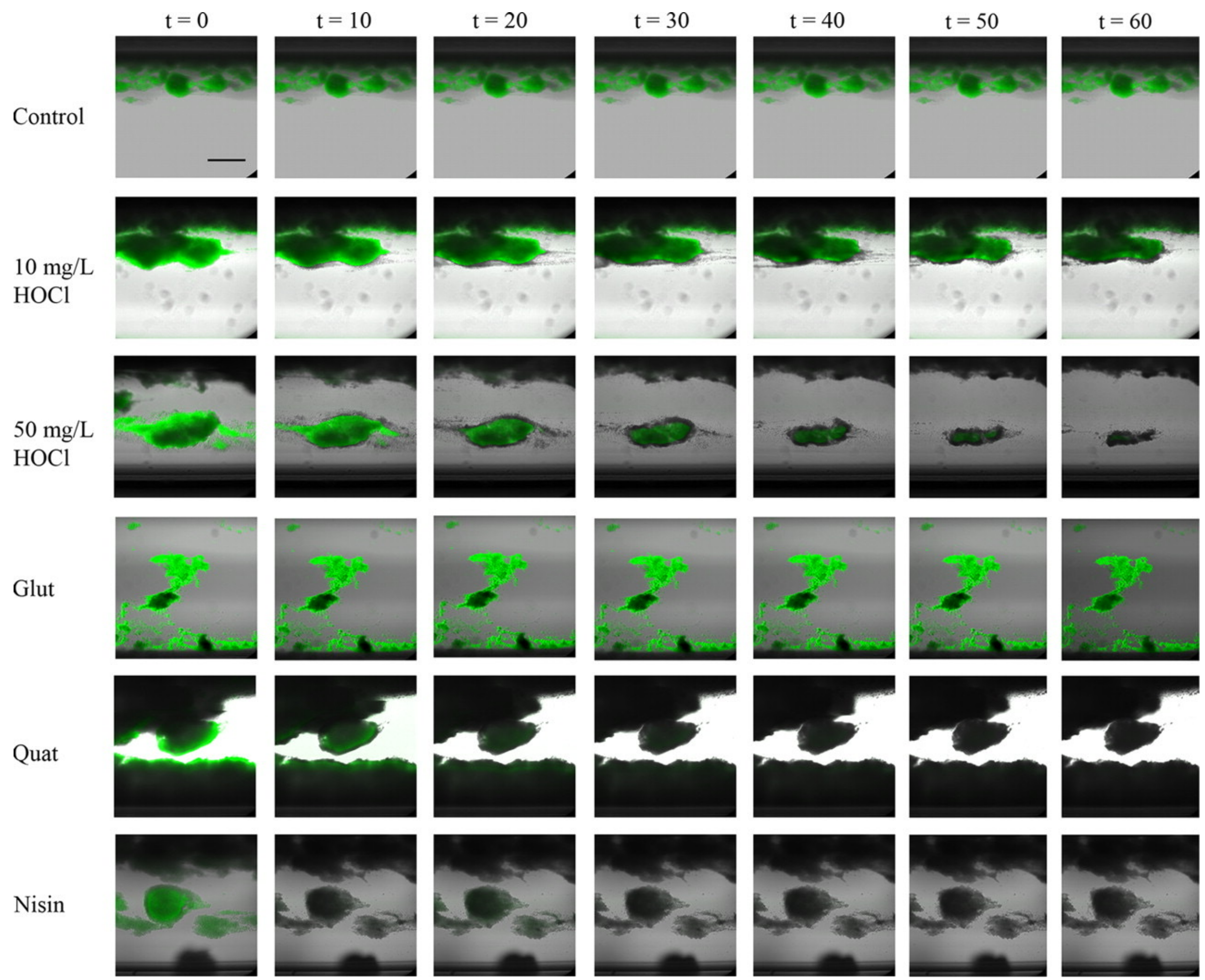




BAC action on *Pseudomonas aeruginosa* Laus 3 biofilm

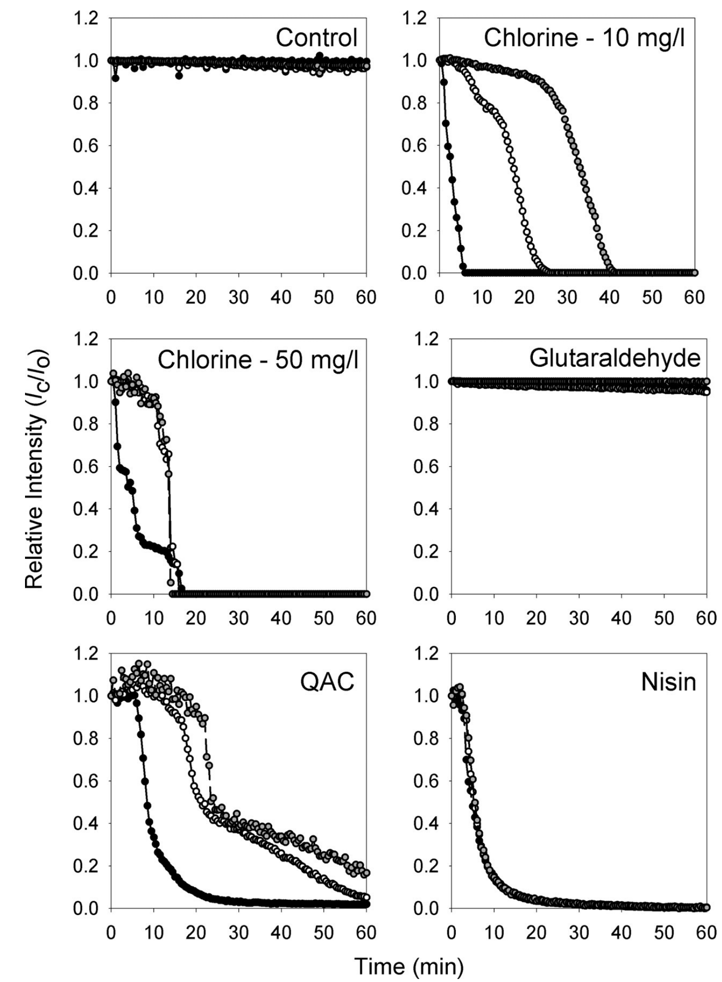
Bridier *et al*. 2011, *AAC*

Direct visualisation of biofilms inactivation



Davison et al., Antimicrobial Agents and Chemotherapy, 2010

Biofilms of *Staphylococcus epidermidis* labelled with Calcein-AM



Periphery zone = black
Median zone = white
Internal zone= grey

Diffusion-reaction limitation in biofilms?

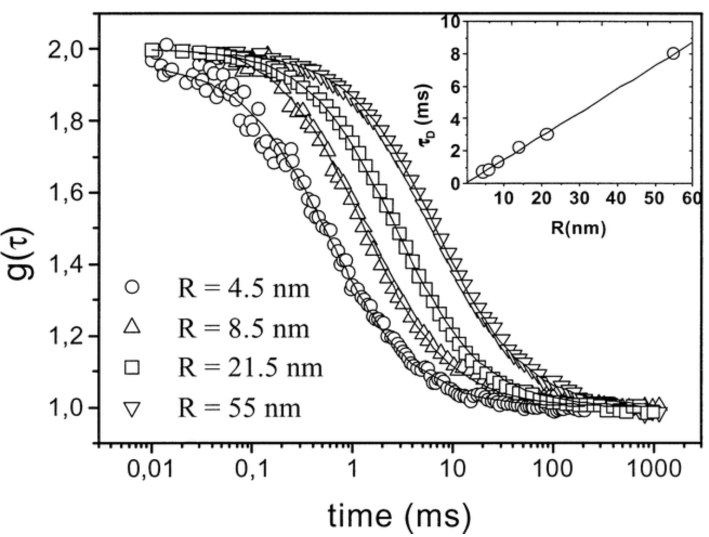
Photochemistry and Photobiology, 2002, 75(6): 570–578

Heterogeneity of Diffusion Inside Microbial Biofilms Determined by Fluorescence Correlation Spectroscopy Under Two-photon Excitation¶

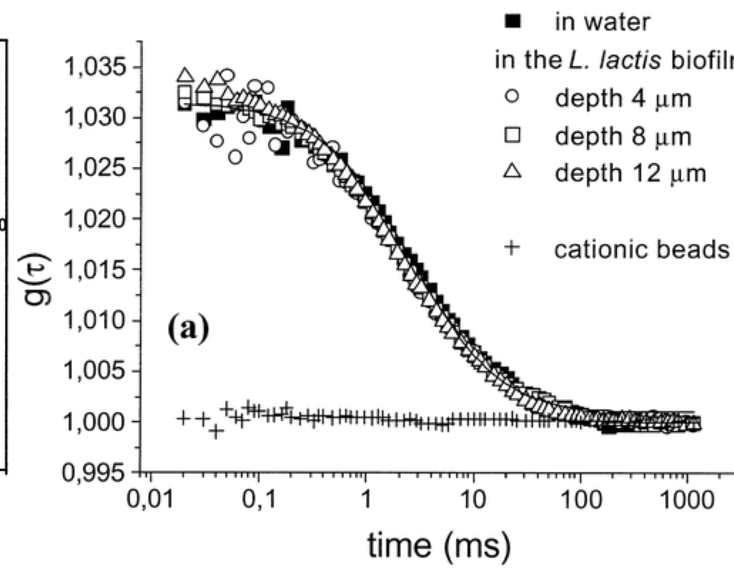
E. Guiot[1], P. Georges[1], A. Brun[1], M. P. Fontaine-Aupart*[2], M. N. Bellon-Fontaine[3] and R. Briandet[3]

[1]Laboratoire Charles Fabry de l'Institut d'Optique, UMR 8501, Orsay, France
[2]Laboratoire de Photophysique Moléculaire, UPR 3361, Orsay, France and
[3]INRA, Unité de Recherche en Bioadhésion et Hygiène des Matériaux, Massy, France

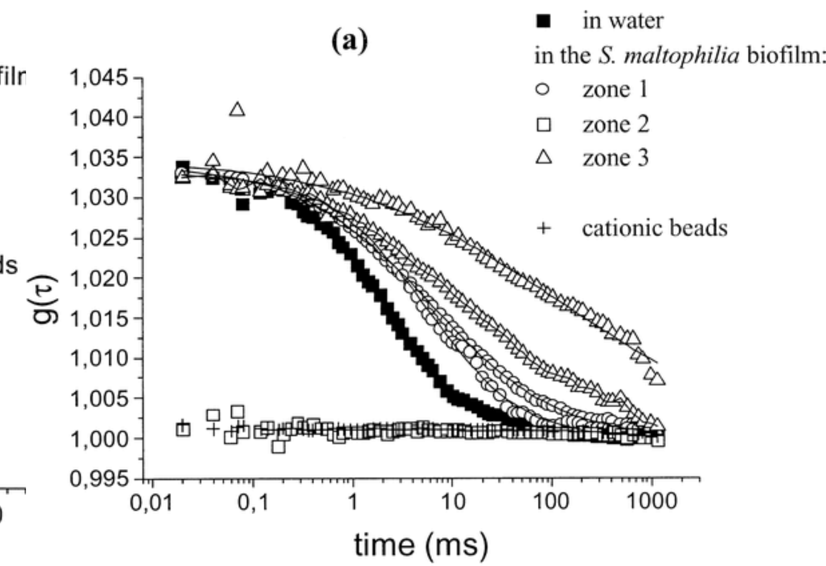
Nanospheres size

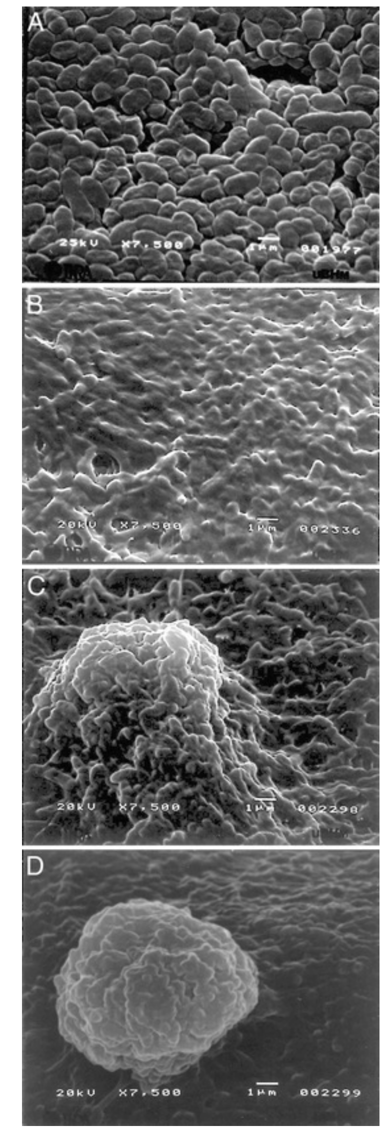


Nanospheres charge in Lactococcus lactis biofilm



Nanospheres charge in Lactococcus lactis biofilm





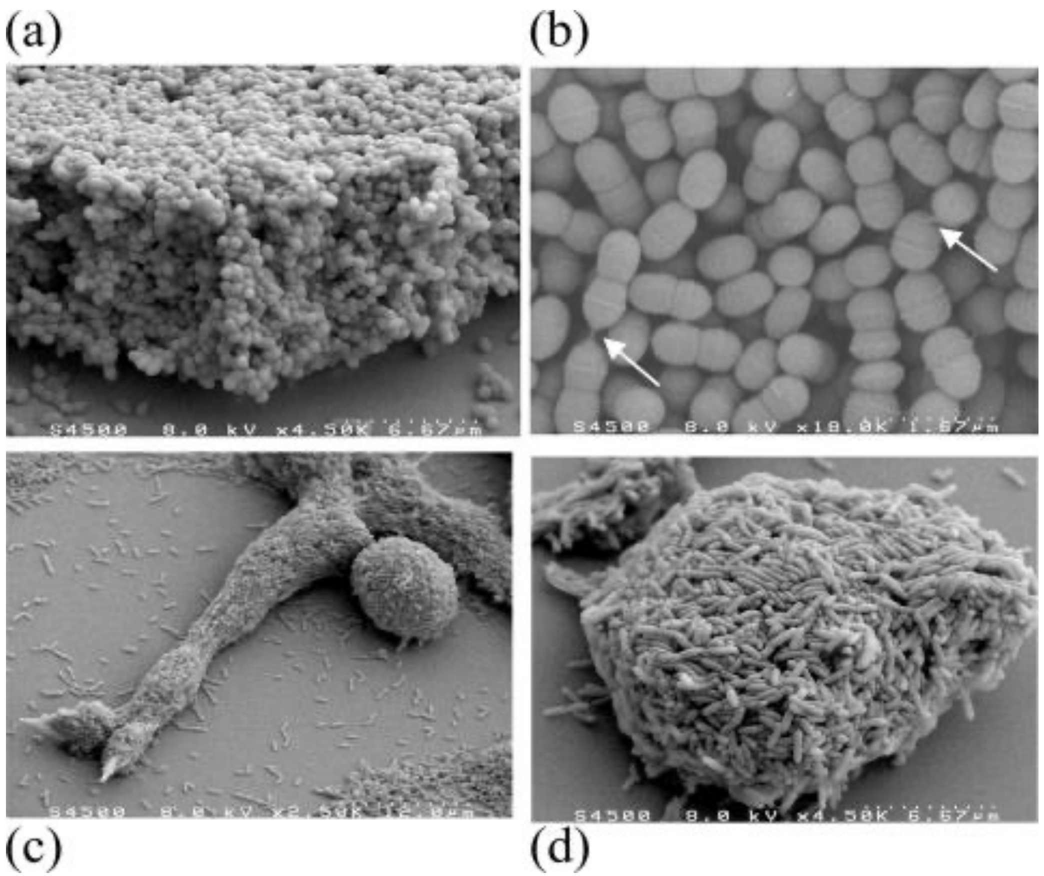
>> Size and charge matter

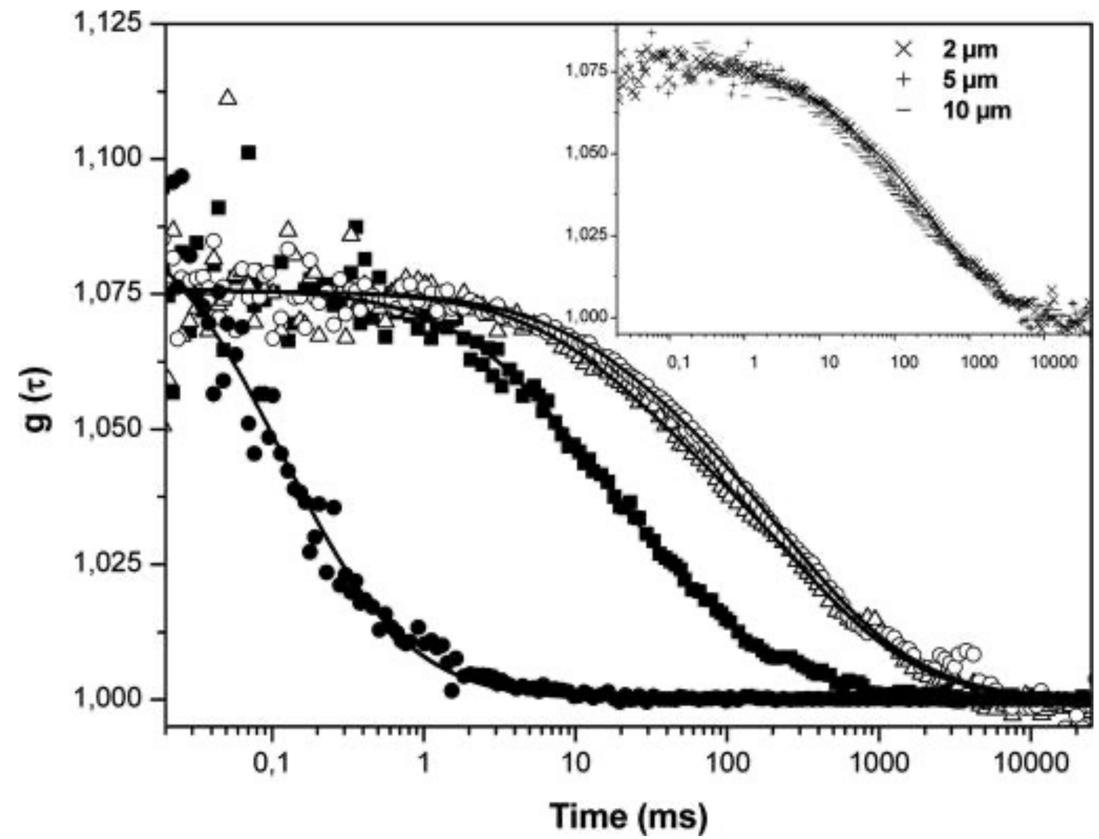
Diffusion-reaction limitation in biofilms?

Applied and Environmental Microbiology®
ENVIRONMENTAL MICROBIOLOGY
April 1, 2008 Volume 74 Issue 7
https://doi.org/10.1128/AEM.02304-07

Fluorescence Correlation Spectroscopy To Study Diffusion and Reaction of Bacteriophages inside Biofilms

R. Briandet[1,5,*], P. Lacroix-Gueu[2], M. Renault[1,5], S. Lecart[3], T. Meylheuc[1,5], E. Bidnenko[4], K. Steenkeste[2], M.-N. Bellon-Fontaine[1,5], M.-P. Fontaine-Aupart[2]





>> even viral particles can diffuse inside in a bifoilm matrix

Diffusion-reaction limitation in biofilms?

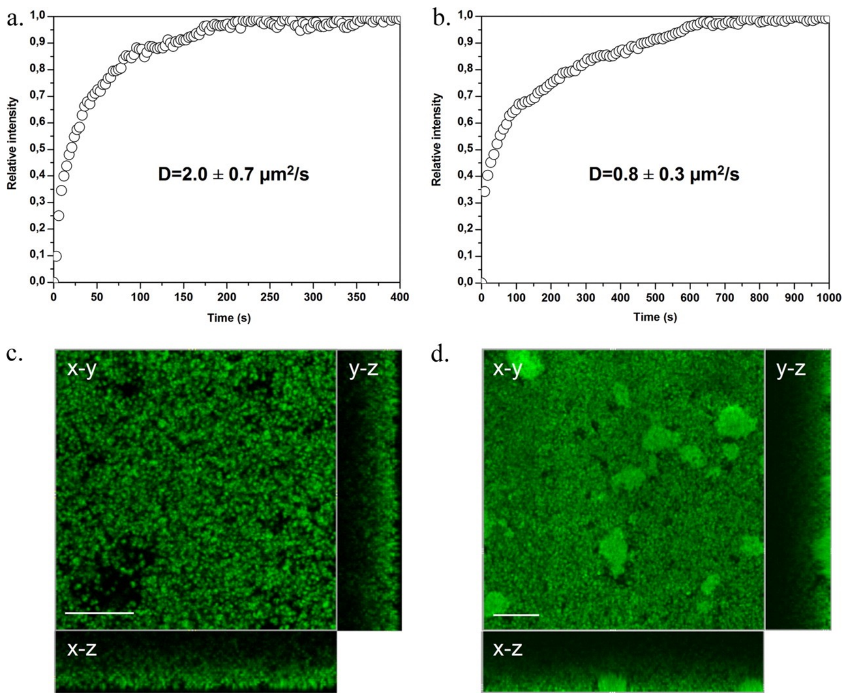
AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

MECHANISMS OF RESISTANCE
June 2012 Volume 56 Issue 6
https://doi.org/10.1128/aac.00216-12

Correlative Time-Resolved Fluorescence Microscopy To Assess Antibiotic Diffusion-Reaction in Biofilms

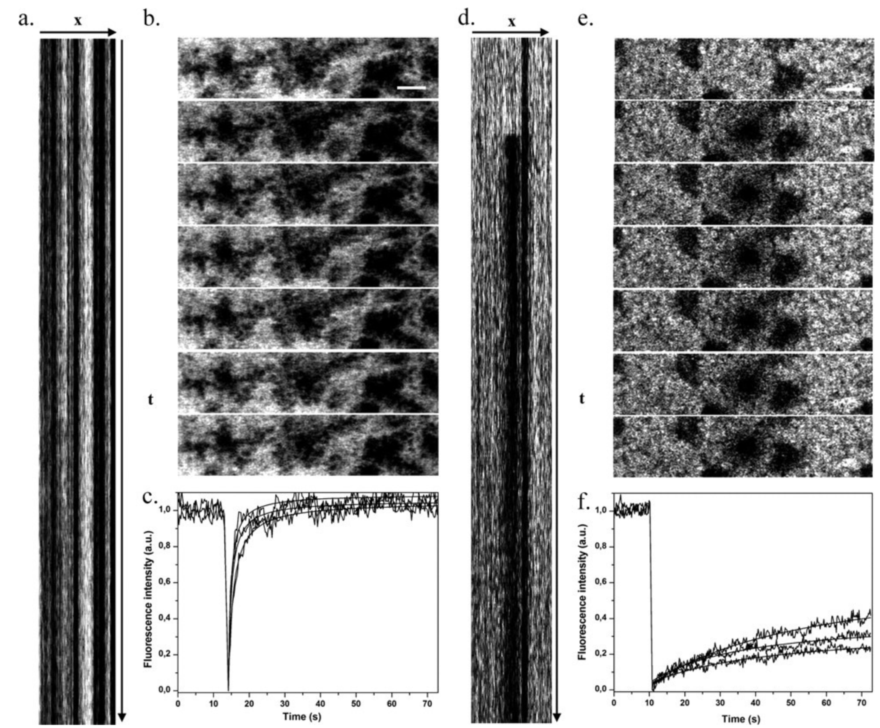
S. Daddi Oubekka[a,b,c], R. Briandet[d,e], M.-P. Fontaine-Aupart[a,b,c], K. Steenkeste[a,b,c]

Time-Lapse (bodipy-vancomycin)



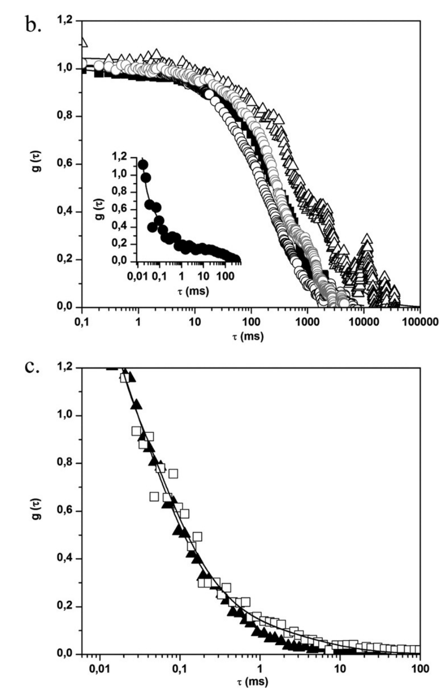
Staphylococcus aureus biofilm

FRAP



Staphylococcus aureus biofilm

FCS

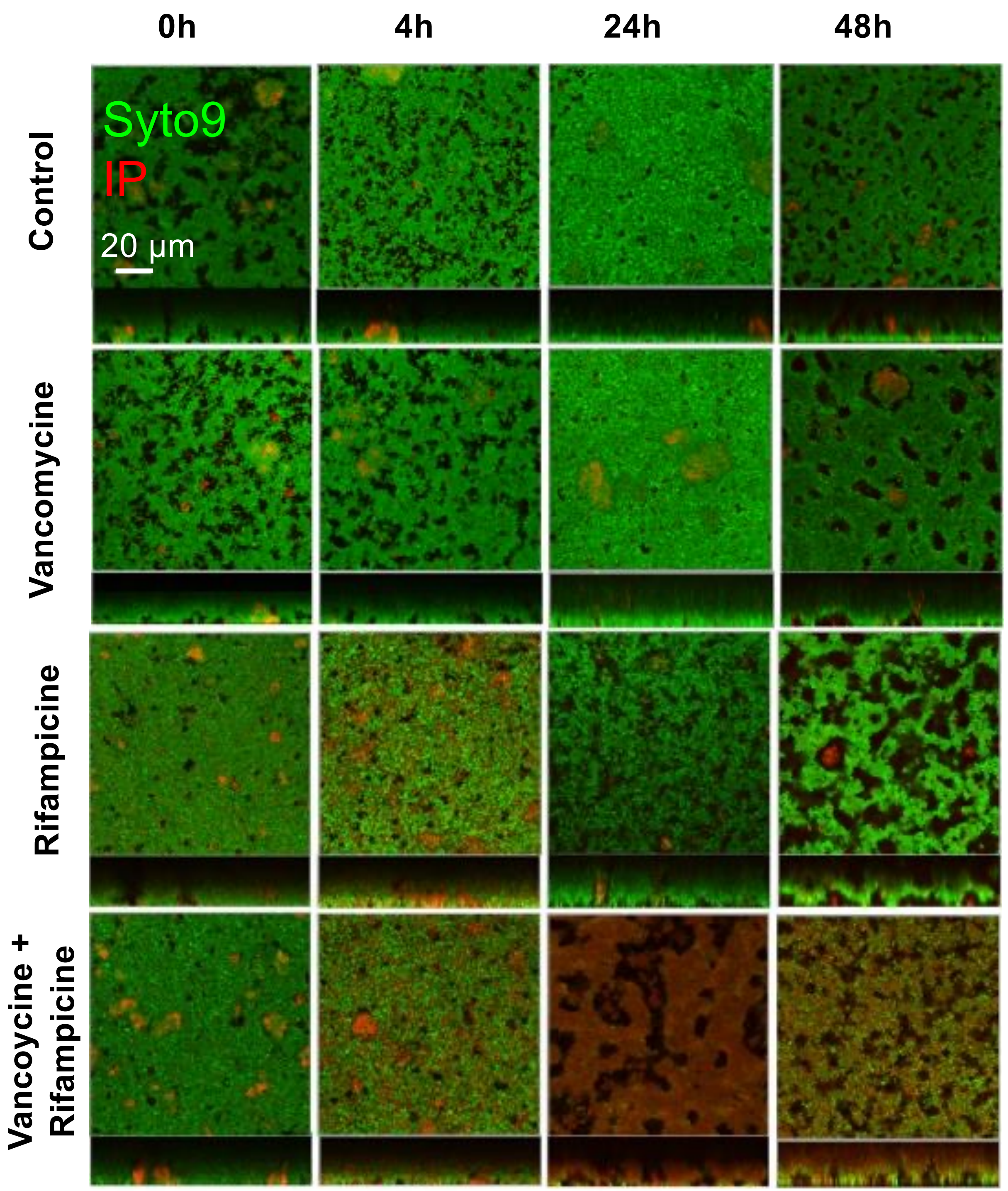


Staphylococcus aureus biofilm

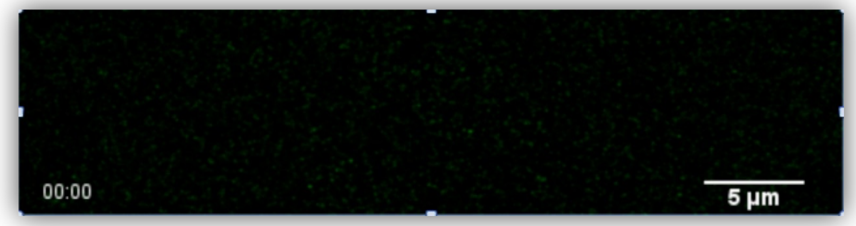
Pseudomonas aeruginosa biofilm (unsensitive strain)

>> at therapeutic concentrations of vancomycin, the biofilm was not an obstacle to the diffusion-reaction of the antibiotic

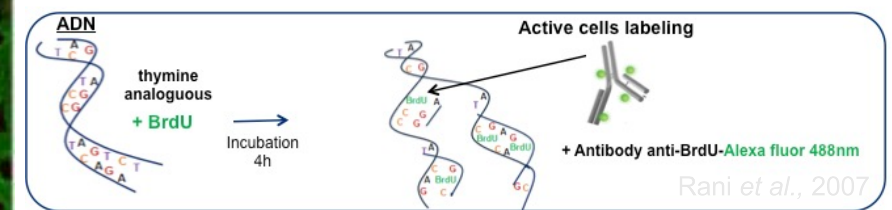
Visualisation of antibiotics action in *S. aureus* biofilms

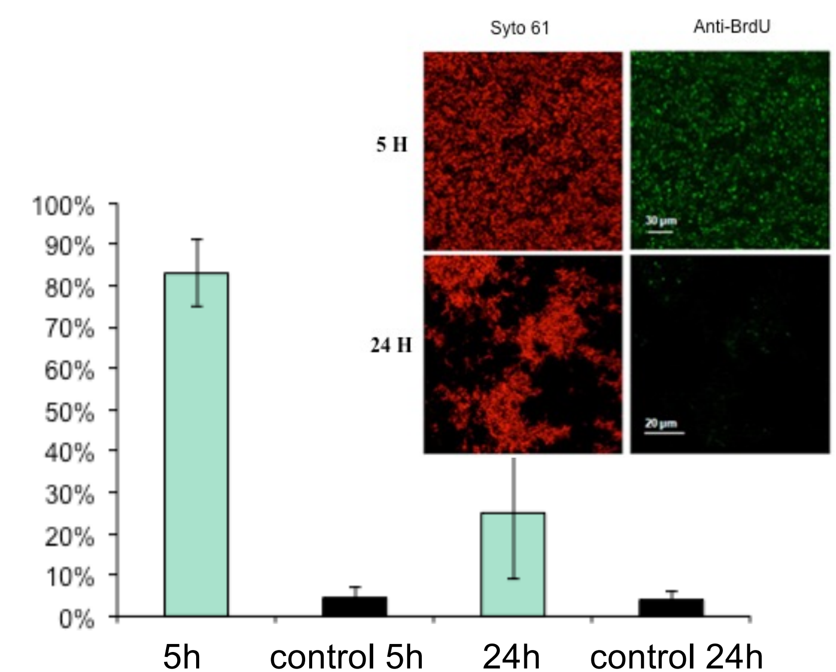


Vancomycin-Bodipy in *S.aureus* biofilm



Synthesis activity in *S.aureus* biofilm

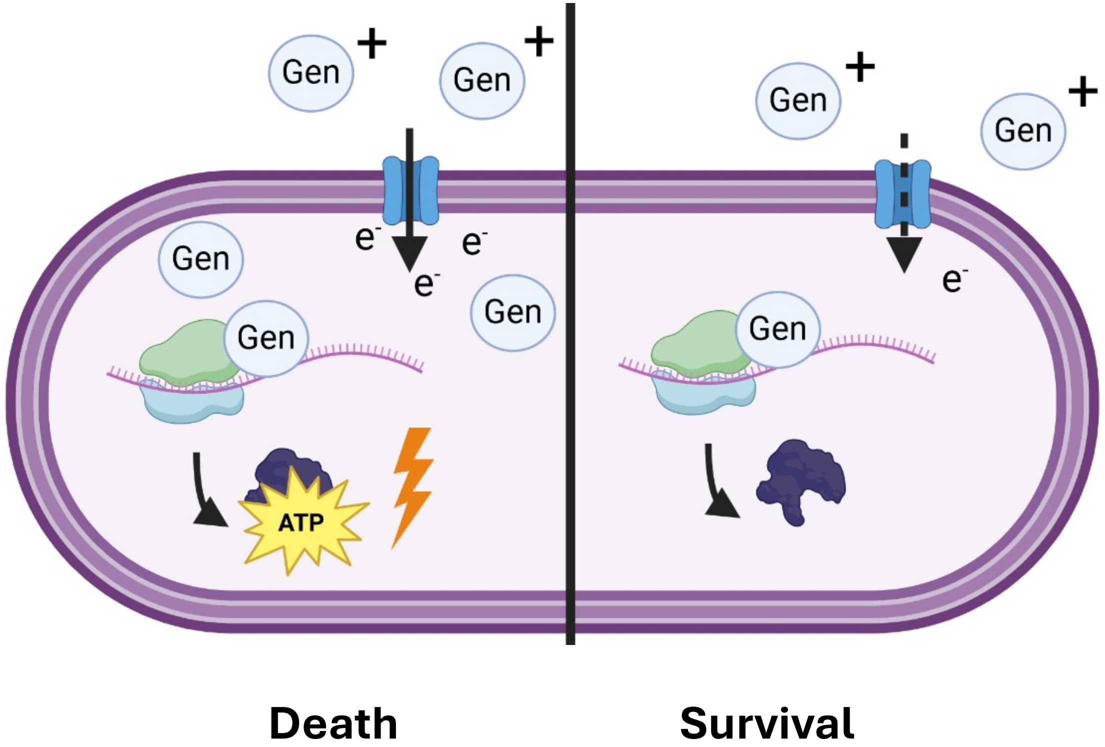


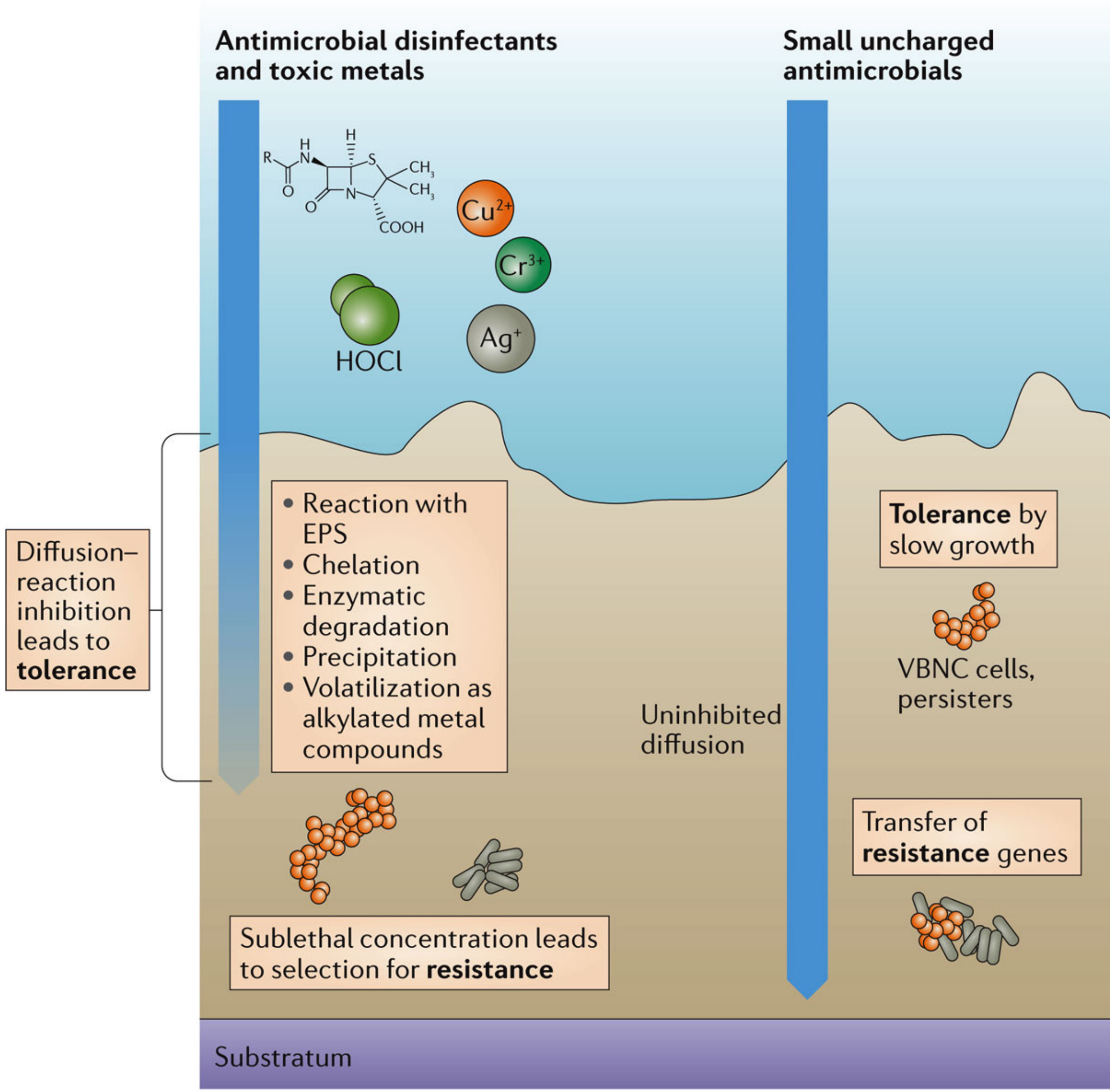


Daddi Oubekka 2012

Slow-growth and antibiotic action in biofilms

Uptake of cationic antimicrobials requires electron transport activity and metabolically active targets

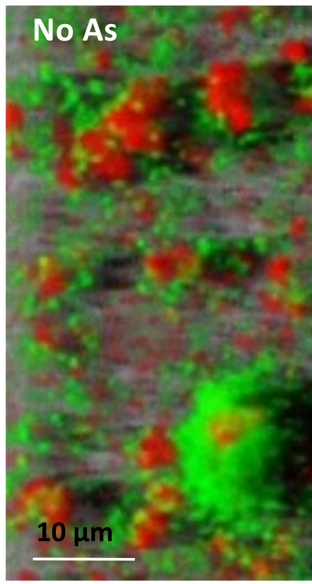


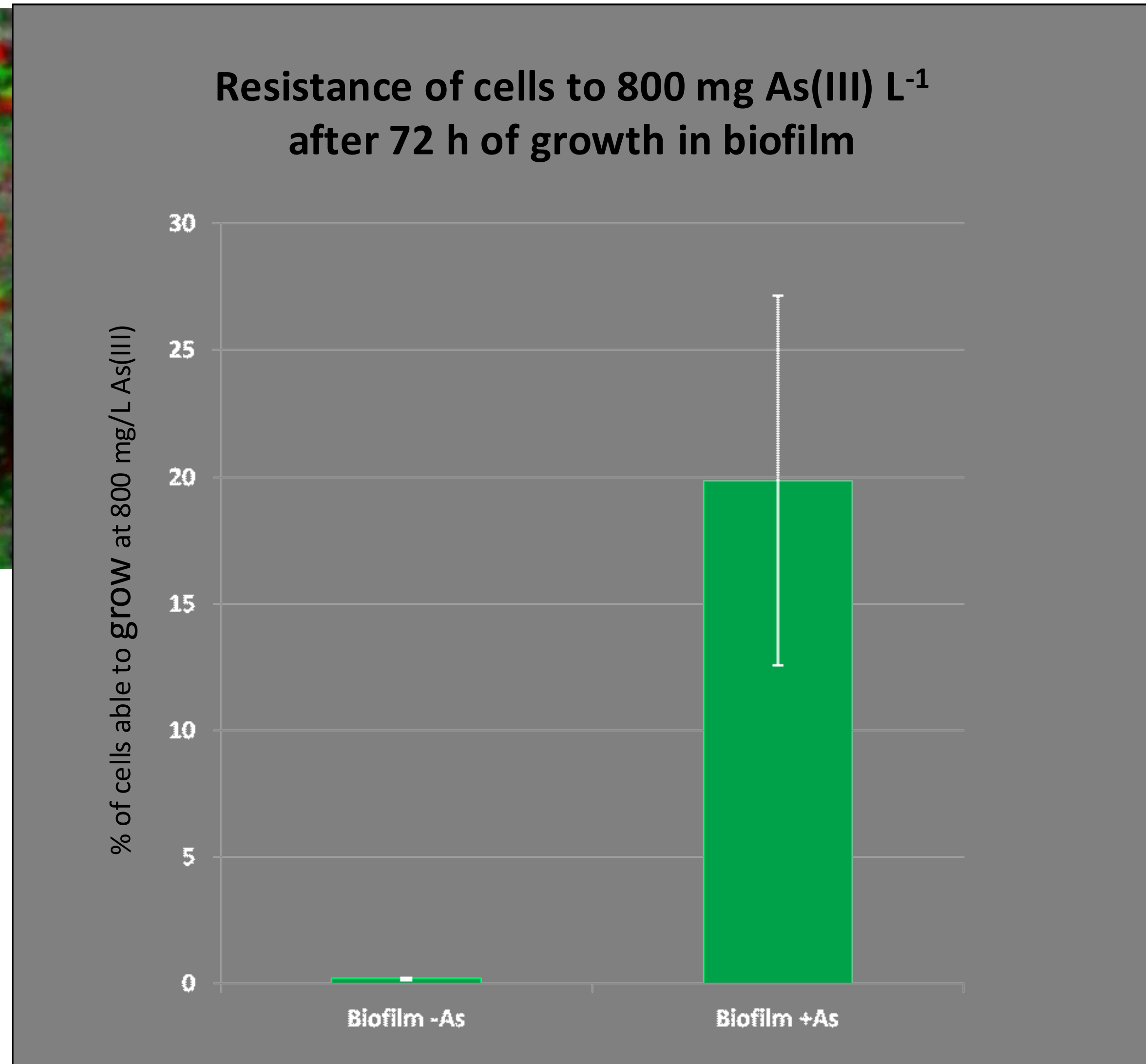


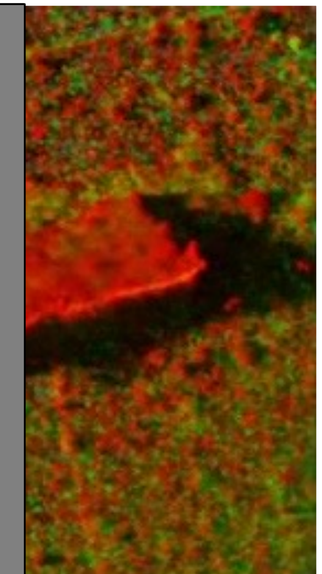
Antimicrobial disinfectants and toxic metals
Small uncharged antimicrobials
H
R
N
H
S
CH3
CH3
O
O
N
COOH
Cu2+
Cr3+
Ag+
HOCl
Diffusion–reaction inhibition leads to tolerance
• Reaction with EPS
• Chelation
• Enzymatic degradation
• Precipitation
• Volatilization as alkylated metal compounds
Uninhibited diffusion
Tolerance by slow growth
VBNC cells, persisters
Transfer of resistance genes
Sublethal concentration leads to selection for resistance
Substratum

Biofilms adaptation to toxic compounds

The case of Thiomonas spp. and arsenic





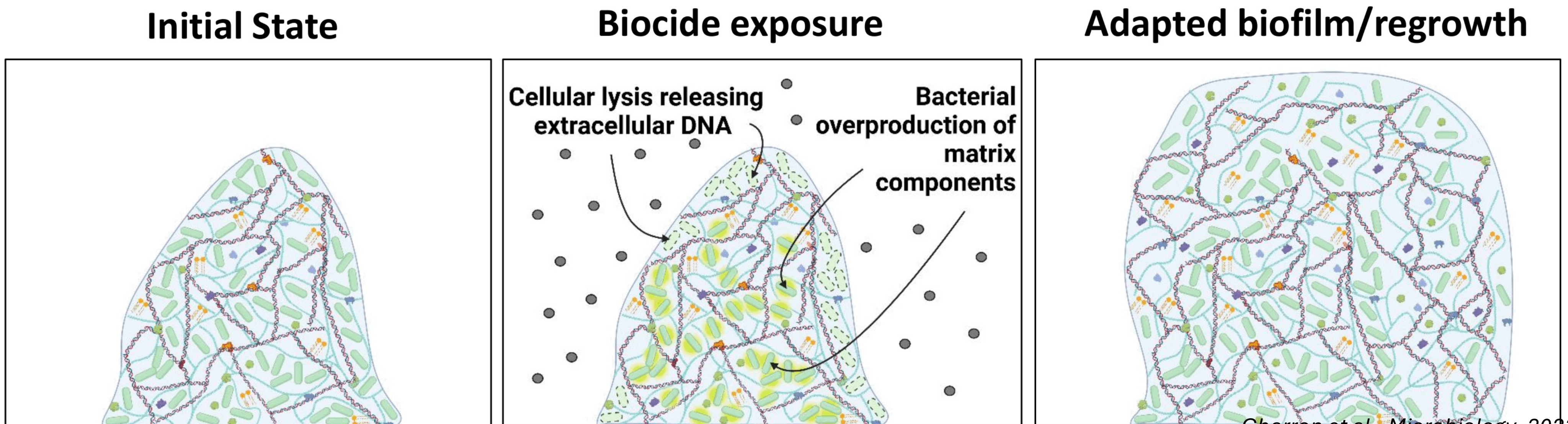


YTO9)

ysaccharides (ConA)

Marchal et al. Plos One 2011

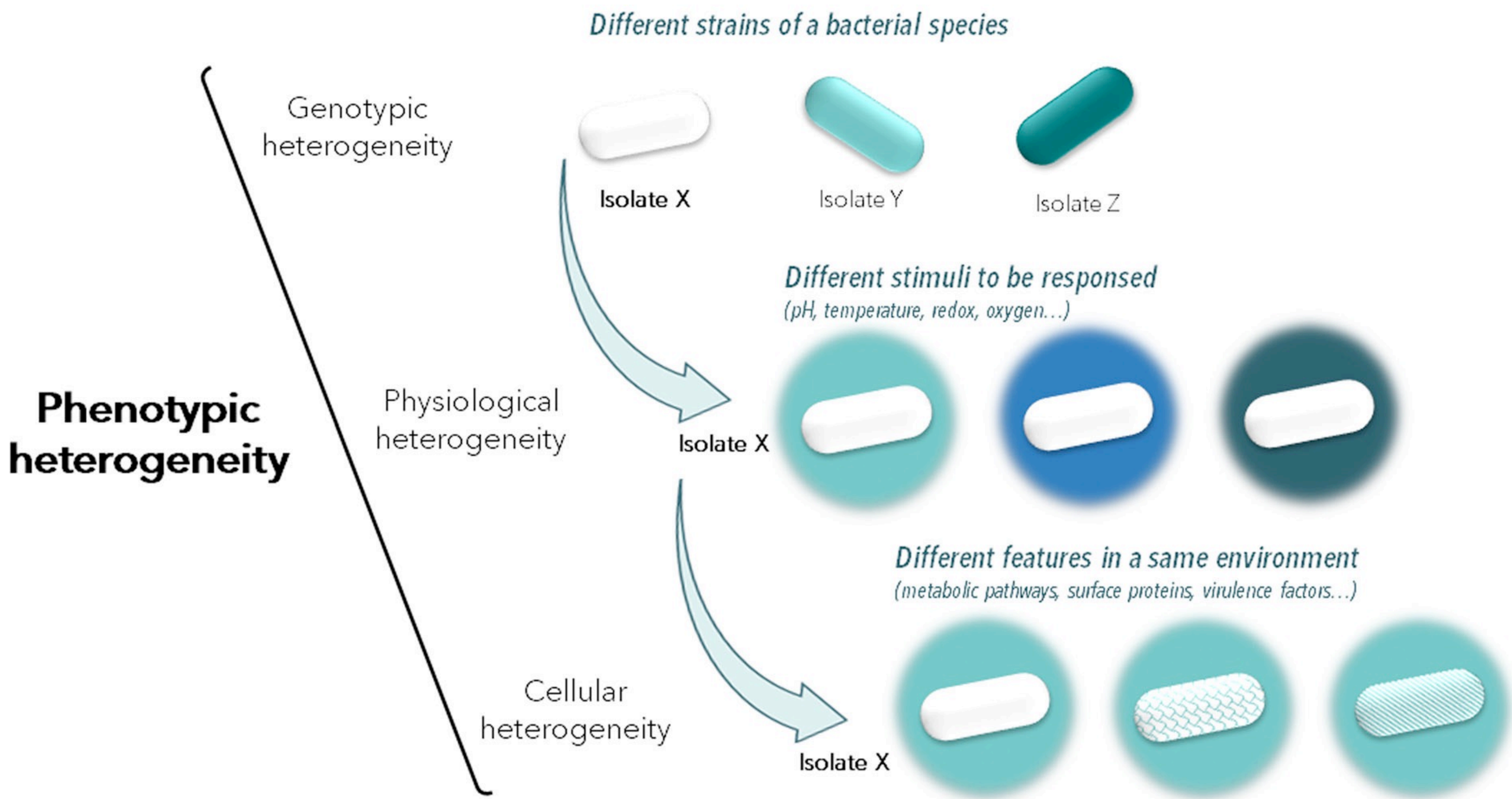
Adaptation related to matrix over-production



Charron et al., Microbiology, 2023

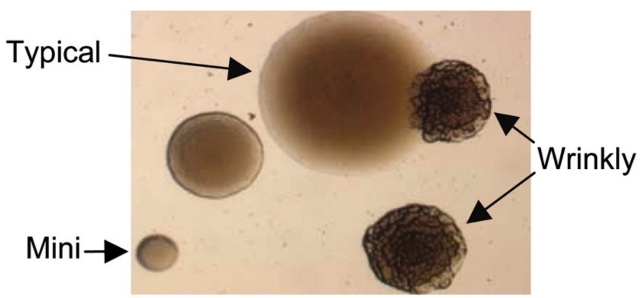
Structural Modulation

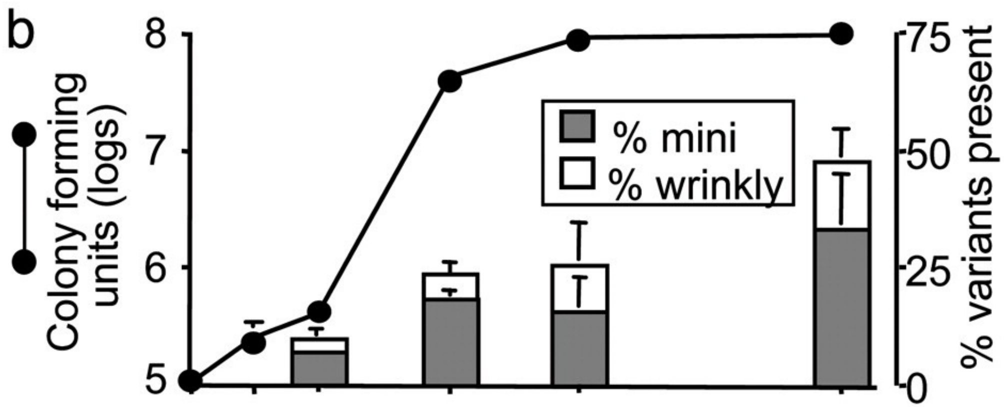
The different levels of phenotypic heterogeneity for a bacterial species

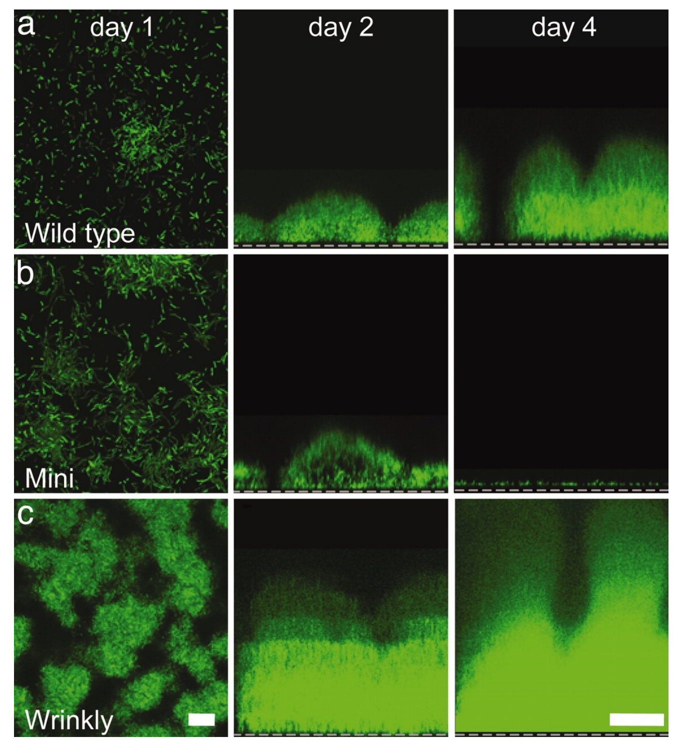


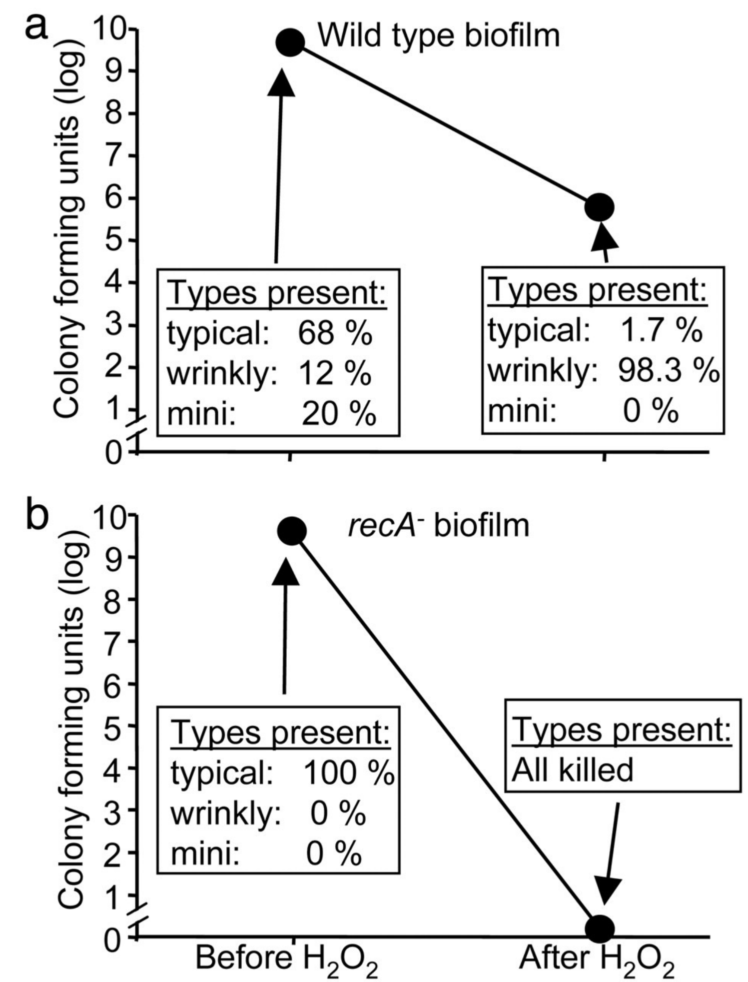
DOI: 10.1111/1541-4337.13020

Self-generated cellular heterogeneity produces "insurance effects" in biofilm communities









Boles et al., PNAS, 2004

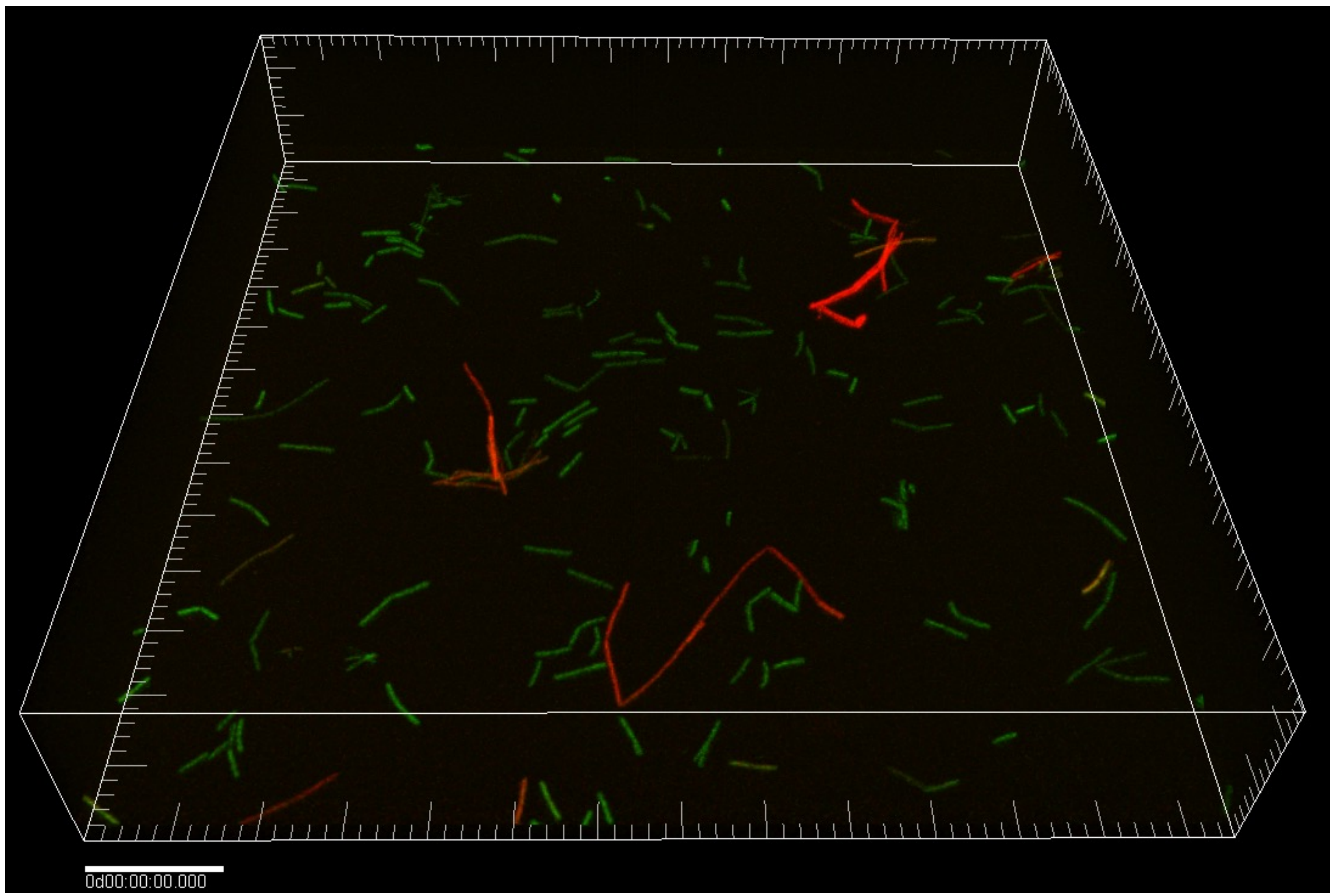
Physiological heterogeneity within biofilms

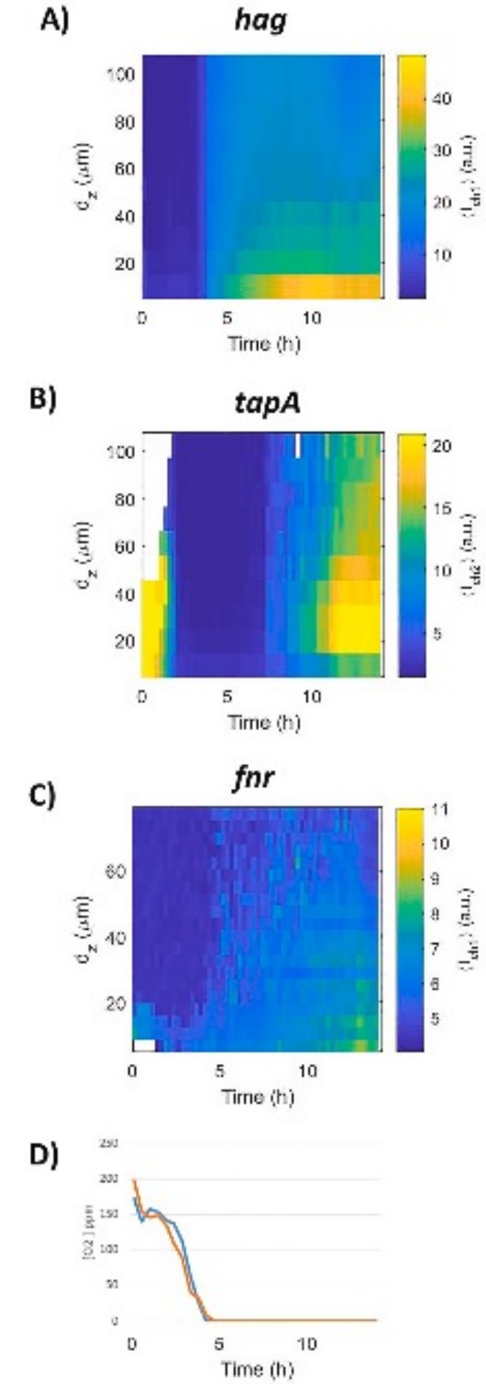
Contents lists available at ScienceDirect
Biofilm
journal homepage: www.sciencedirect.com/journal/biofilm

The coordinated population redistribution between *Bacillus subtilis* submerged biofilm and liquid-air pellicle☆

Pilar Sanchez-Vizuete[a,1], Yasmine Dergham[a,b], Arnaud Bridier[c], Julien Deschamps[a], Etienne Dervyn[a], Kassem Hamze[b], Stéphane Aymerich[a], Dominique Le Coq[a,d], Romain Briandet[a,*]

B.subtilis Phag-GFP PtapA-mKate2





Physiological heterogeneity within biofilms

nature communications

Article

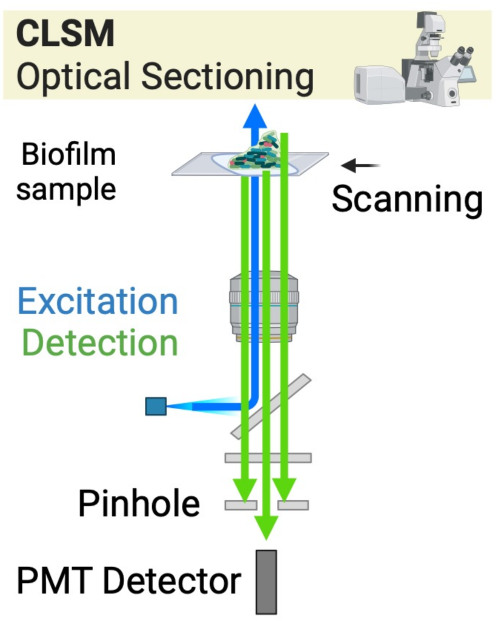
https://doi.org/10.1038/s41467-023-43386-w

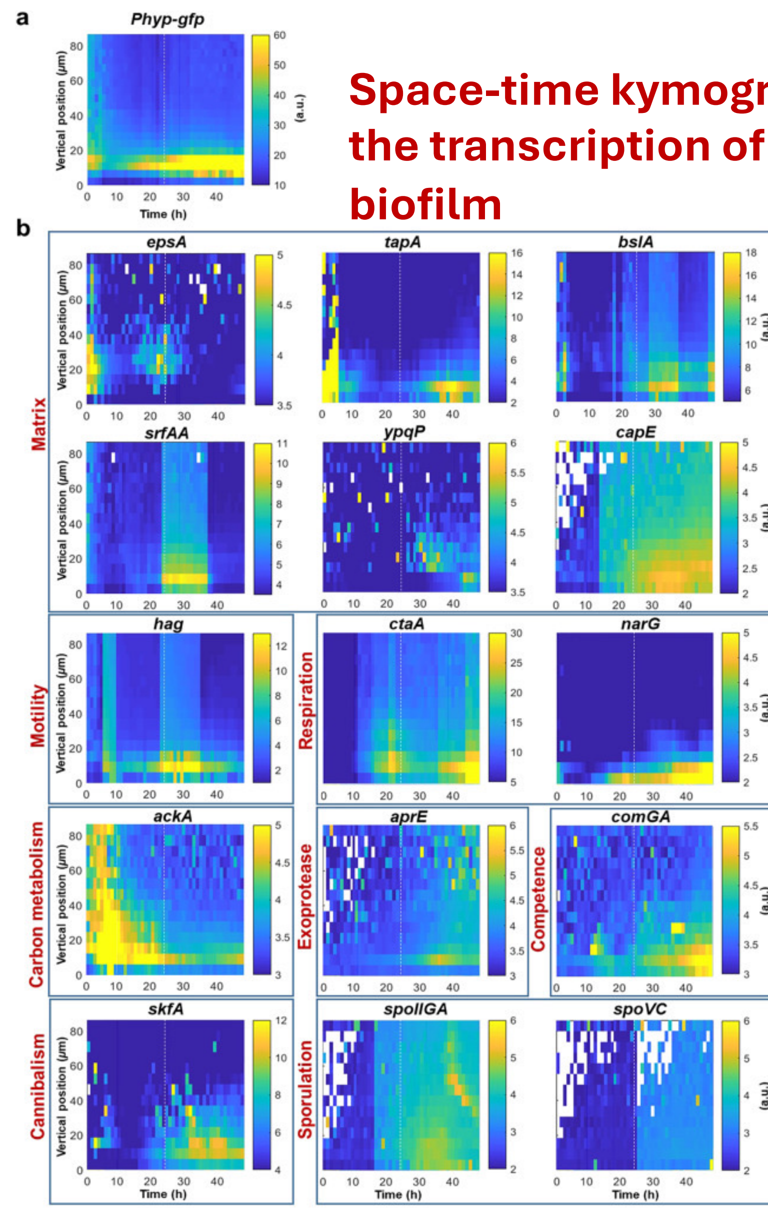
Direct comparison of spatial transcriptional heterogeneity across diverse *Bacillus subtilis* biofilm communities

Received: 9 January 2023

Accepted: 8 November 2023

Yasmine Dergham[1,2], Dominique Le Coq[1,3], Pierre Nicolas[4], Elena Bidnenko[1], Sandra Dérozier[4], Maxime Deforet[5], Eugénie Huillet[1], Pilar Sanchez-Vizuete[1], Julien Deschamps[1], Kassem Hamze[2] & Romain Briandet[1]



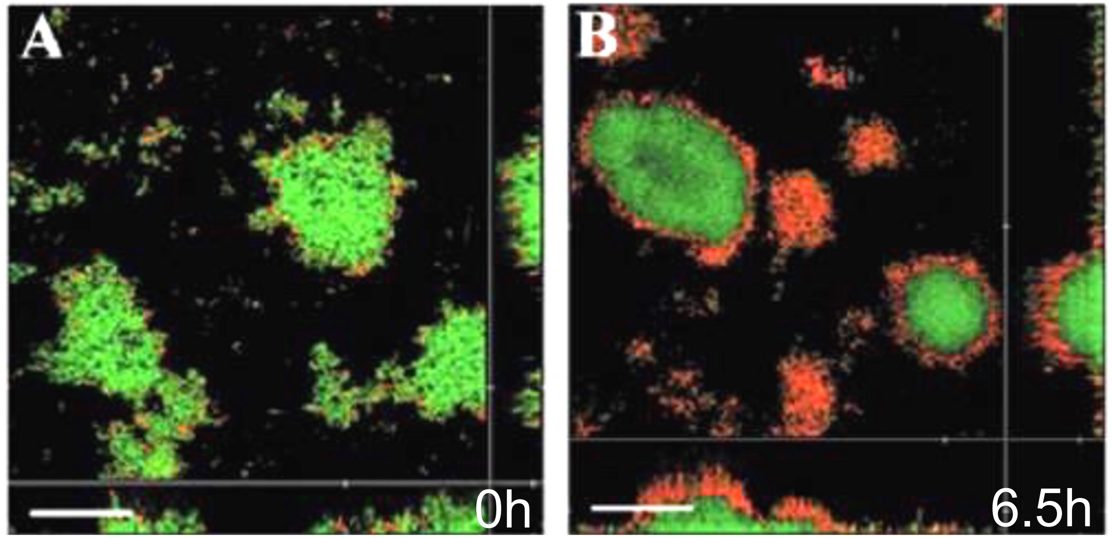


Space-time kymographs of fluorescent reporters for the transcription of 15 genes in the submerged biofilm

Spatial heterogeneity of antimicrobial tolerance within biofilms

Colistine action (25 µg/mL)

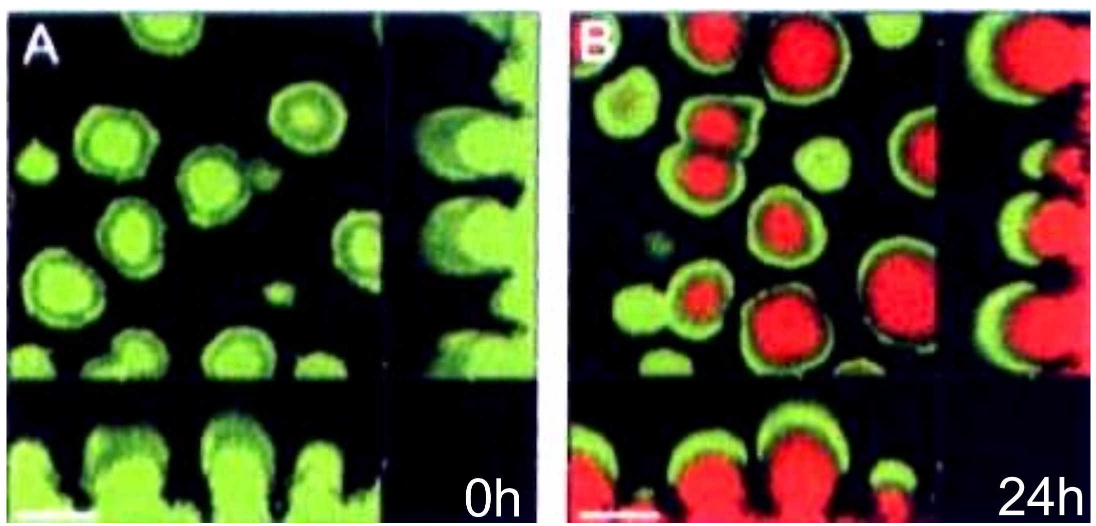
Escherichia coli



GFP / Iodure Propidium

Folkesson A et al. *PLOS One*, Vol3, Issue 4,1- 11; S. 2008

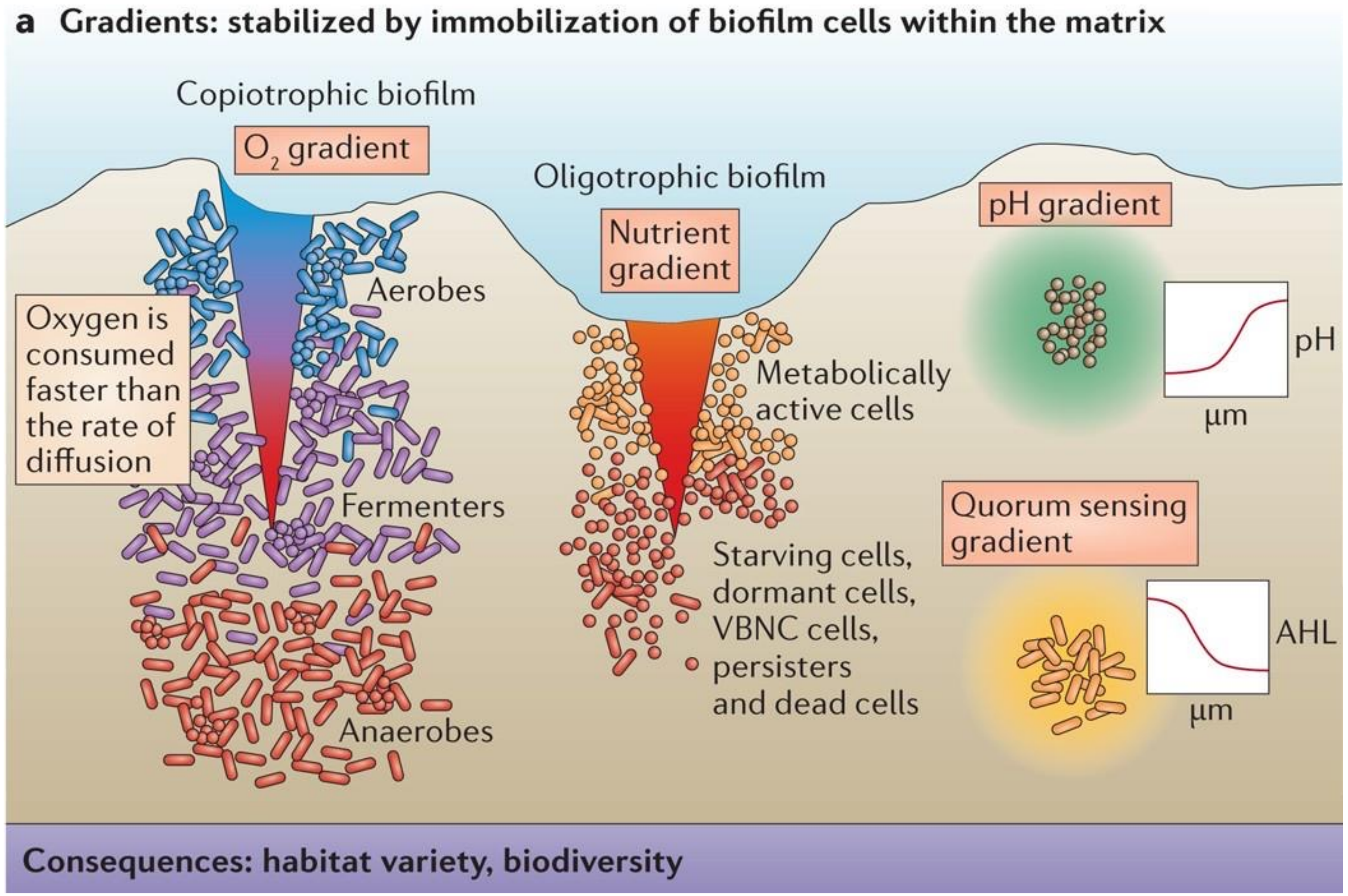
Pseudomonas aeruginosa



→ Structure related mechanisms of biofilm tolerance

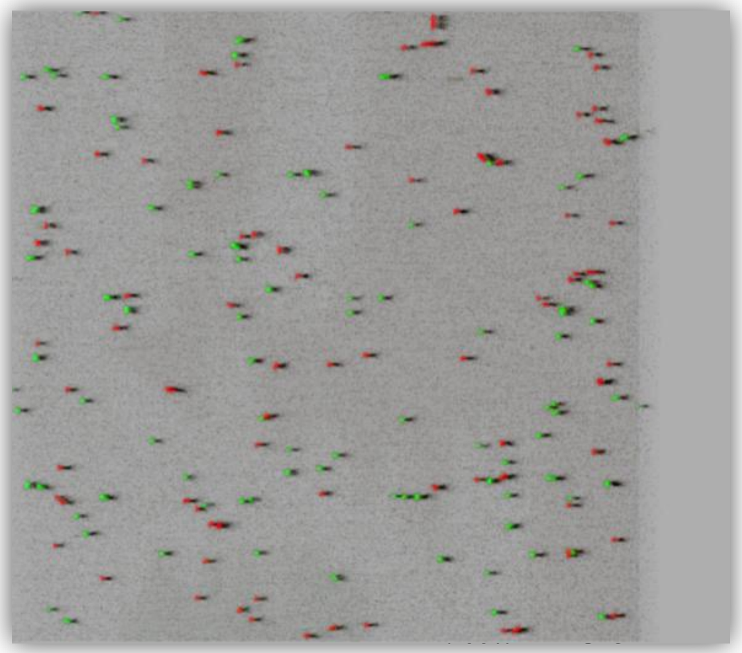
Pamp SJ et al. *Molecular Microbiology*, Vol68, 223-240; 2008

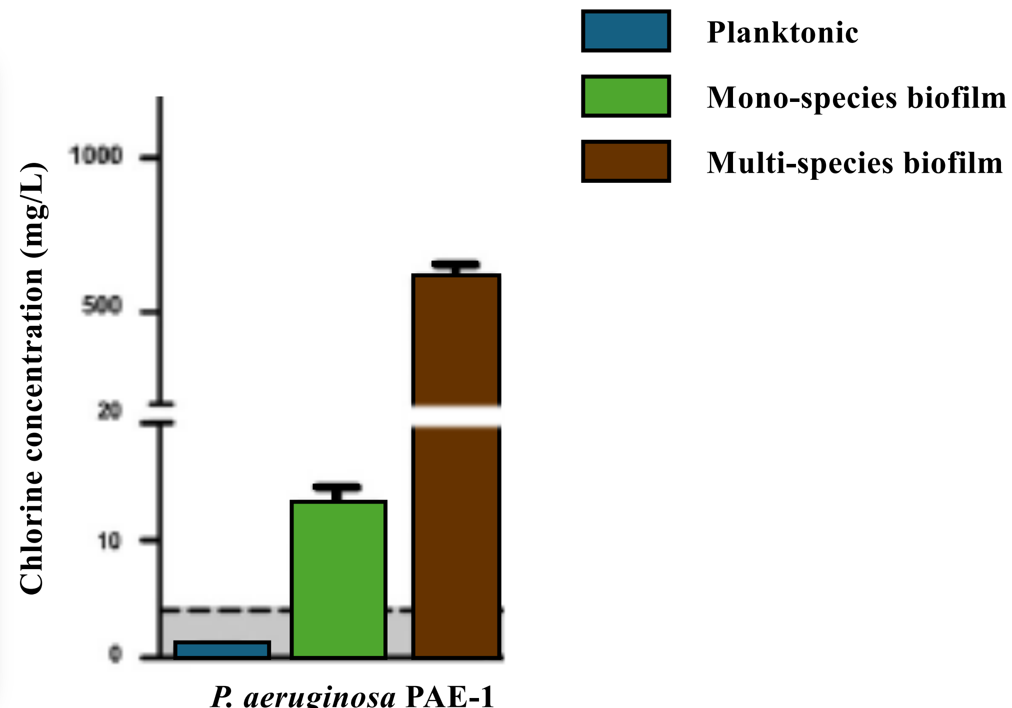
Biofilm lifestyle gives emerging properties



Flemming et al., Nature Reviews Microbiology, 2016

Hyper-tolerance to biocide in multispecies biofilms





(Adapted from Schwering *et al.* 2013)

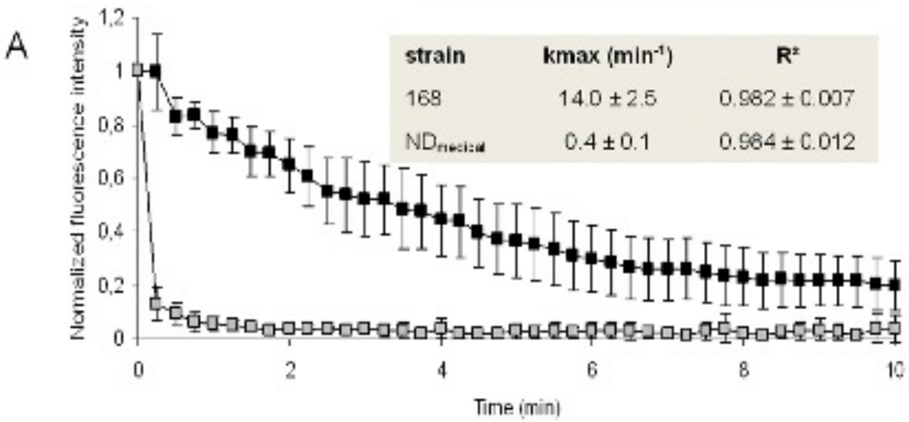
- The presence of different species in the biofilm can alter tolerance to biocides

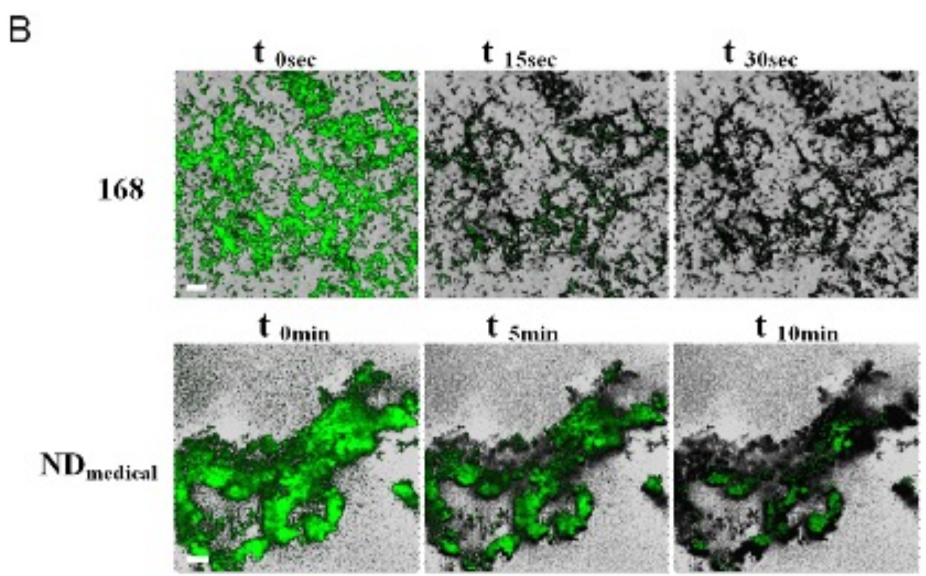
Hyper-tolerance to biocide in multispecies biofilms

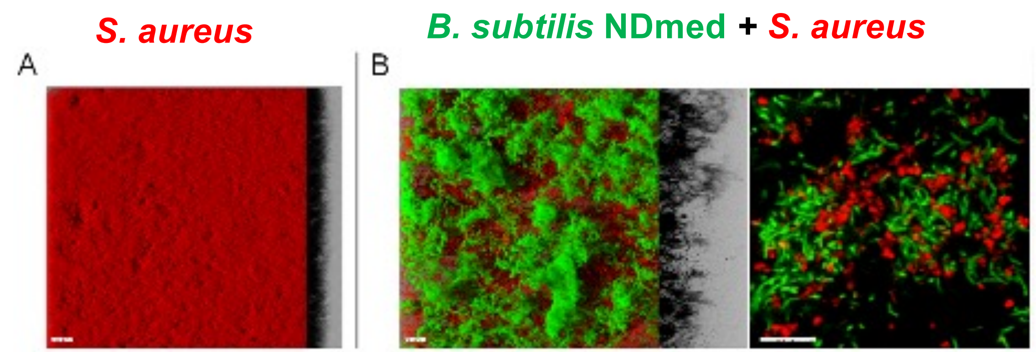
OPEN ACCESS Freely available online PLOS ONE

Biofilms of a *Bacillus subtilis* Hospital Isolate Protect *Staphylococcus aureus* from Biocide Action

Arnaud Bridier[1,2], Maria del Pilar Sanchez-Vizuete[1,2], Dominique Le Coq[1,2,3], Stéphane Aymerich[1,2], Thierry Meylheuc[1,2], Jean-Yves Maillard[4], Vincent Thomas[5], Florence Dubois-Brissonnet[1,2], Romain Briandet[1,2]*



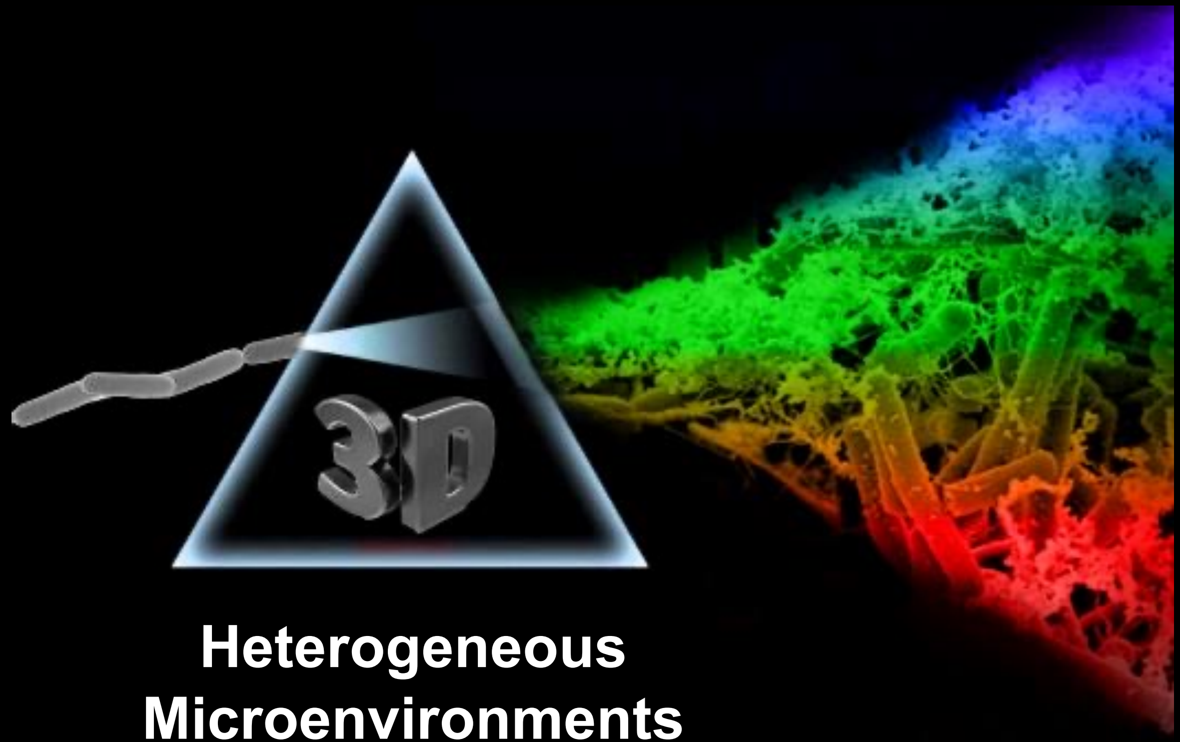




Bactericidal activity of water and 0.35% PAA on single and mixed species biofilms after 5 min of treatment.

		log (CFU/well)	
	Strain	**Water**	**PAA (0.35%)**
Single species biofilm	*B. subtilis* 168	7.6±0.2	–
	B. subtilis NDmedical	7.7±0.1	3.9±0.6
	S.aureus AH478	9.3±0.1	–
Mixed species biofilm	*B. subtilis* 168	7.5±0.5	–
	S.aureus RN4220	8.2±0.4	–
	B. subtilis NDmedical	7.3±0.3	3.9±0.3
	S.aureus RN4220	8.4±0.1	2.6±0.5

Biofilms & Spatially Organized Communities `



Diversification of cell types

-Patterns of gene expression, single-cell physiology

-Heterogeneity of cell behaviors (growth, survival, virulence, social phenotypes)

>>Emerging community functions

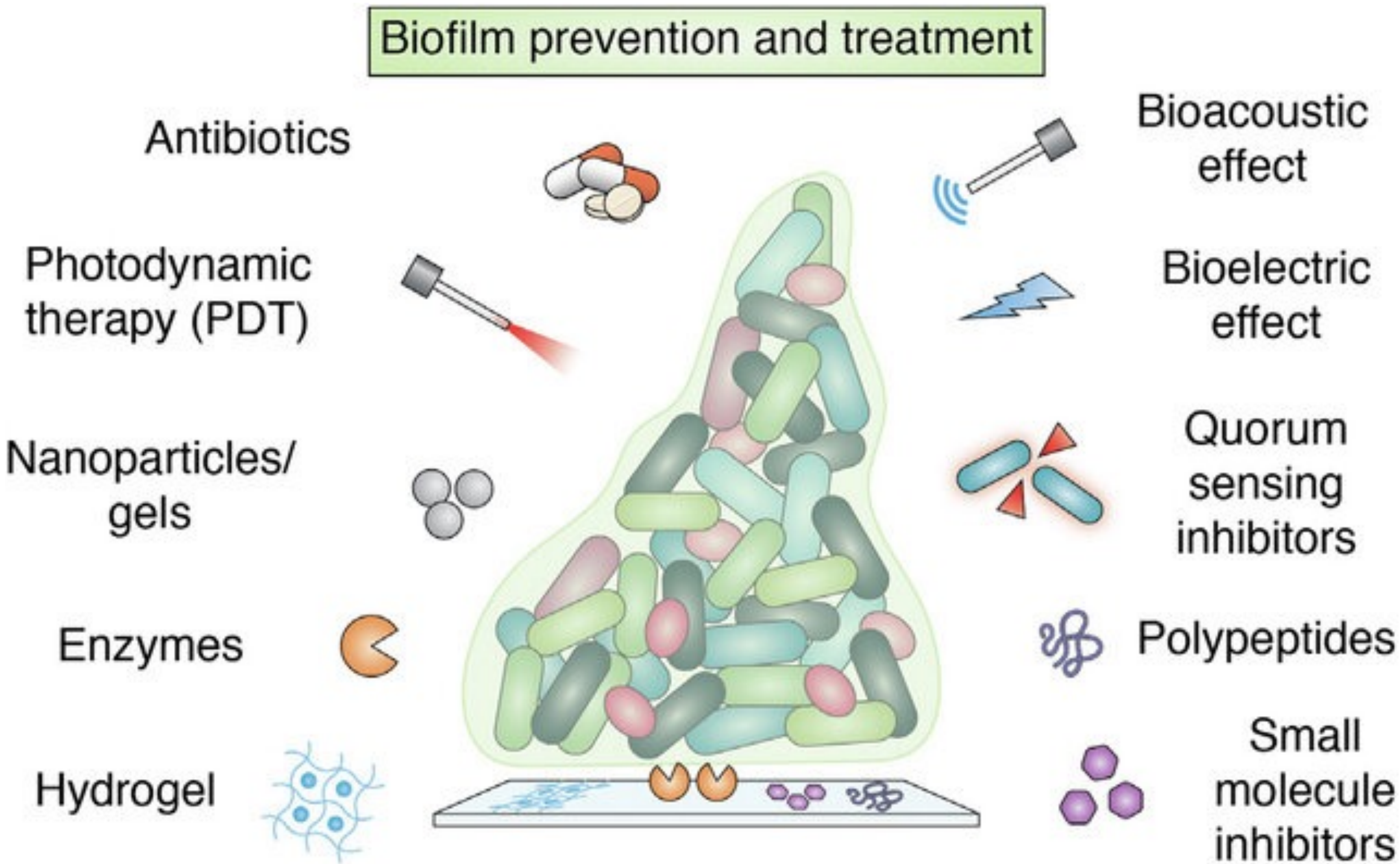
Heterogeneous Microenvironments

-gradients (gas, nutrients, inhibitors, QS)

-substratum microdomains

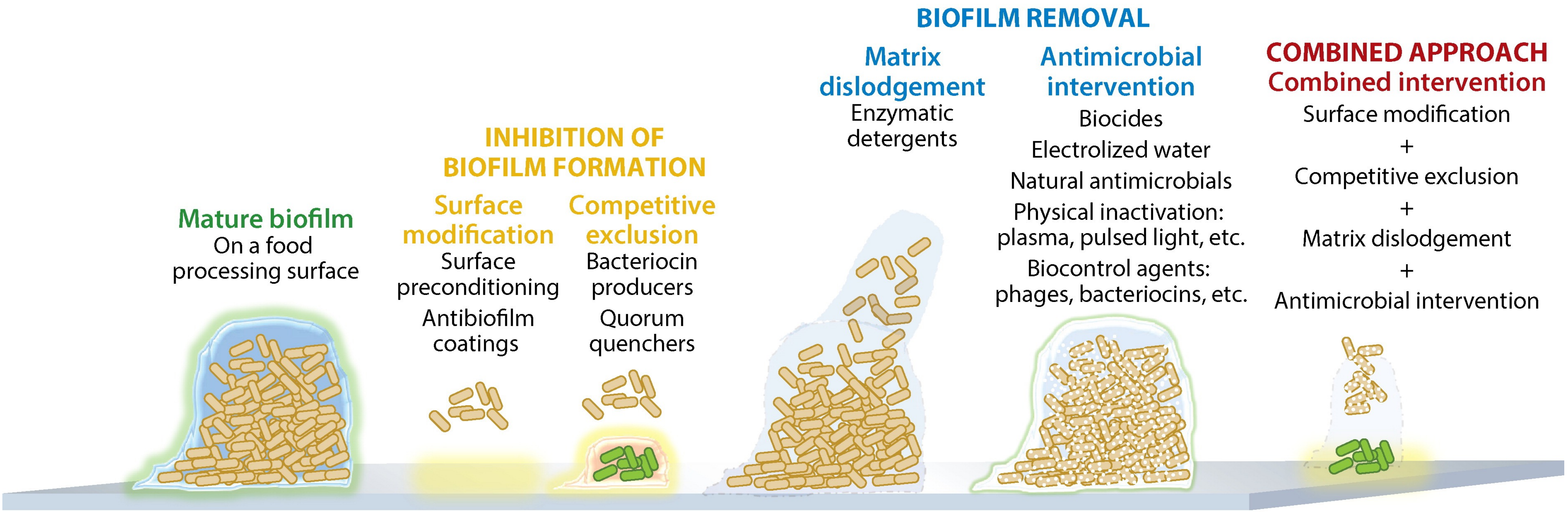
-background microbiota & predators

Combatting biofilms



10.1016/j.jbc.2022.102352

Combined approaches to control biofilms?



Alvarez-Ordóñez A, et al. 2019.
Annu. Rev. Food Sci. Technol. 10:173–95

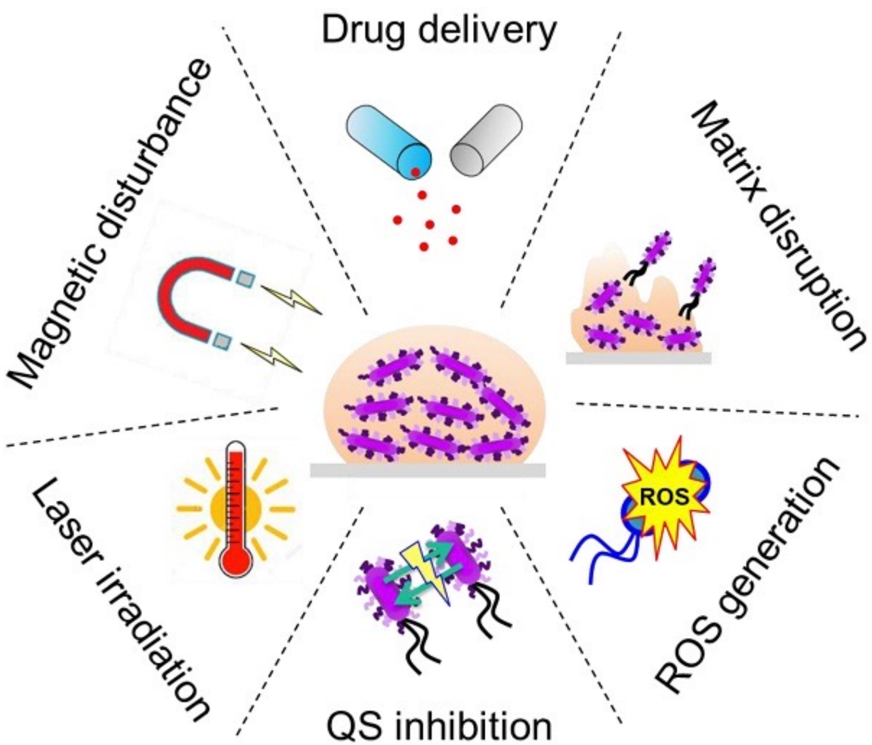
Recent advances in nanotechnology for eradicating bacterial biofilm

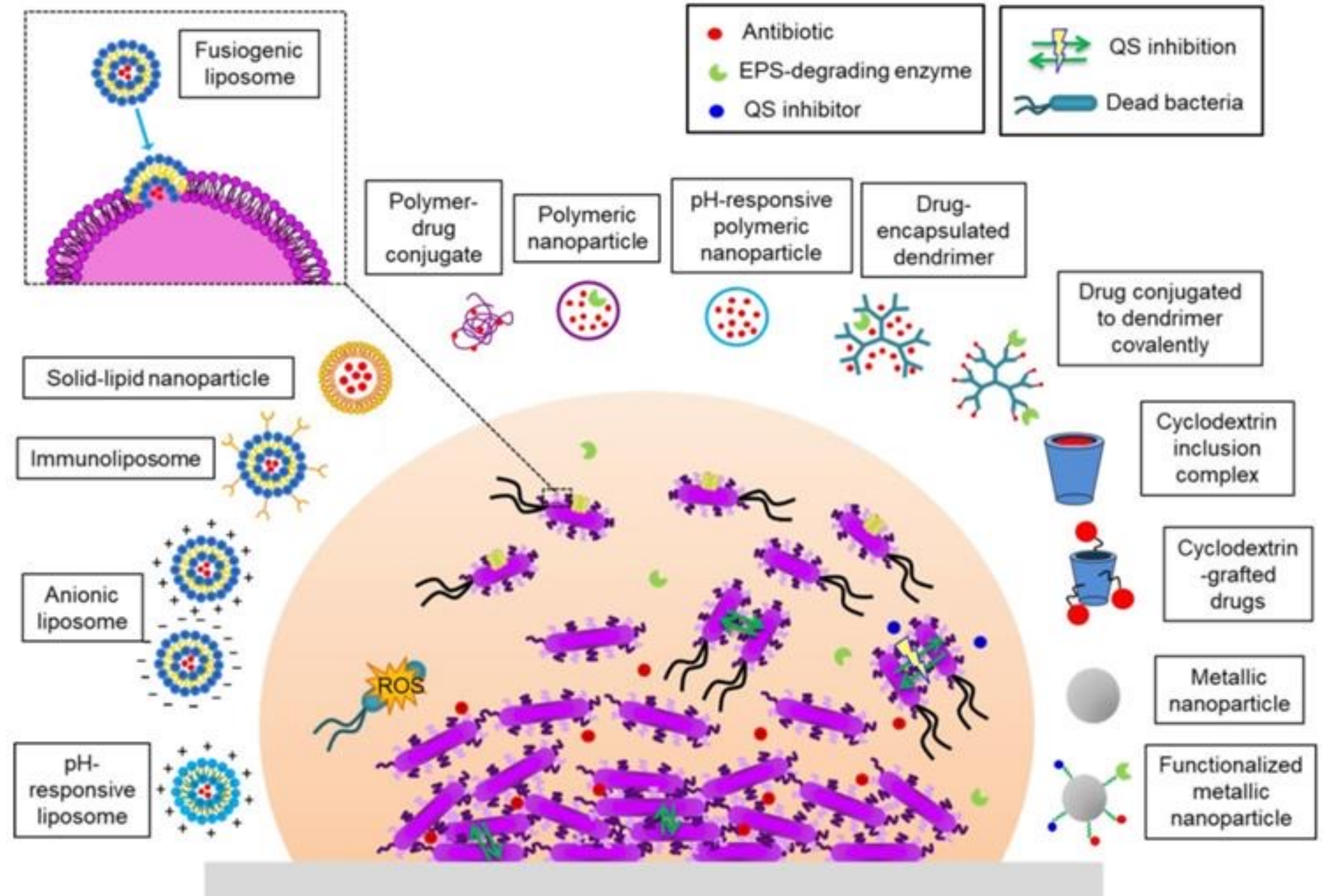

IVYSPRING INTERNATIONAL PUBLISHER
Theranostics
2022; 12(5): 2383-2405. doi: 10.7150/thno.67296

Review

Recent advances in nanotechnology for eradicating bacterial biofilm

Célia Sahli[1], Sergio E. Moya[2], John S. Lomas[1], Christine Gravier-Pelletier[3], Romain Briandet[4] and Myryana Hémadi[1]✉





Sahli C, et al. Recent advances in nanotechnology for eradicating bacterial biofilm. Theranostics 2022; 12(5):2383-2405.

Materials Today Chemistry 35 (2024) 101920

Contents lists available at ScienceDirect

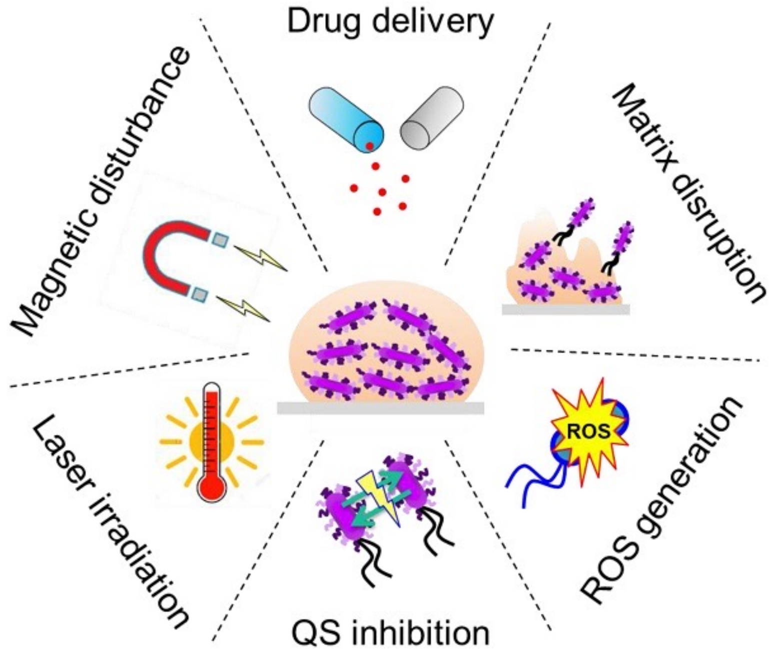
Materials Today Chemistry

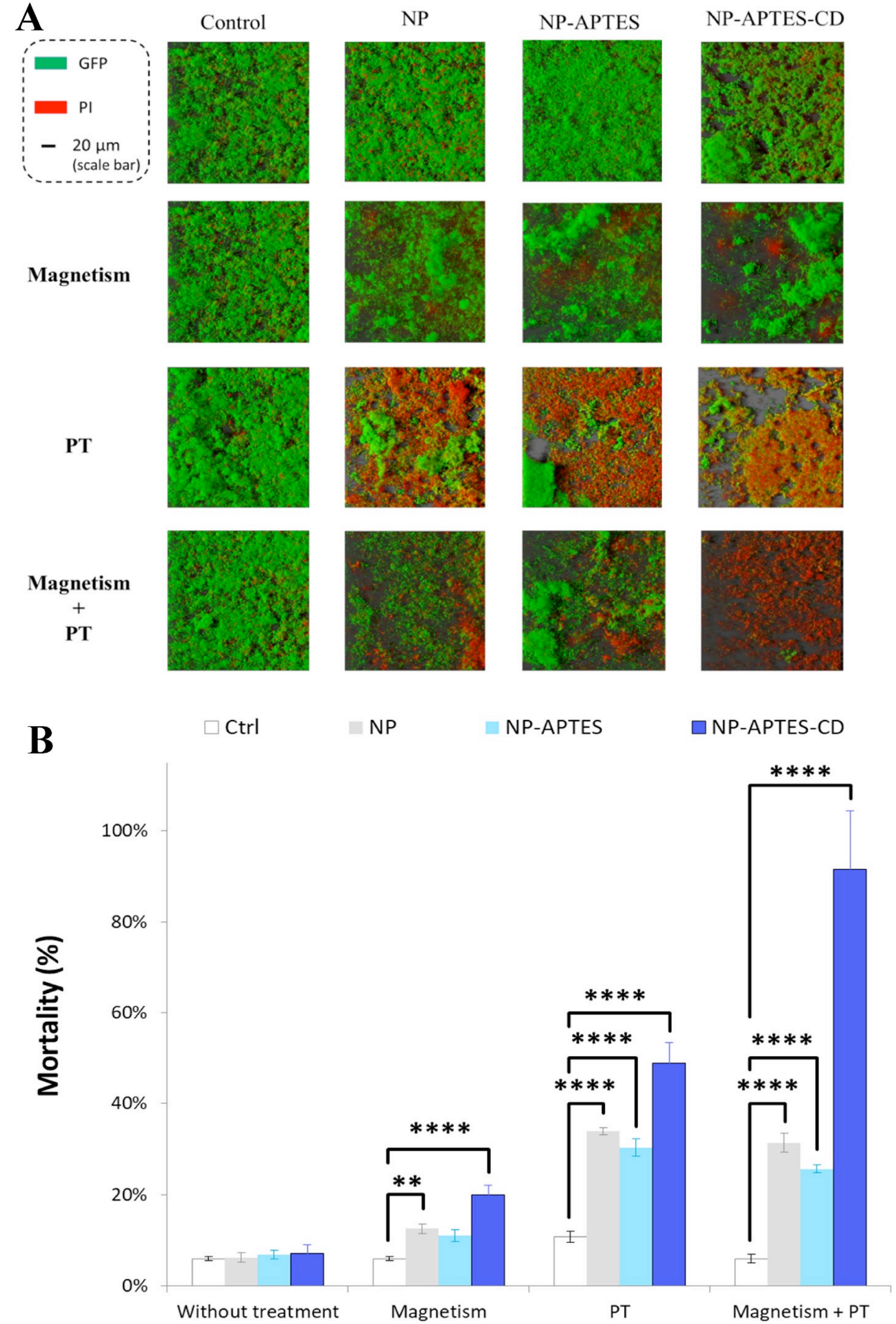
journal homepage: www.journals.elsevier.com/materials-today-chemistry/

Dual therapy for the eradication of bacterial biofilms: Iron oxide nanoparticles and carbon dots as magnetic actuator and photothermal agents

Célia Sahli [a], Julien Deschamps [b], Laurent Royon [c], John S. Lomas [a], Romain Briandet [b], Miryana Hémadi [a,*]

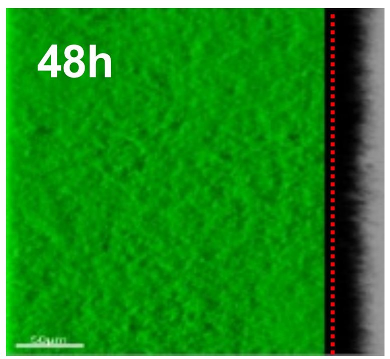
[a] Université Paris Cité, CNRS-UMR 7086, ITODYS, 75013, Paris, France
[b] Université Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, 78350, Jouy-en-Josas, France
[c] Université Paris Cité, CNRS-UMR 8236, Laboratoire Interdisciplinaire des Energies de Demain (LIED), 75013, Paris, France



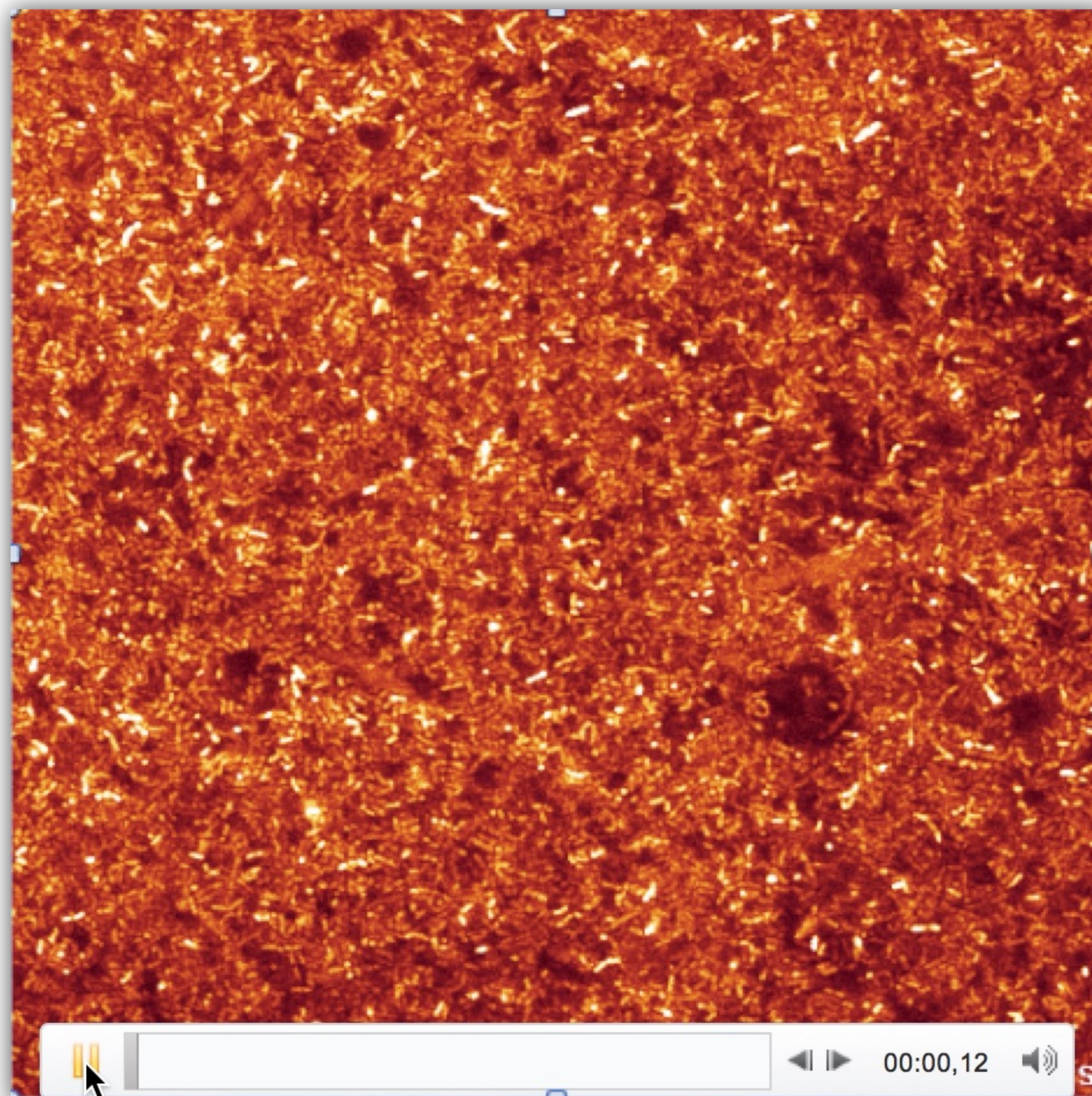


Confocal microscope images (**A**) and mortality (**B**) of *B. subtilis* NDmed) biofilm in contact with IONP, NP-APTES or NP-APTES-CD, under magnetic actuation, PT or both. Bars indicate standard deviations (**:

INFILTRATION OF THE BIOFILM MATRIX BY STEALTH SWIMMERS

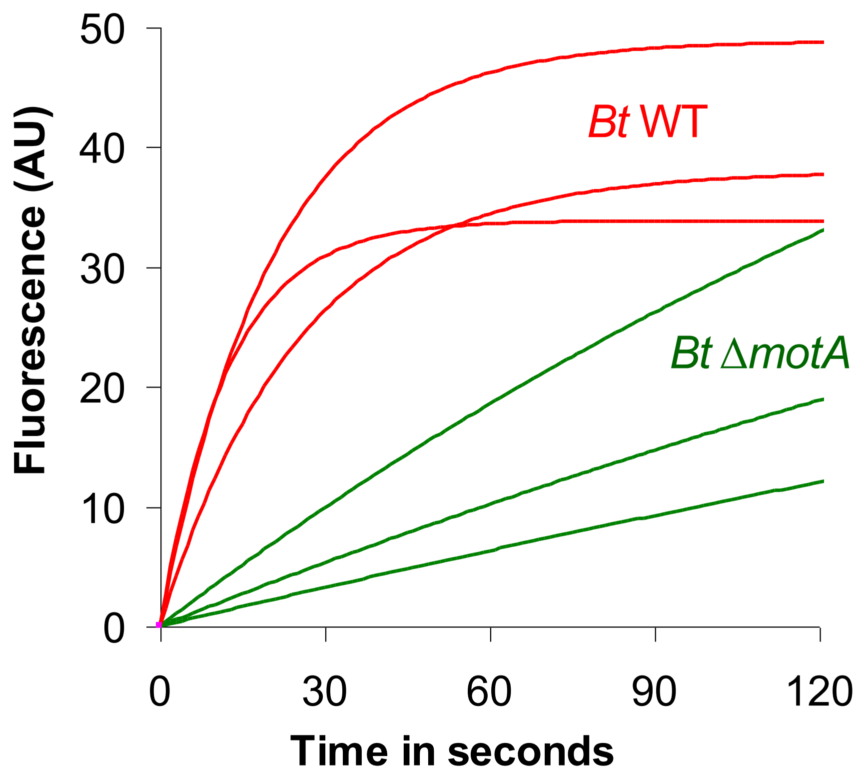


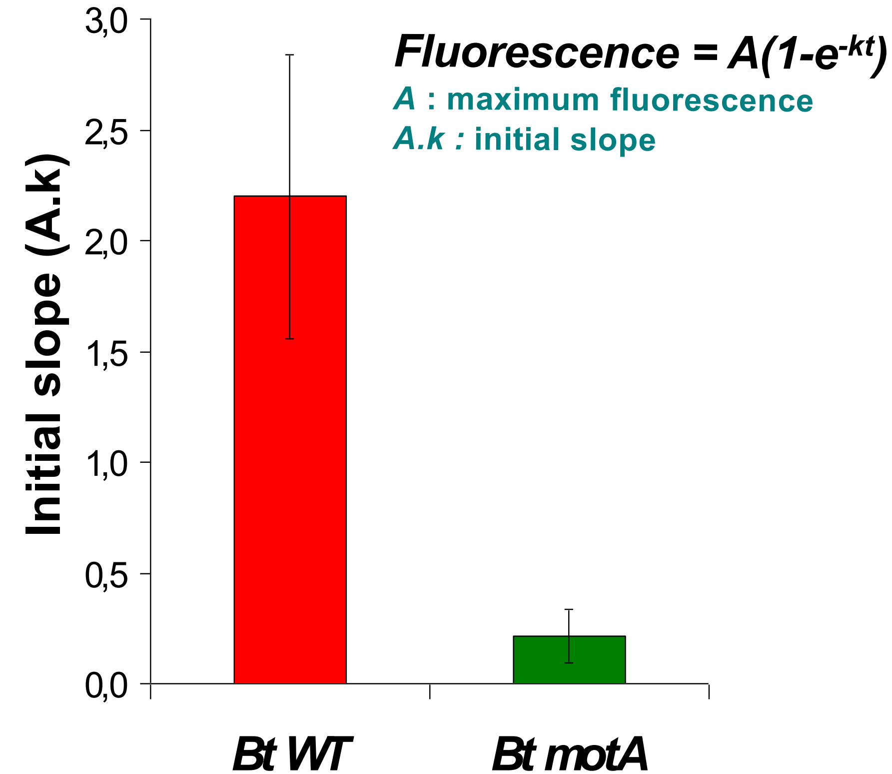
Bacillus thuringiensis 407
GFP



Houry et al. **PNAS**

Initial penetration of macromolecules in biofilms is facilitated by swimmer bacteria



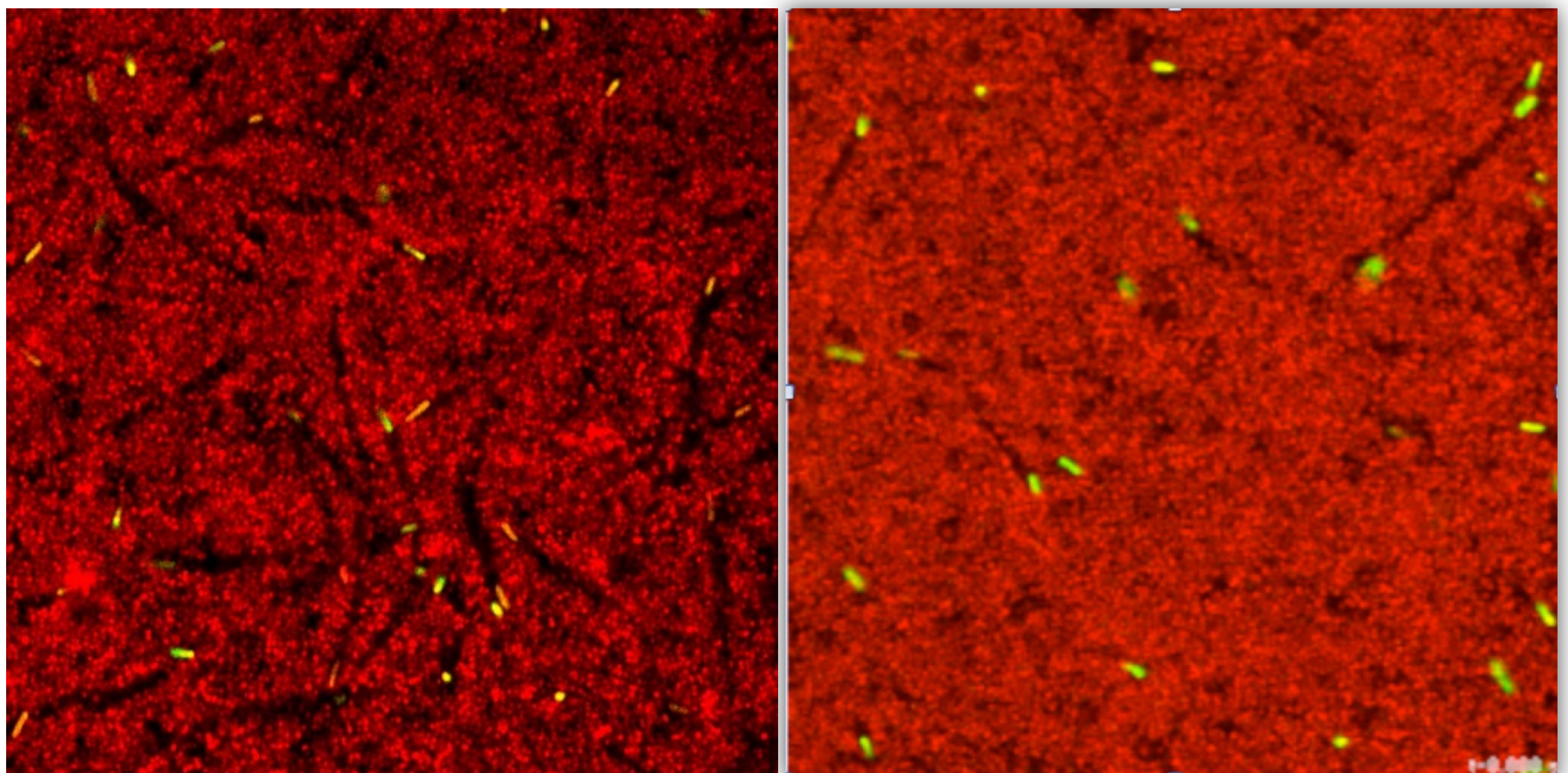


Gradual penetration of FITC-Dextran 250 KDa in the basal layer (z= 5µm) of *B. thuringiensis* biofilms

Houry et al. PNAS

Motile bacilli penetrate biofilms comprising a heterologuous species

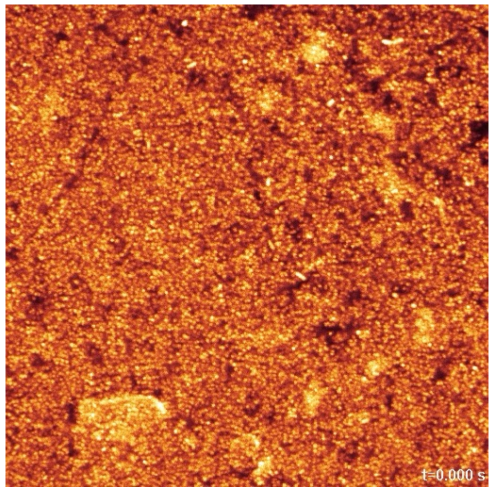
S.aureus biofilms (red) + *B. thuringiensis swimmers* (green)



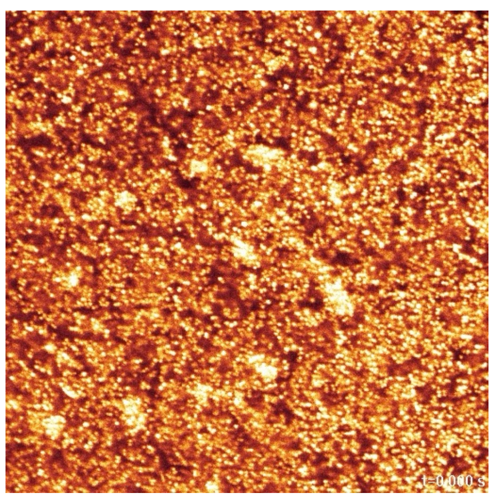
Houry et al. PNAS

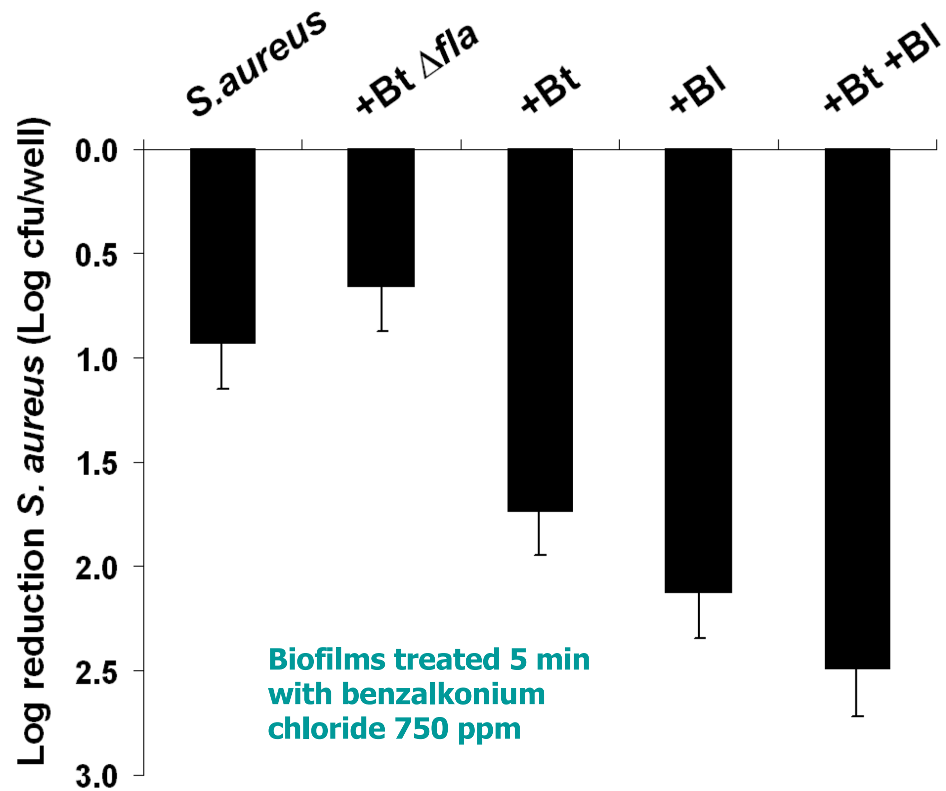
SWIMMERS INFILTRATION SENSITIZES *S. AUREUS* BIOFILMS TO CHEMICAL ANTIMICROBIALS

***S.aureus* + *B. thuringiensis* (Bt)**



***S.aureus* + *B. licheniformis* (Bl)**



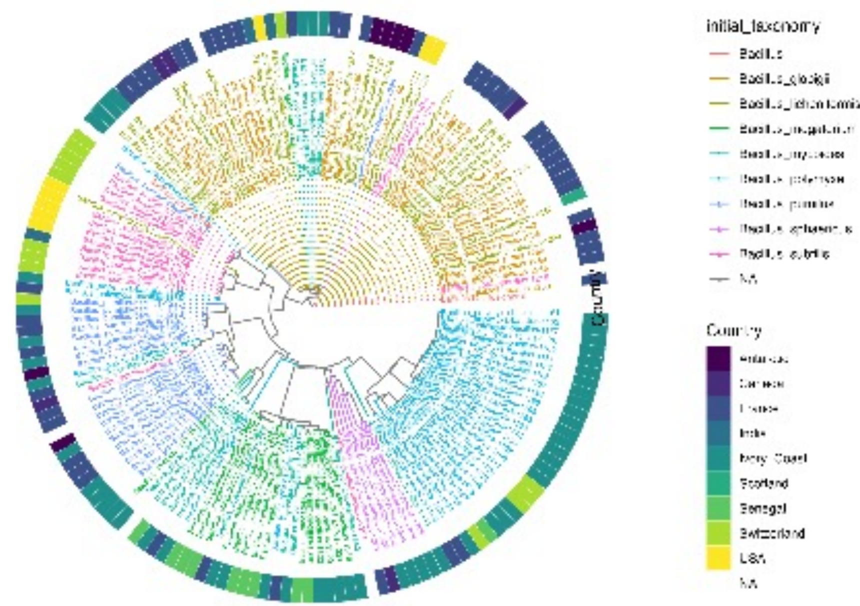


- **Applications of motility to pathogenic biofilm elimination**

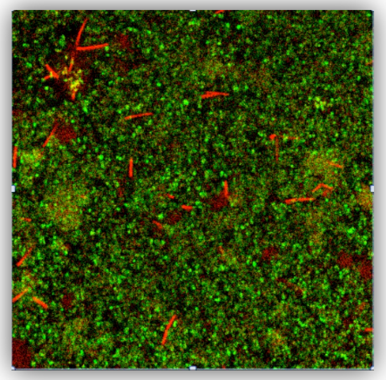
Houry et al. PNAS

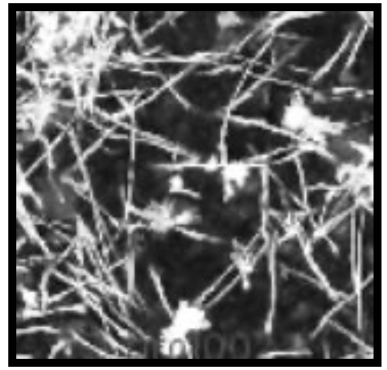
WHAT ABOUT NATURAL STRAINS?

~400 bacillus isolates from the Delaporte collection

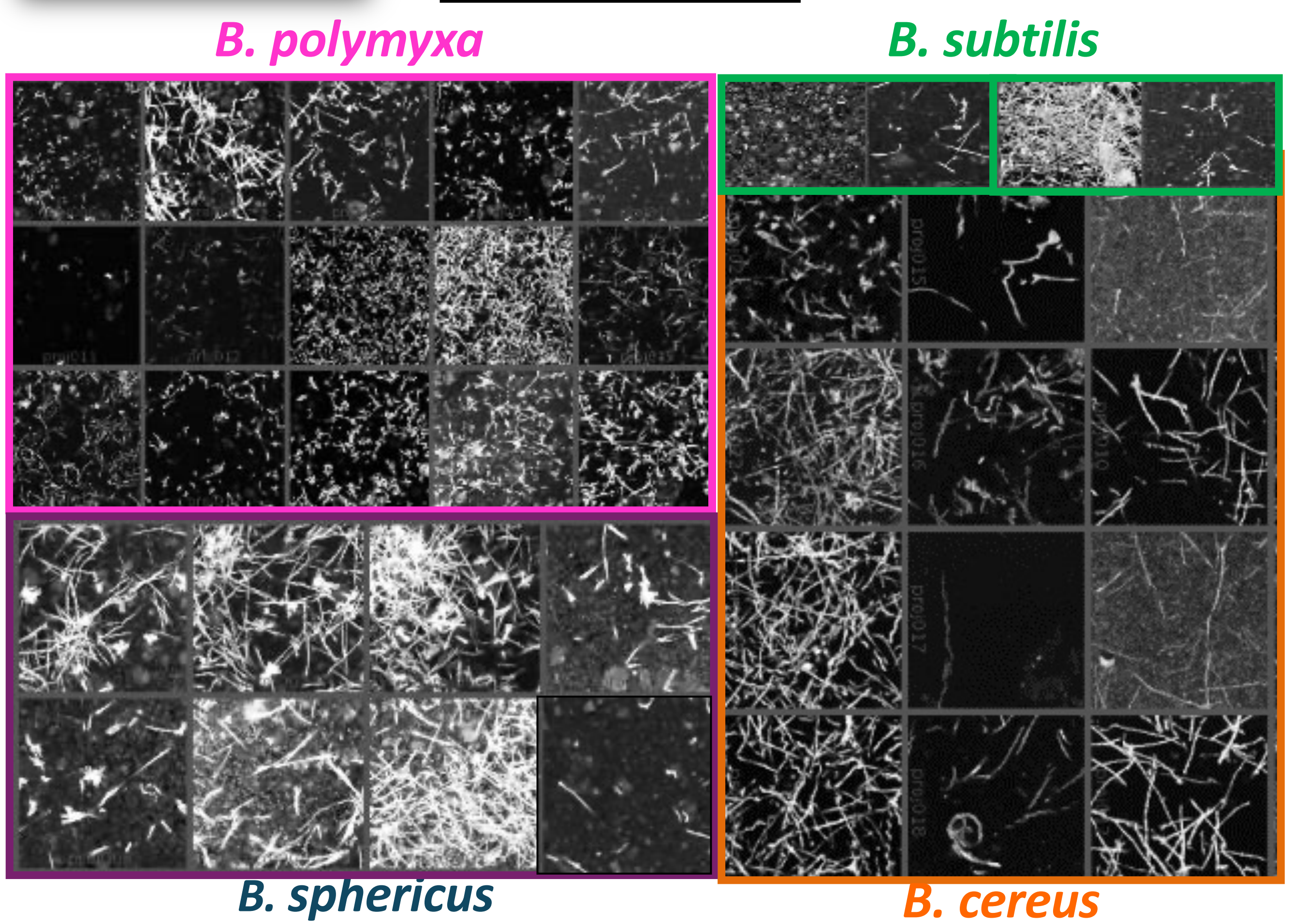


Inter/ Intra species variability in swimming patterns

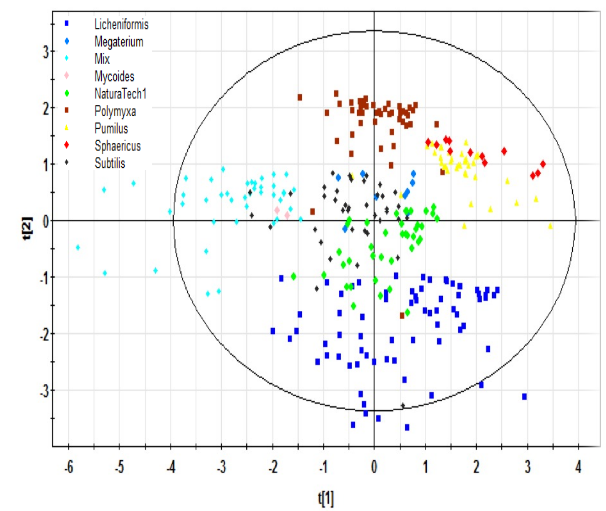




Trajectories projections for 30 seconds



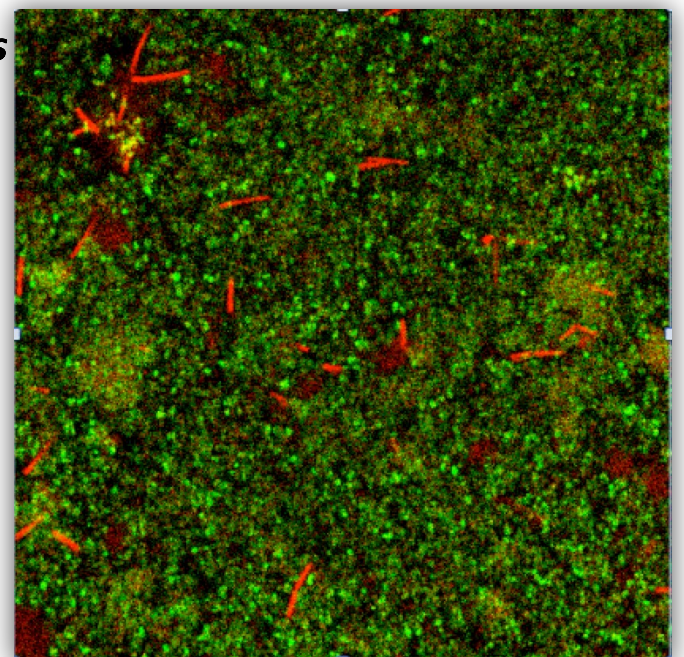
125 strains x 5 projections of 30s videos



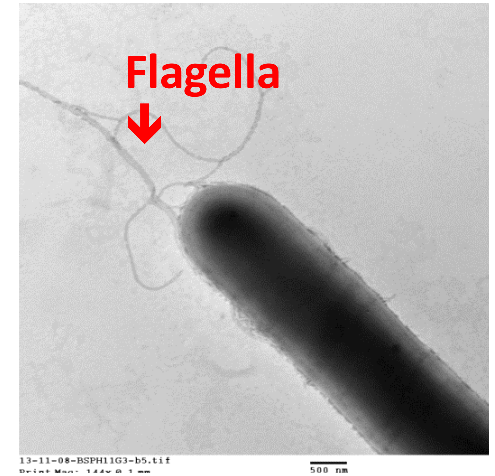
Li et al. IEEE Intern Symp Biomed Imaging 2014

Screening 125 natural motile bacilli for their ability to invade *S. aureus* biofilm

B. sphaericus

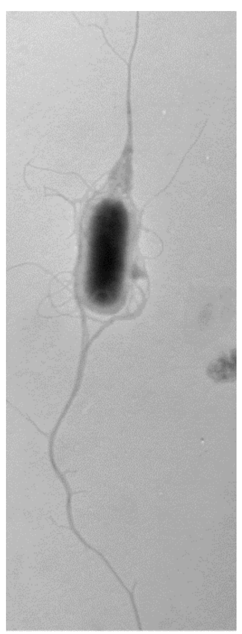


2 µm

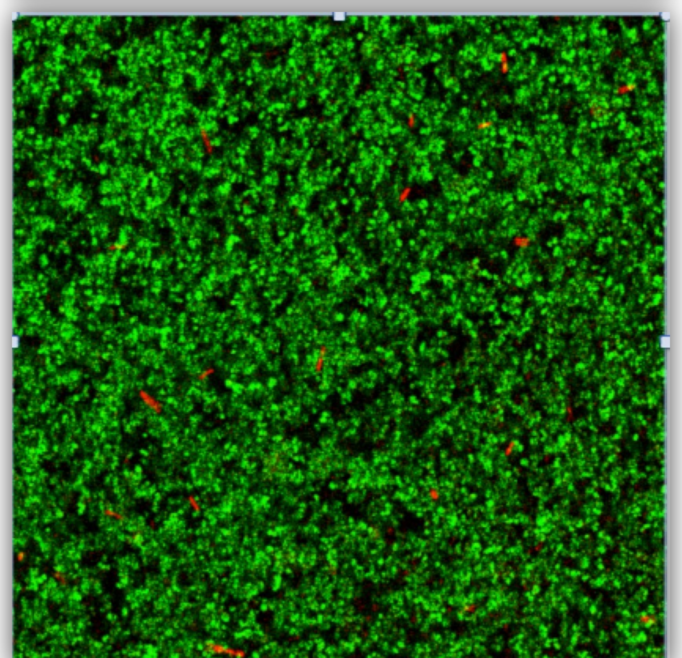


0.5 µm

B. lichenifomis



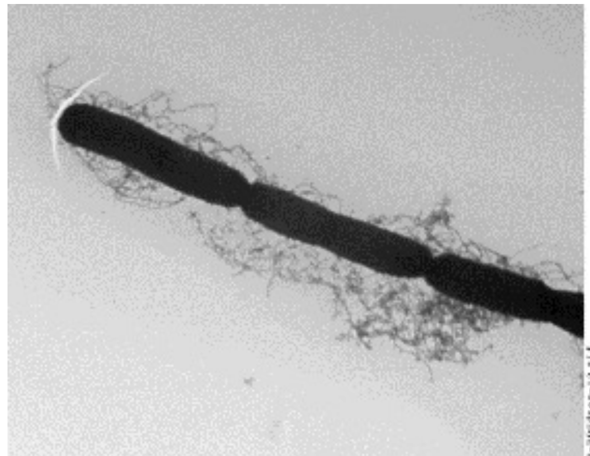
2 µm

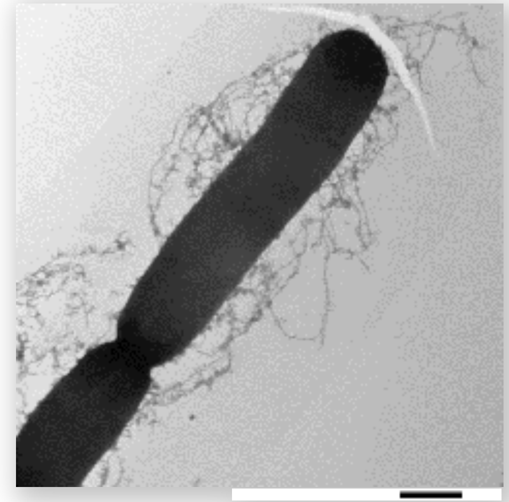


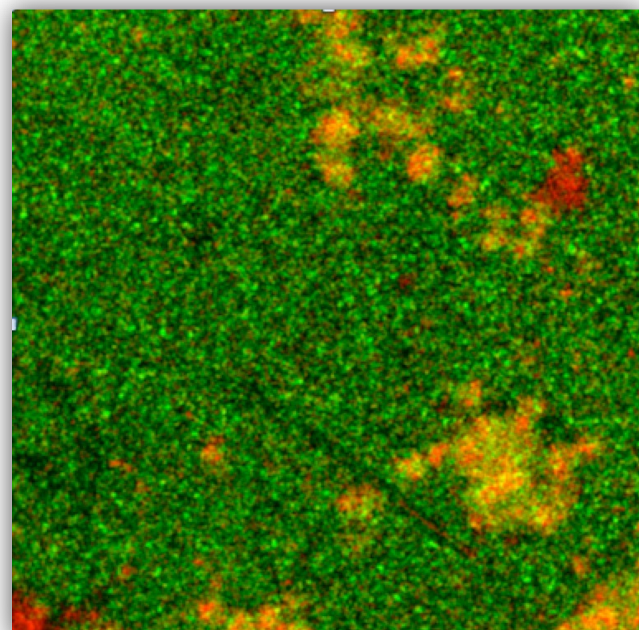
S. aureus-GFP **biofilms (Green)**

Bacilli swimmers – staining **(Red)**

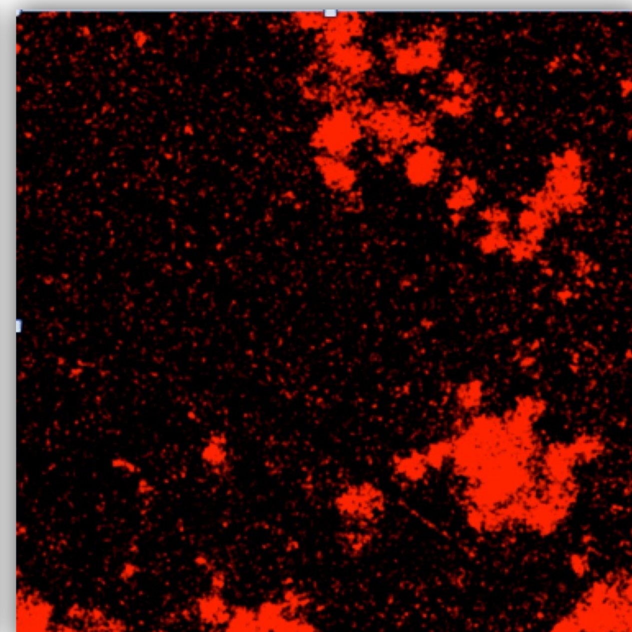
Bacilli chains swimming in the matrix



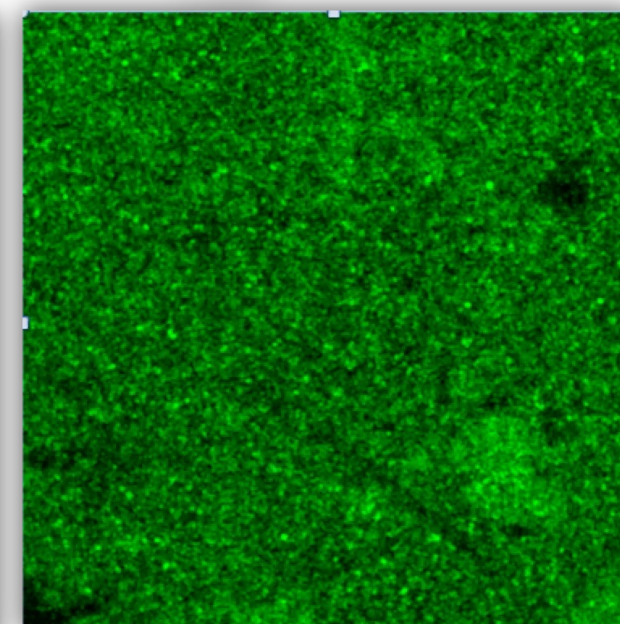




B. cereus merged channel

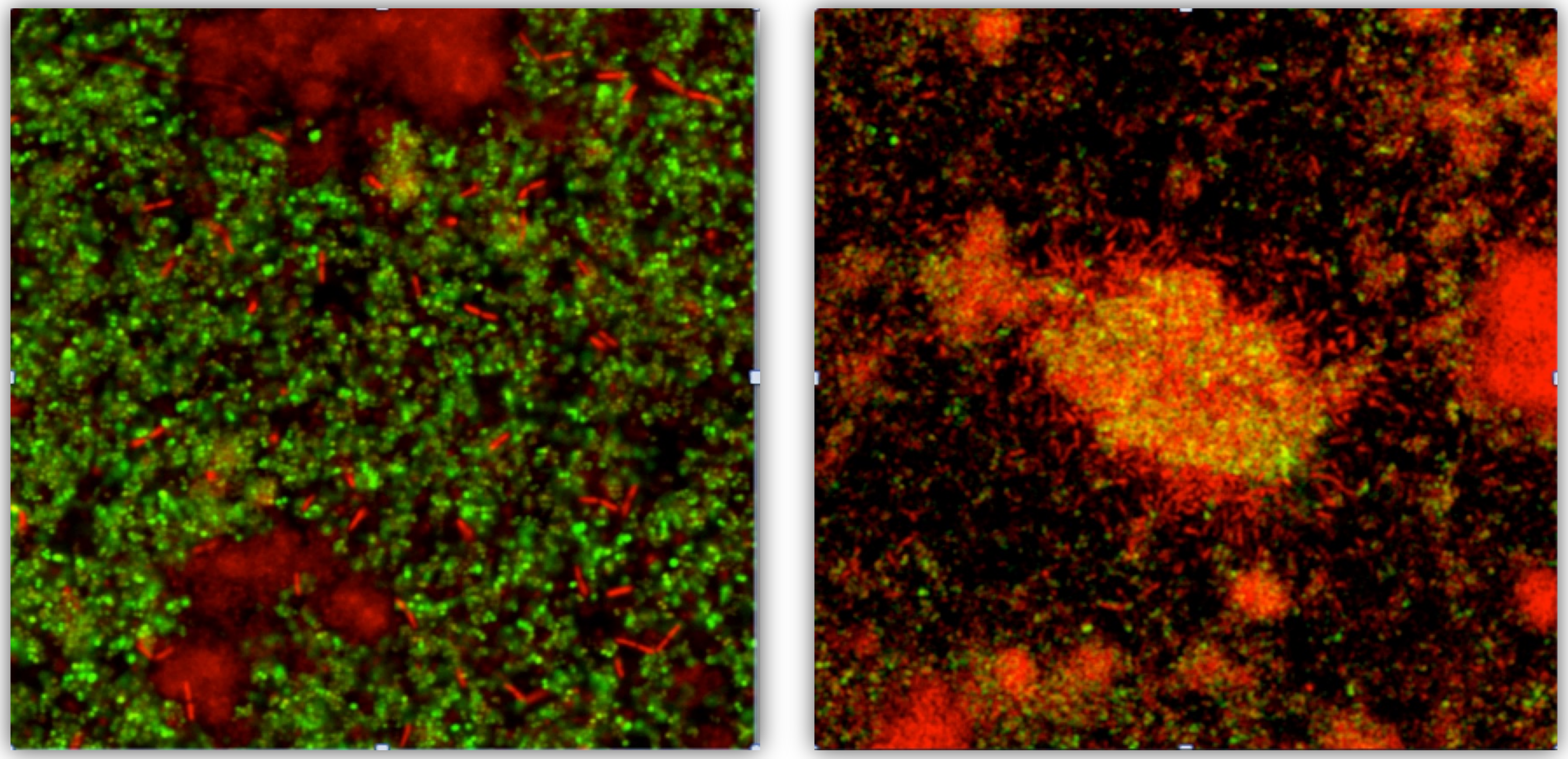


Swimmers channel



S. aureus-GFP Biofilm channel

S.aureus biofilm eDNA pockets

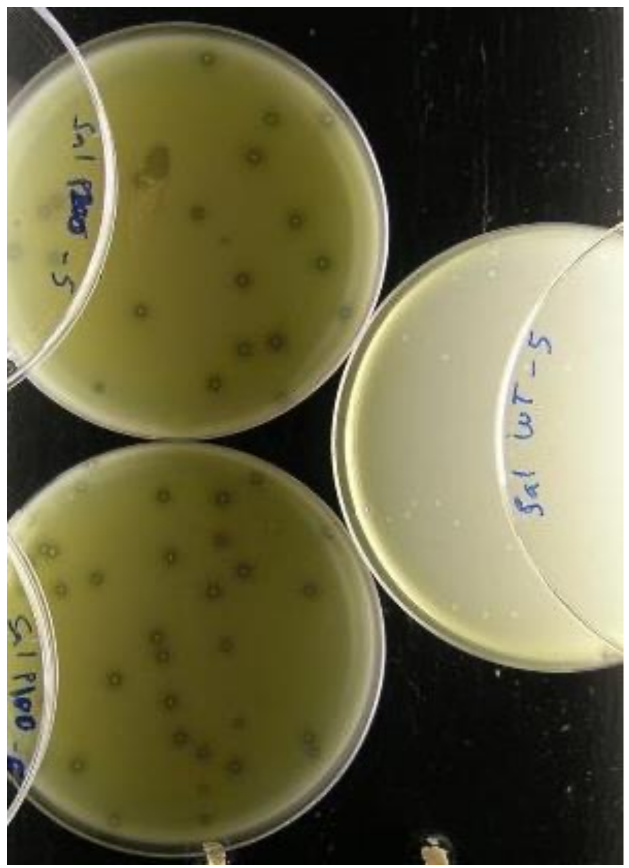


S. aureus GFP eDNApockets

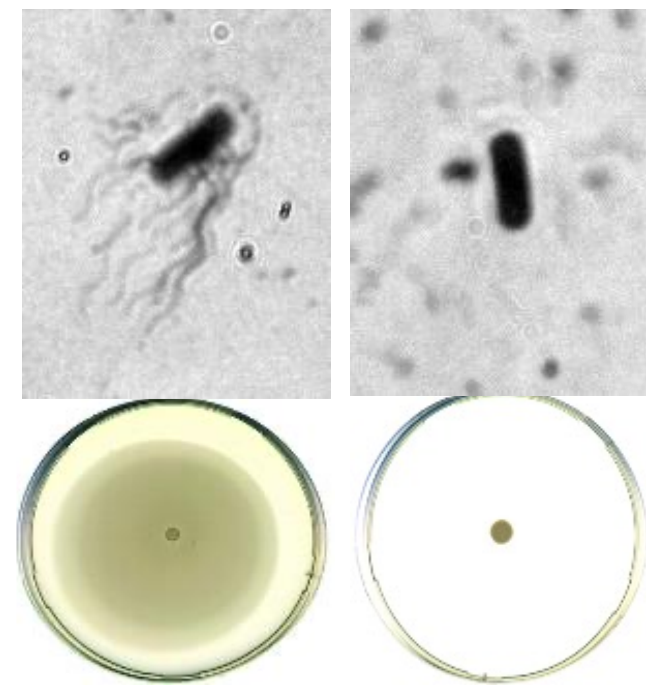
Bacillus spp 1B15 *Bacillus spp* 10C2

Can stealth swimmers deliver self-produced antimicrobial in the target biofilm ?

Lysostaphin (Lys+)

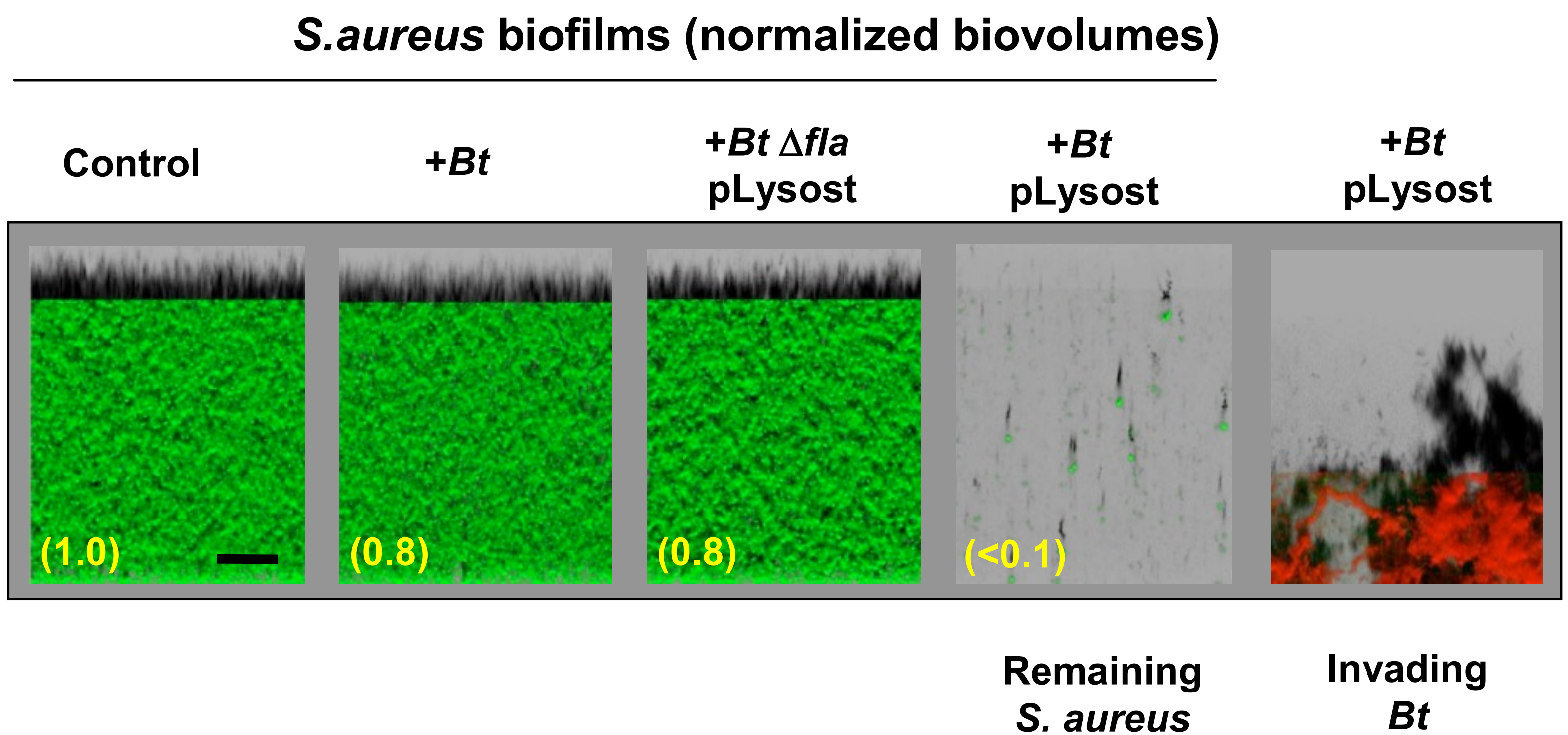


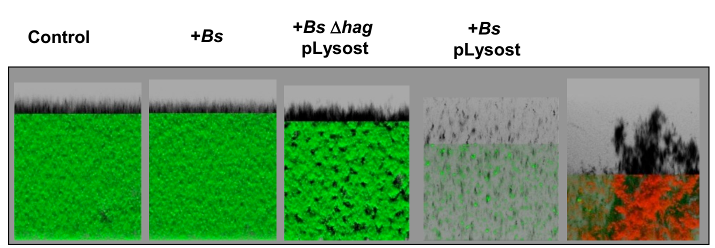
Bt WT   Bt *Δfla*



Motility +   Motility -

S. AUREUS BIOFILMS ARE DISRUPTED AND SUPPLANTED BY MOTILE BACILLI EXPRESSING LYSOSTAPHIN





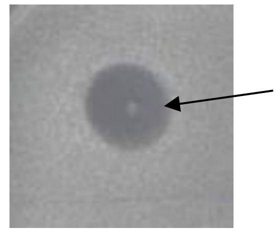
Similar results with *B. subtilis*

Houry et al. PNAS

Natural swimmers producing antimicrobials active on *S. aureus* biofilms

Agar invasion assay on S. aureus

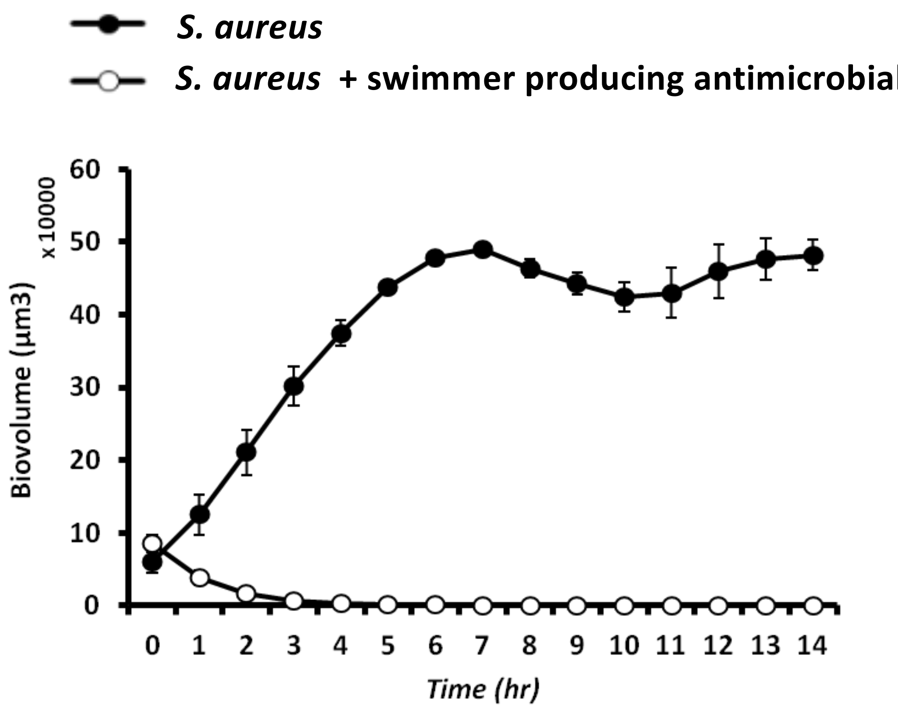
no antimicrobial production

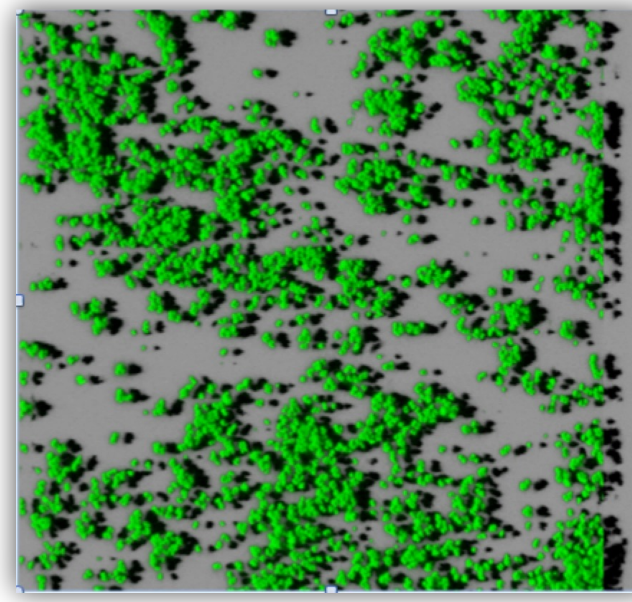


Inhibition zone

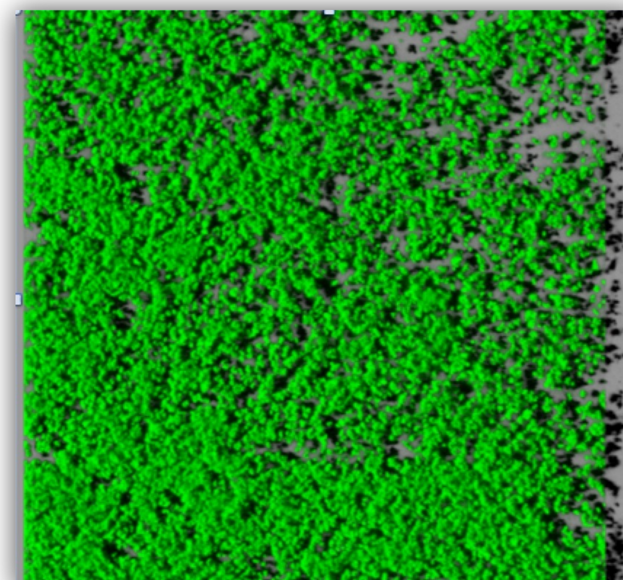
antimicrobal production

- **32 positive among 125 bacillus tested**



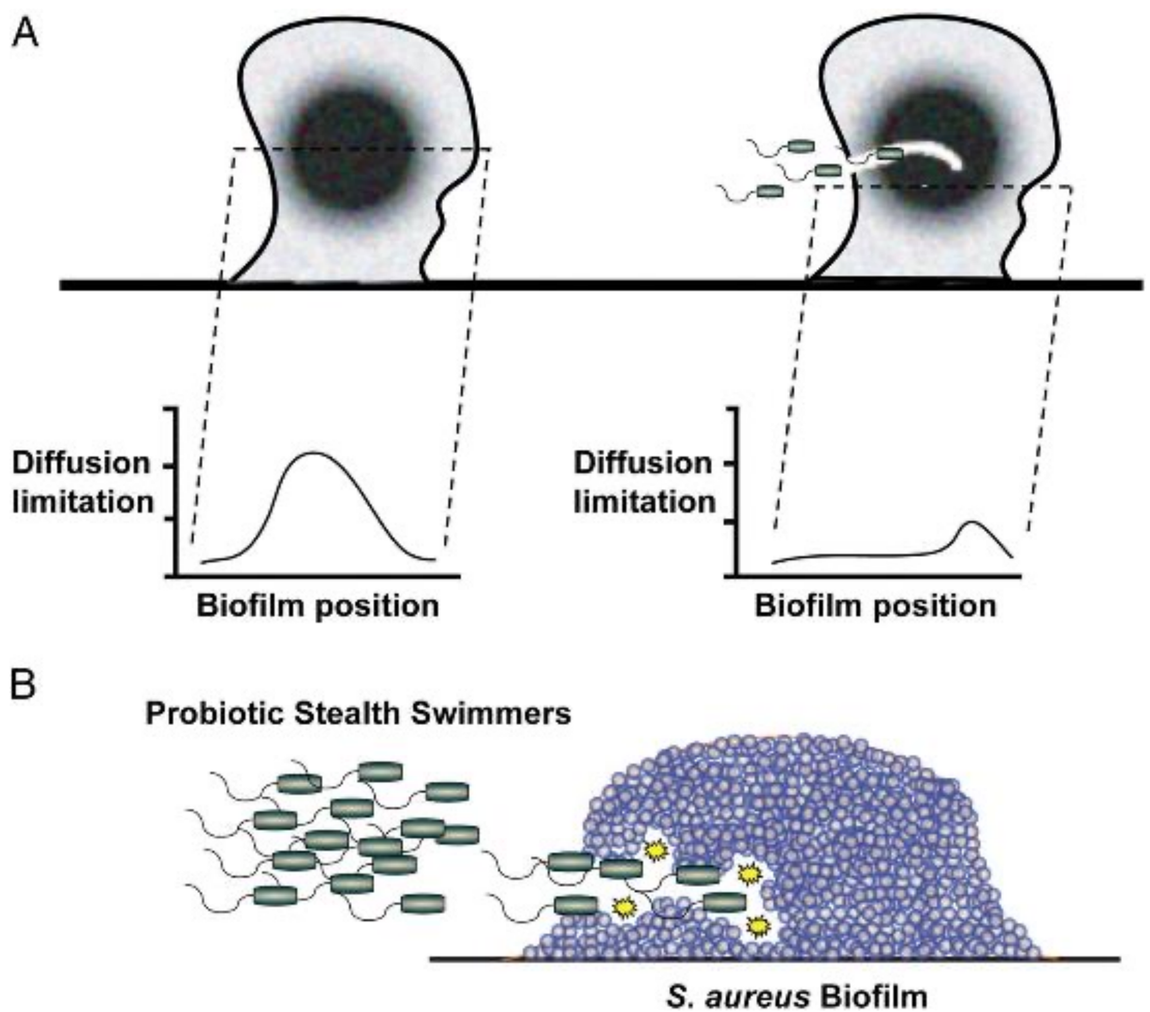


***S. aureus-GFP* biofilms growth (1 Image / hr for 15h)**



6 hrs Biofilm + selected swimmer producing antimicrobial (1 Image / hr for 15h)

Stealth swimmers in bacterial biofilms



-Role of a minor bacterial population in biofilms dynamics and ecology

-Applications of motility to pathogenic biofilm elimination

Boles B R , Horswill A R PNAS Commentary 2012;109:12848-12849

Should we aim for complete eradication or ecological management of biofilms ?