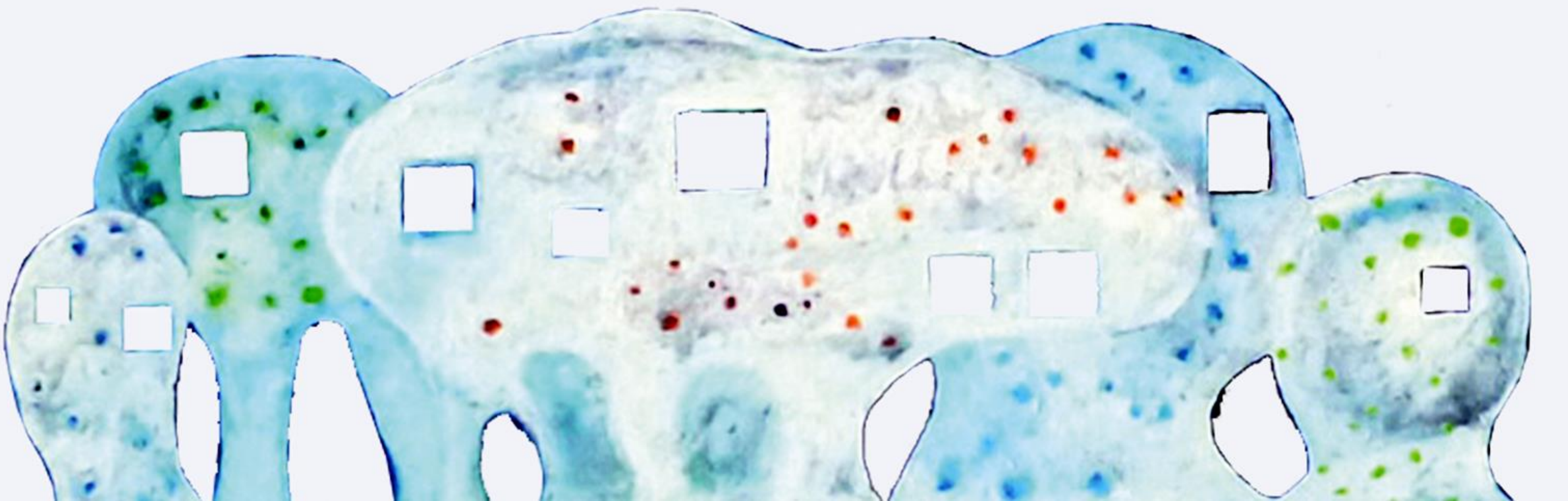
Biofilms in food industry - *structure, function and control strategies*

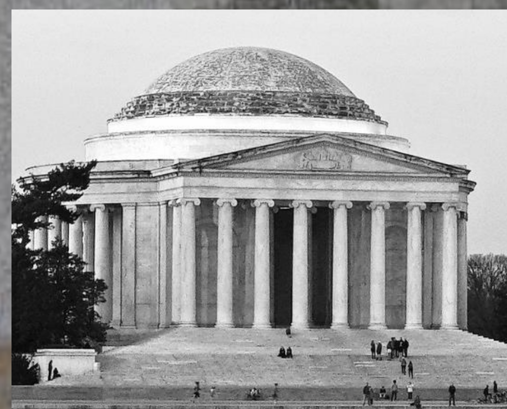



S4500 2.0 kV x700
S4500 2.0 kV x7.00K 4.29µm
S4500 0.8 kV x3.00K 10.0µm
S4500 0.8 kV x4.00K 7.50µm
INRA MIMA2 Imaging Center
S4500 0.8 kV x600 50.0µm
S4500 0.8 kV x4.00K 7.50µm

Biofilms everywhere !

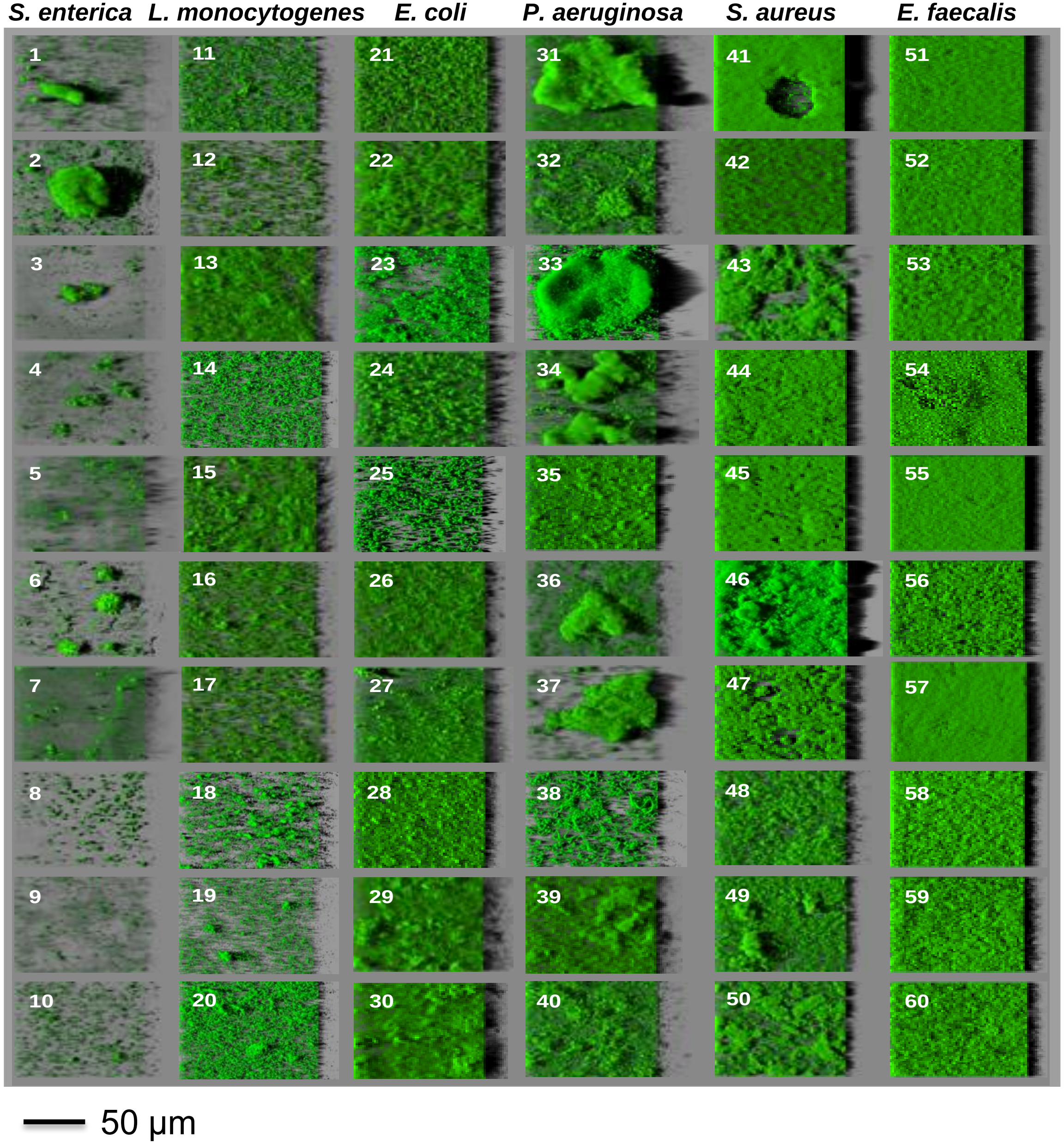


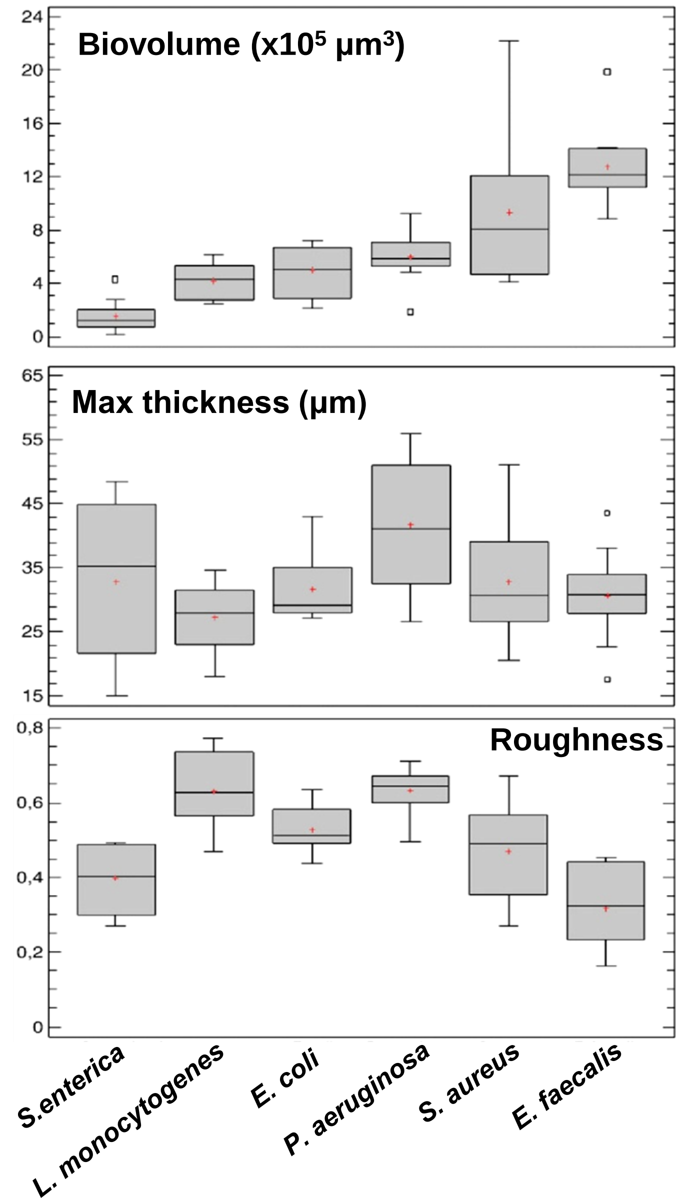







Axenic biofilms structural diversity

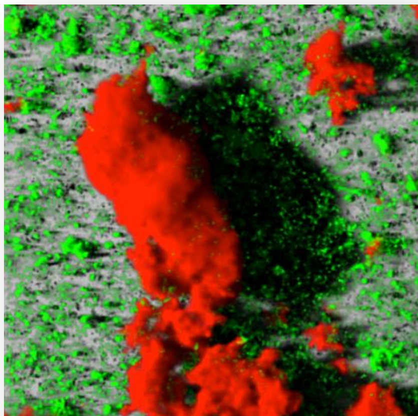




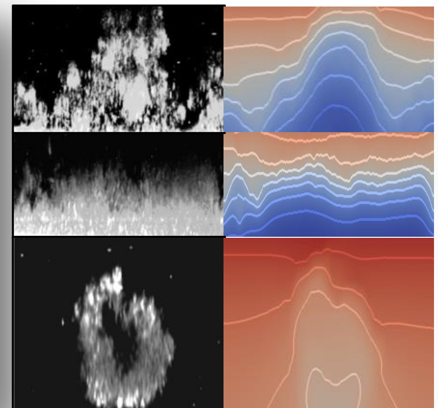
Bridier et al. *J. Microbio. Methods*, 2010

3D-driven heterogeneity

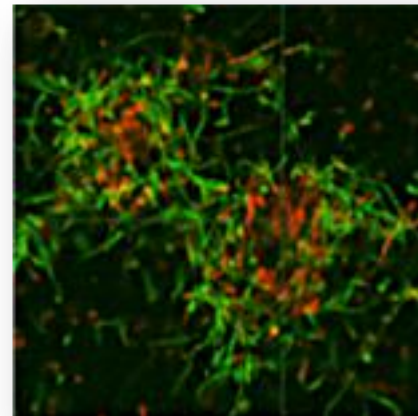
Matrix - EPS



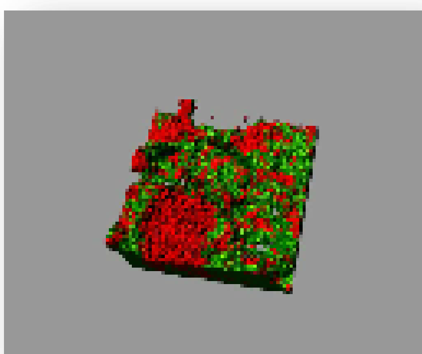
Diffusion-reaction



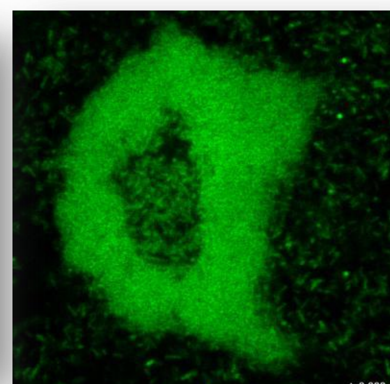
Gene expression



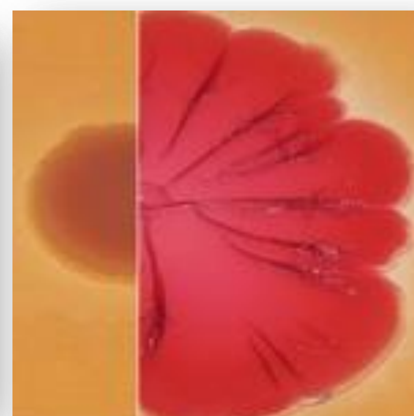
Localized cell death



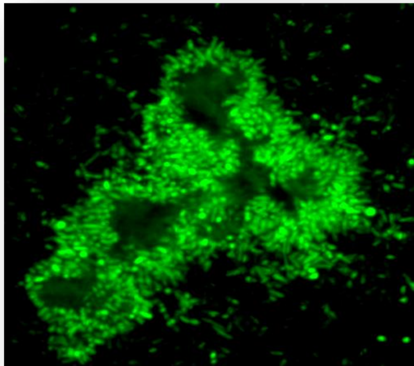
Dispersion



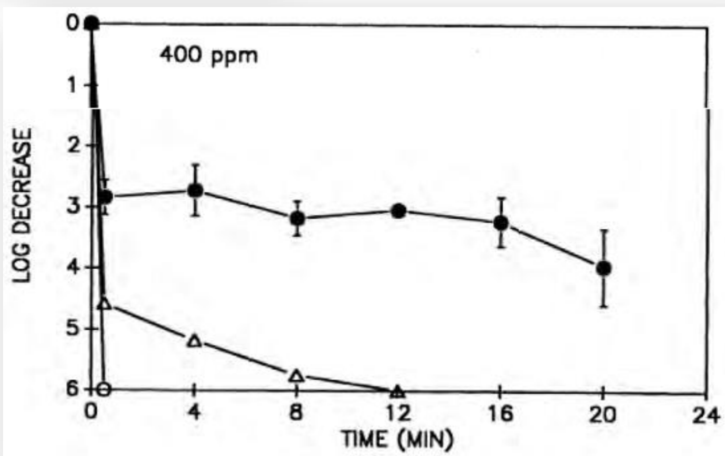
Genetic variations



The challenge of a collective resistance

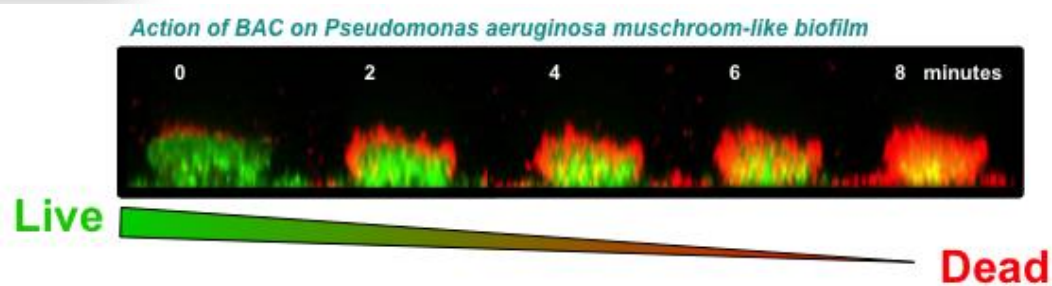


Disinfectants	Ratio of active concentration between biofilms and free cells
Oxidizing Agents	5 - 600
Quaternary Ammonium	10 - 1000



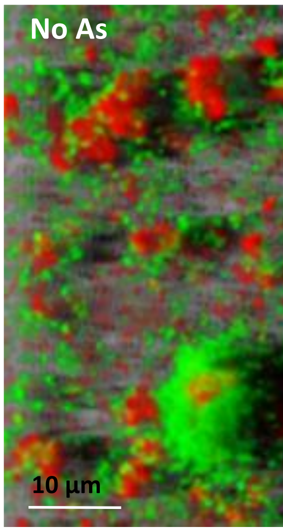
Activity of BAC on *Listeria monocytogenes*
free cells (O),
adherent cells (Δ),
biofilm cells (●)

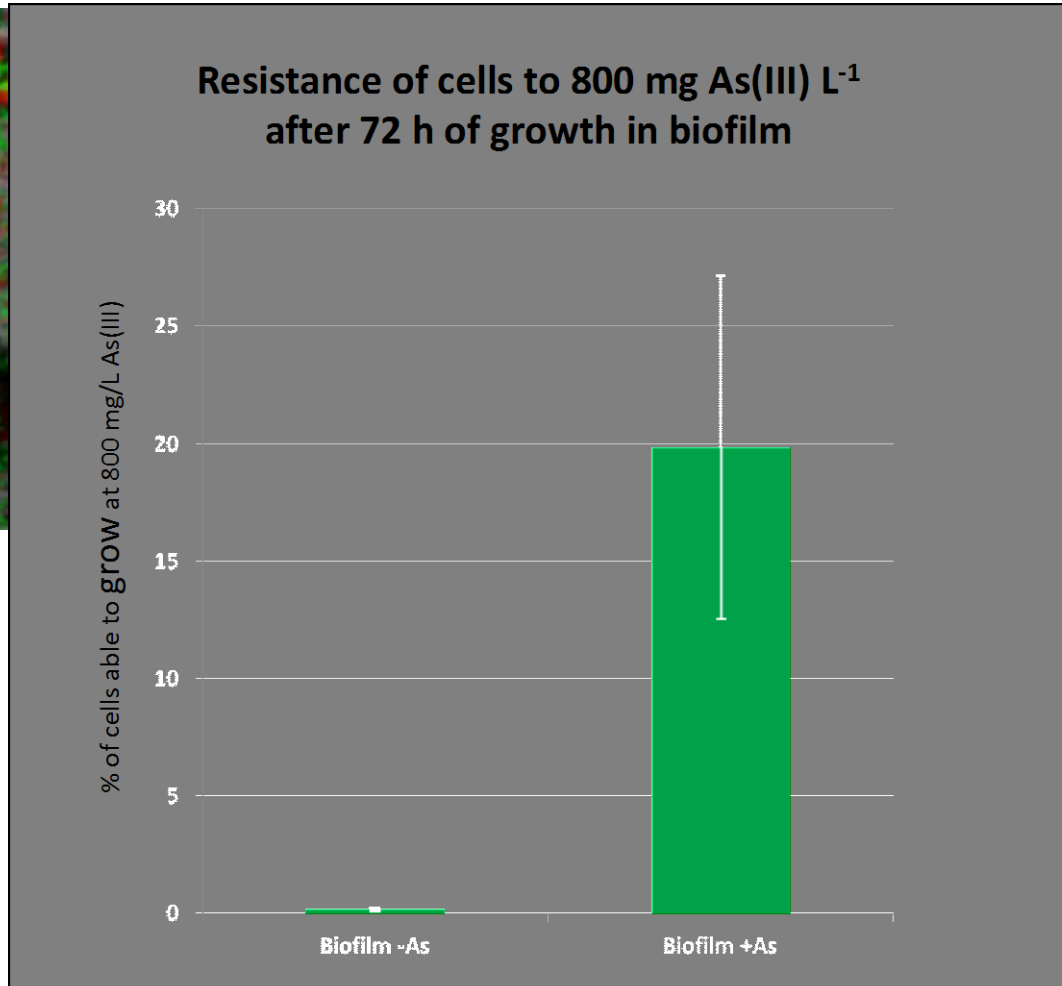
Frank and Koffi ,1990

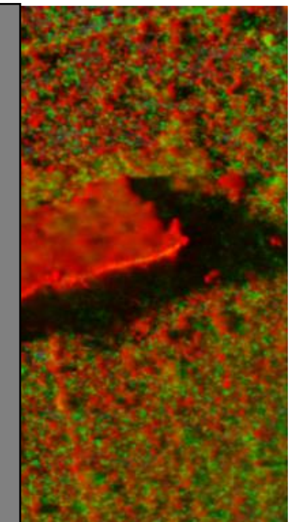


***Structural adaptation* to toxic compounds**

The case of Thiomonas spp. and arsenic



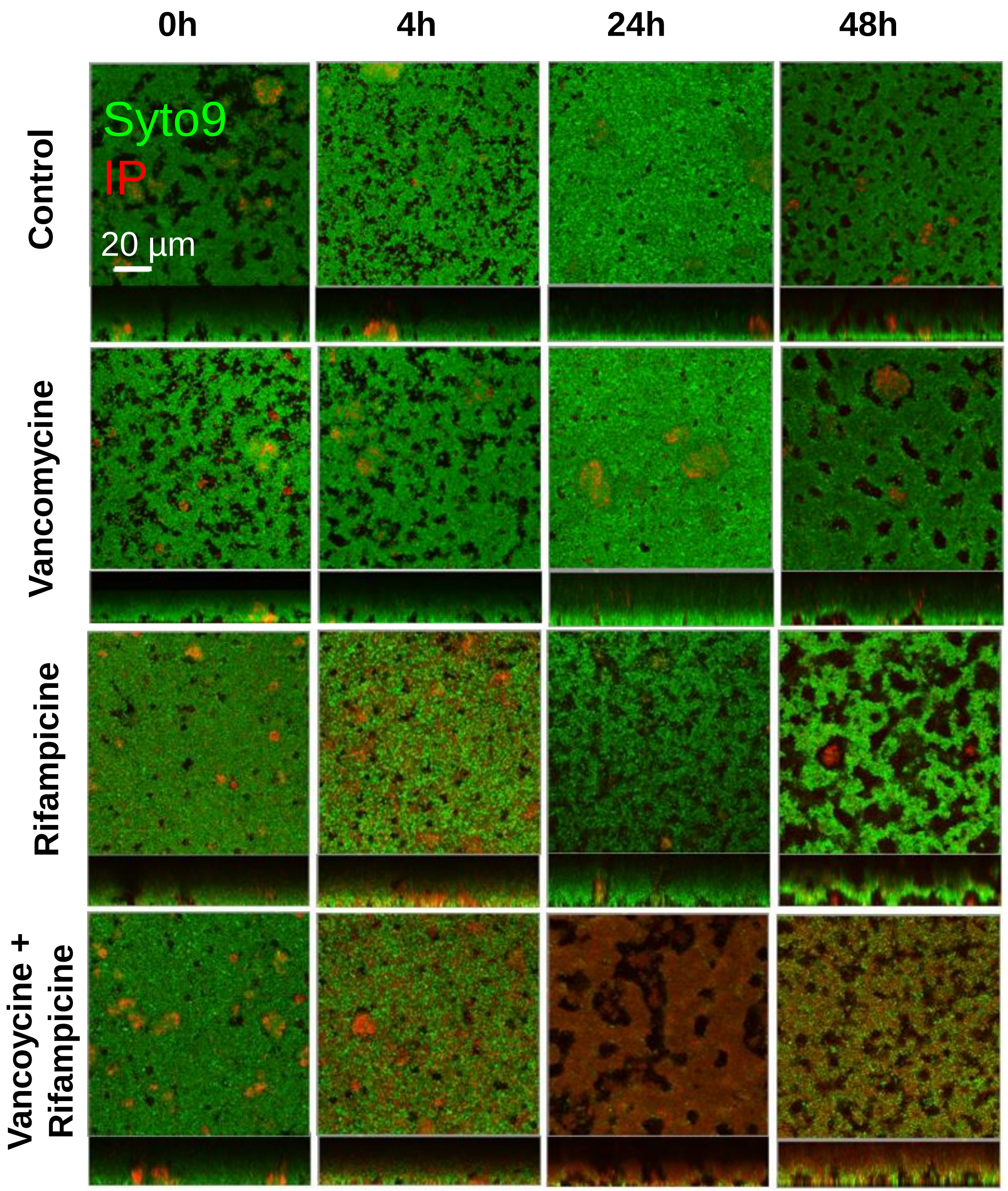




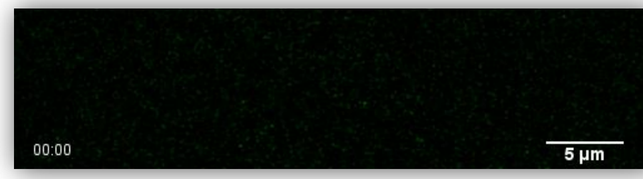
YTO9)
ysaccharides (ConA)

Marchal et al. Plos One 2011

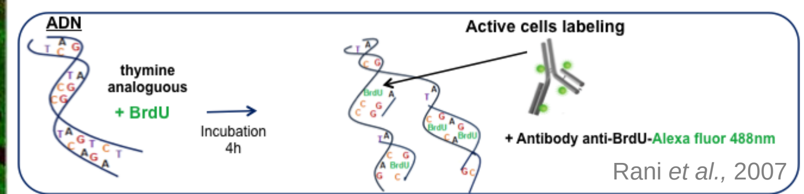
Resistance *vs* tolerance: a case study with antibiotics

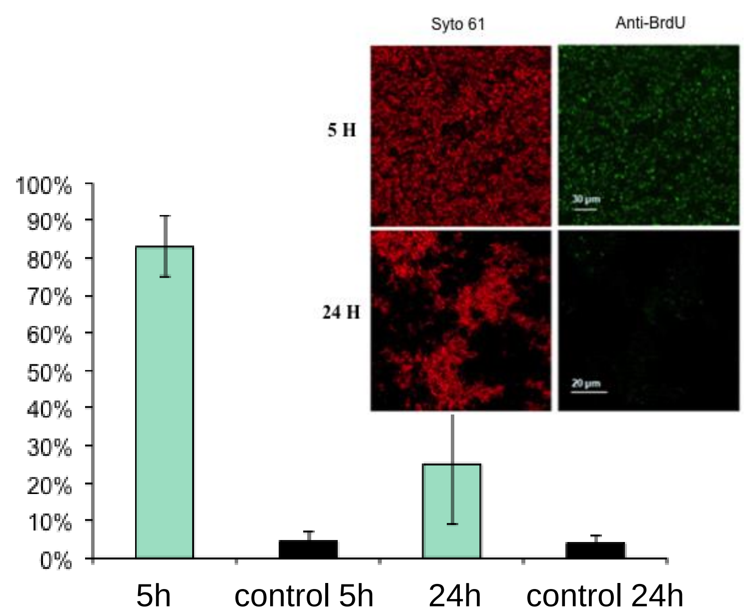


Vancomycin-Bodipy in *S.aureus* biofilm



Synthesis activity in *S.aureus* biofilm

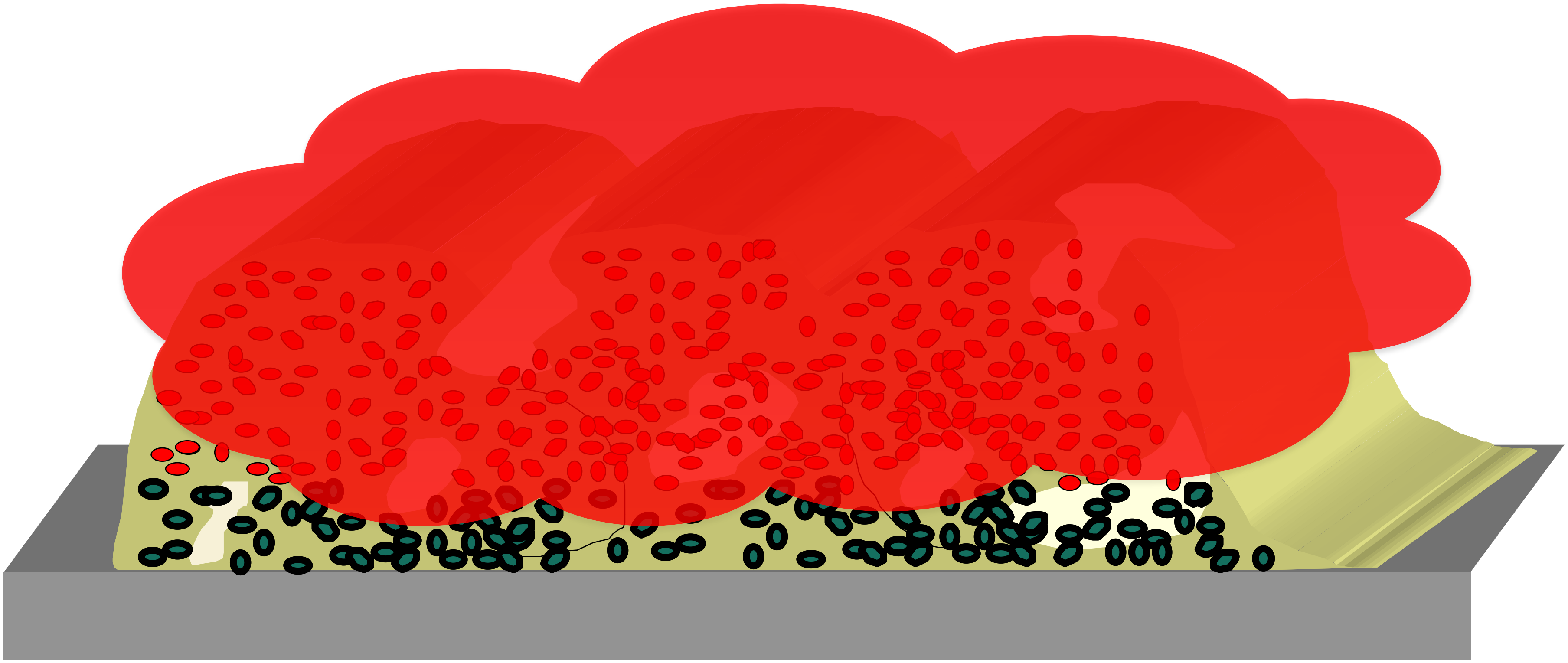




Samia Daddi Oubekka 2012

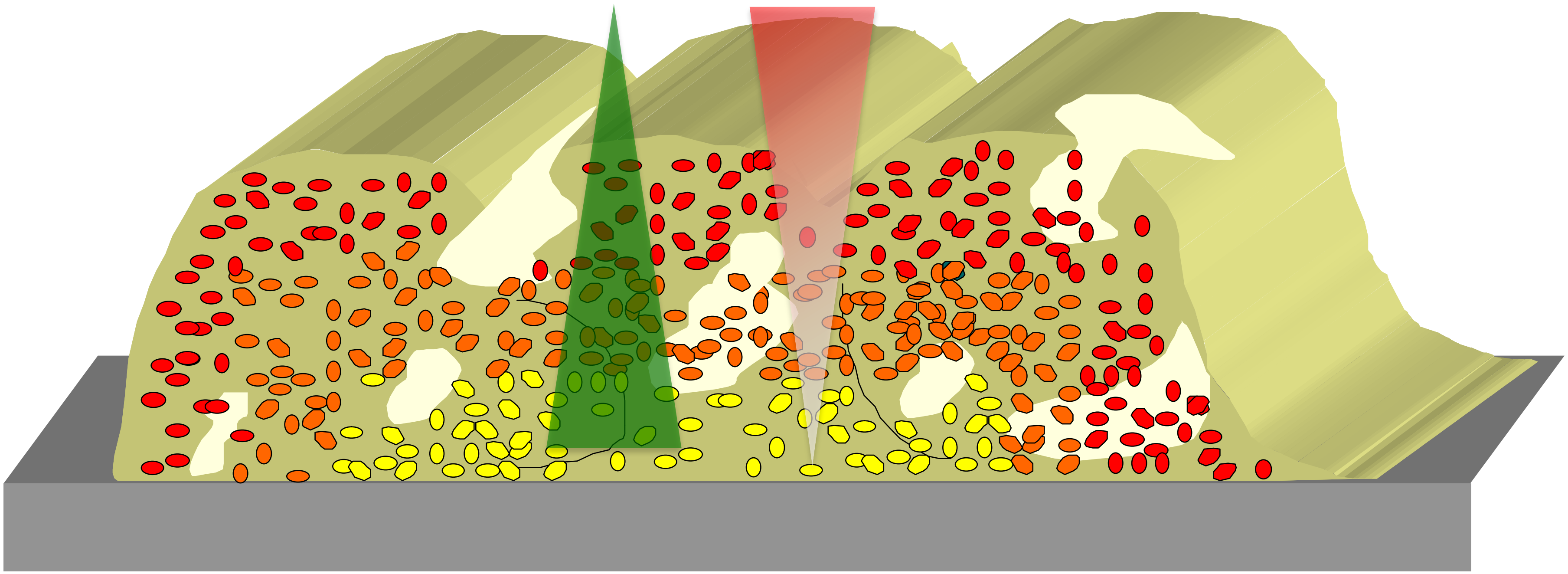
Relation biofilm architecture – tolerance to biocides

→ Diffusion – reaction limitation



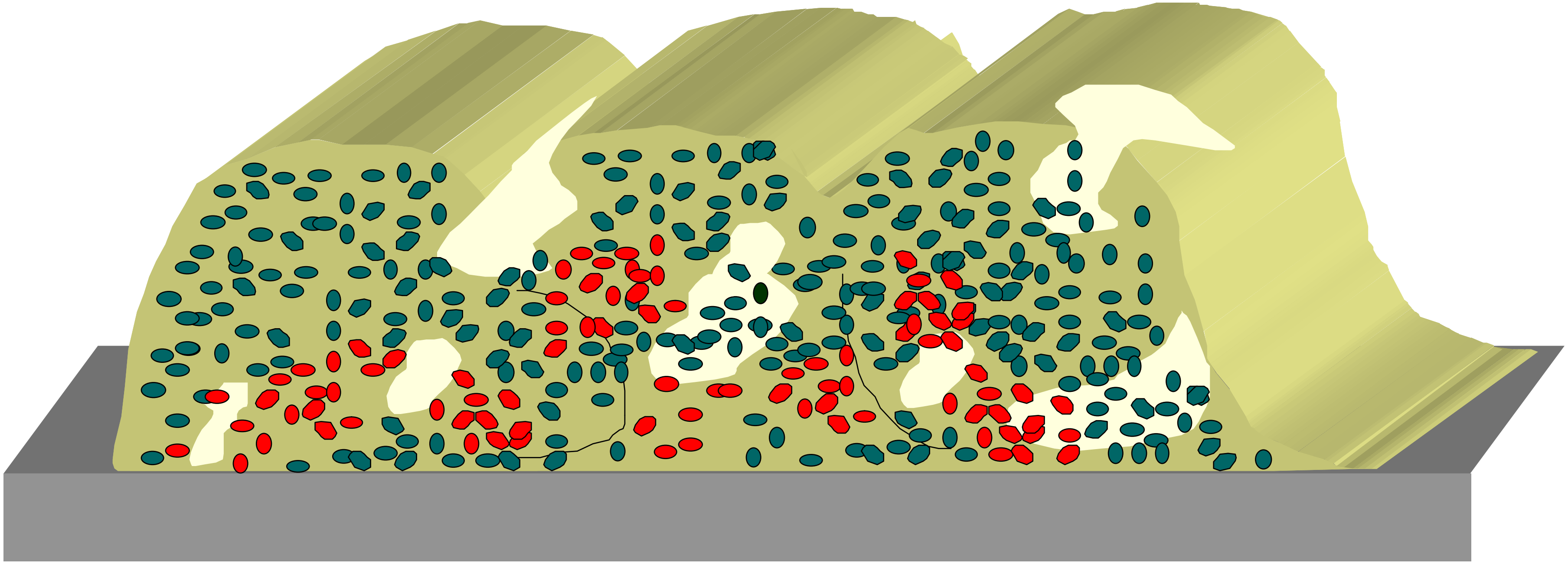
Relation biofilm architecture – tolerance to biocides

→ Stress response, slow growth, persisters

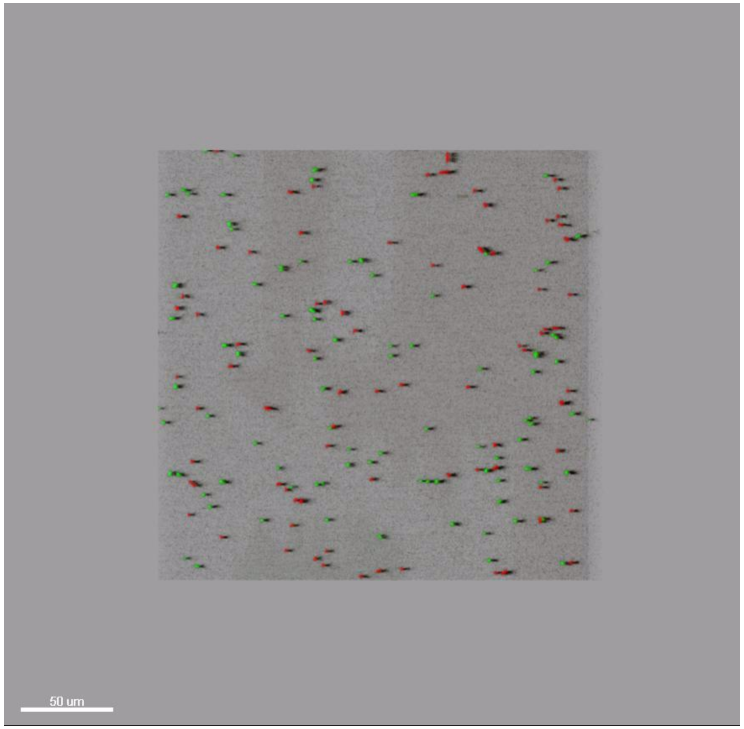


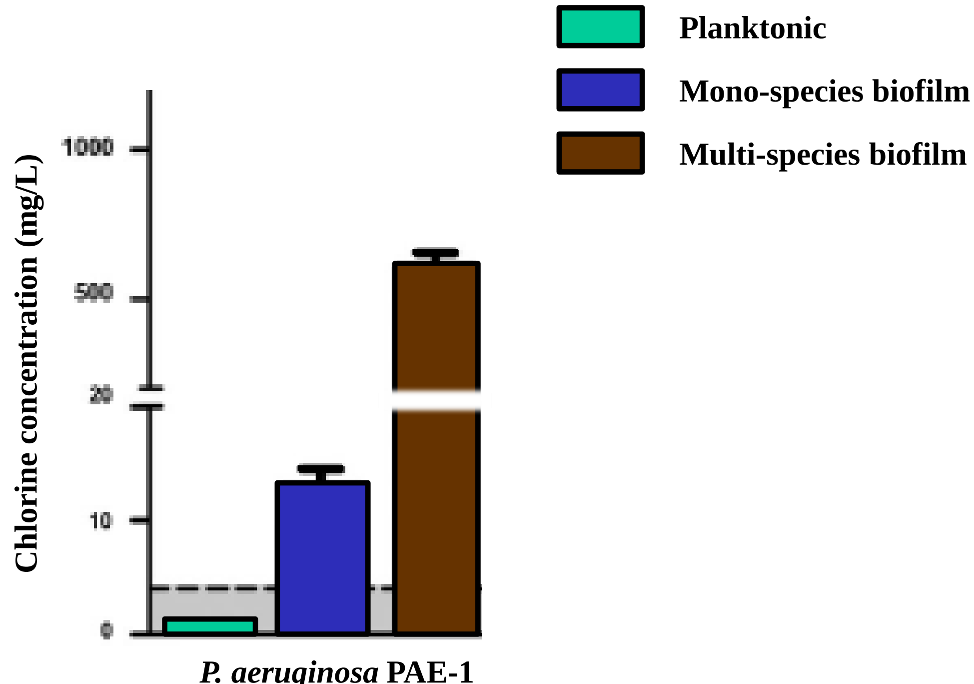
Relation biofilm architecture – tolerance to biocides

→ Emergence of resistant mutants



Hyper-tolerance to biocide in multispecies biofilms



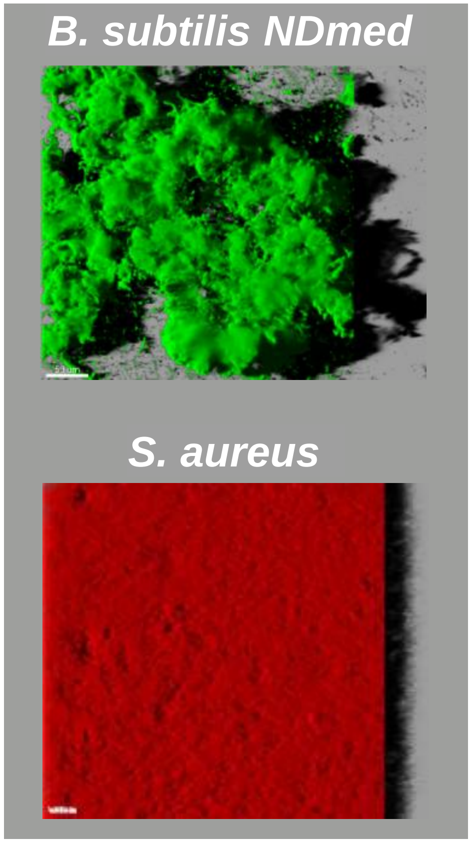


(Adapted from Schwering *et al.* 2013)

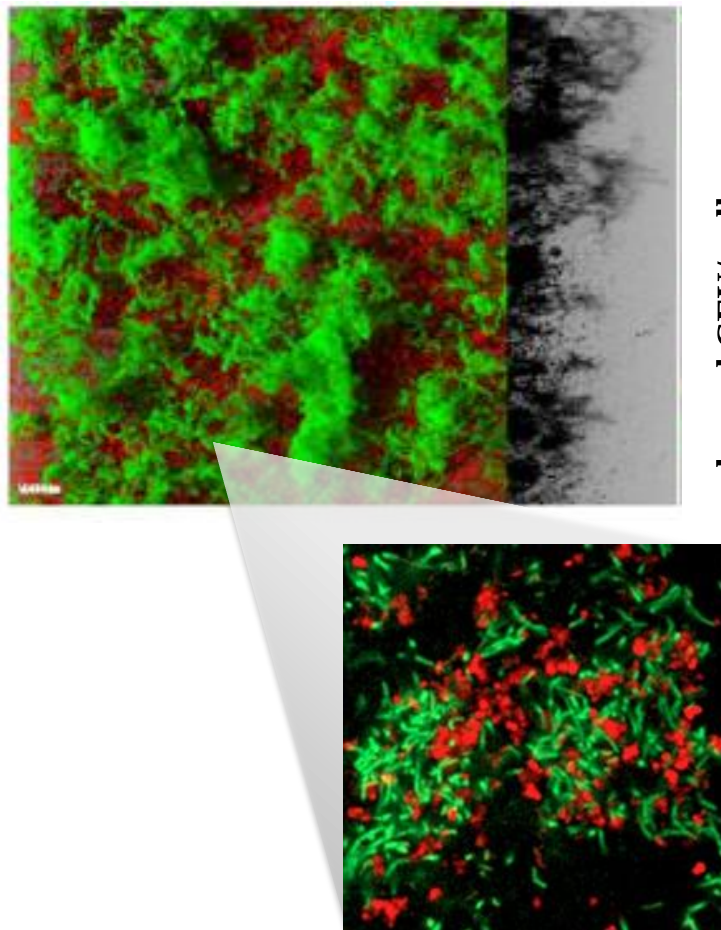
> The presence of different species in the biofilm can alter tolerance to biocides:

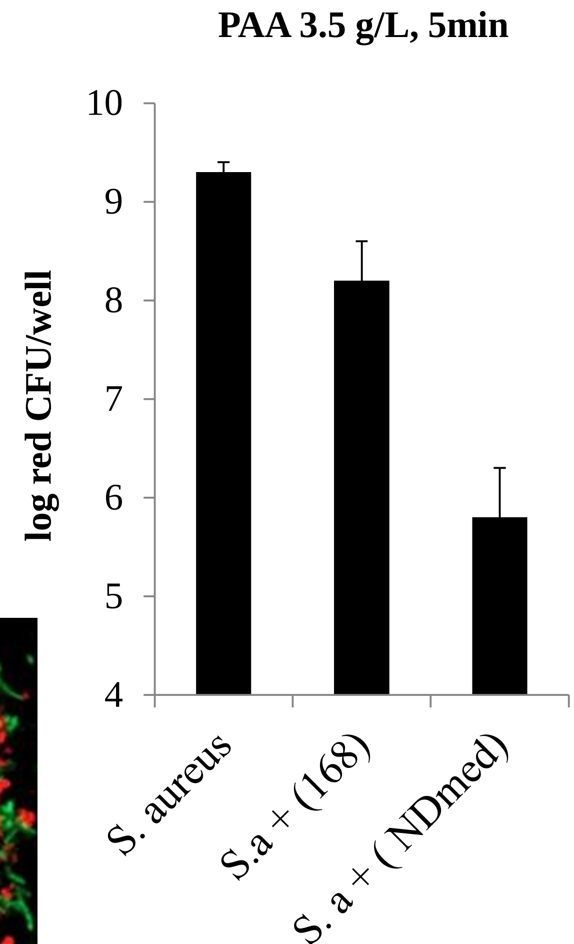
- Increase/ modification of the matrix
- Modification of architecture/ gradients
- Share of "public goods"
- Cell-cell communication (QS)
- Undescribed mechanisms ?

Protection of *Staphylococcus aureus* in mixed species biofilms with *Bacillus subtilis* NDmed



B. subtilis* NDmed + *S. aureus

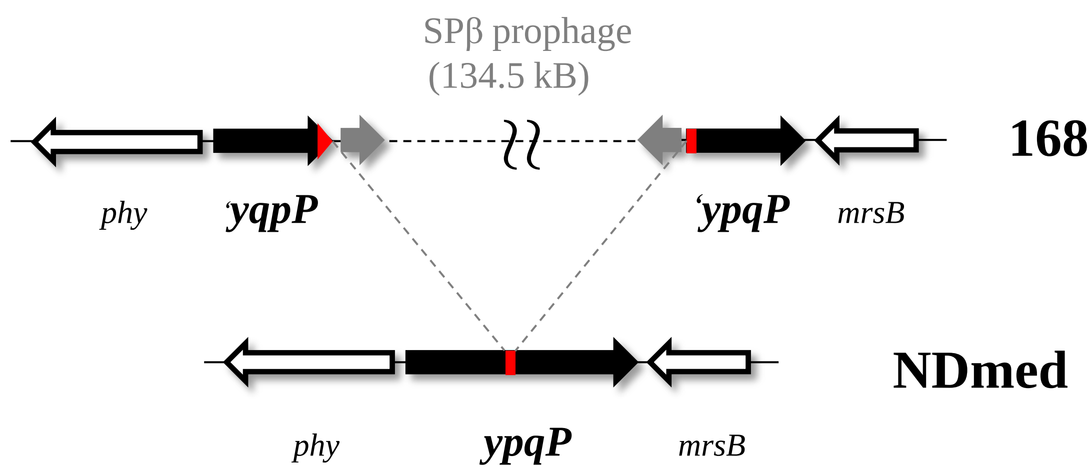




Bridier et al., Plos One 2011

ypqP is disrupted in the lab strain 168 but not in the natural strain NDmed

A



B

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168   MPKQQTAELKPFFHNKTVLVTGGTGSIGSQIVKRLLMLTPKQVIVFSKDDSKQYVMSQKYAEDKRLLFVLGDVRDHRRVNQVMKGVDIVFHAAALKQVPT
NDmed MPKQQTAELKPFFHNKTVLVTGGTGSIGSQIVKRLLMLTPKQVIVFSKDDSKQYVMSQKYAEDKRLLFVLGDVRDHRRVNQVMKGVDIVFHAAALKQVPT 100

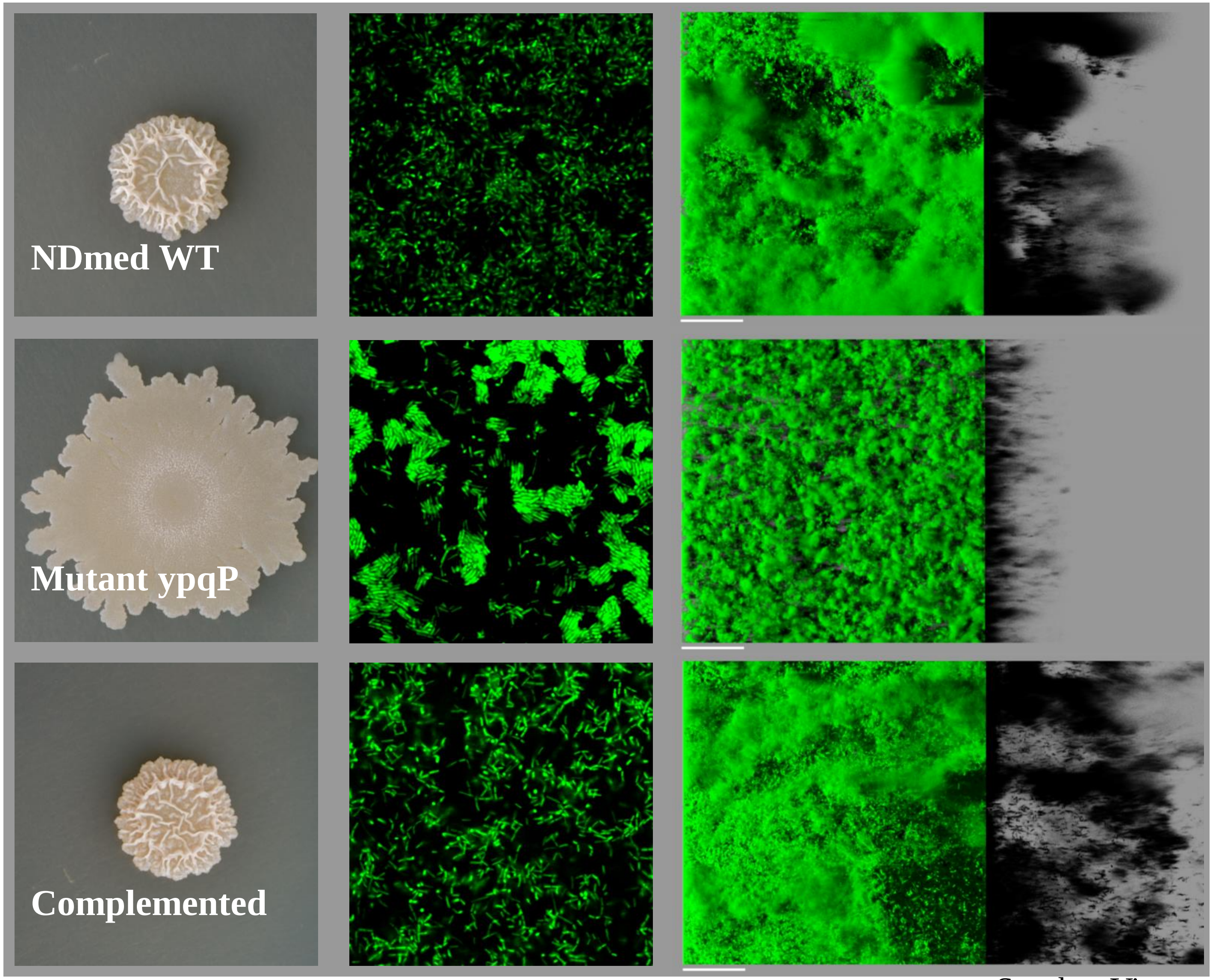
168   CEDHPFEAIQTNLIGGQNVVEAALSHRVQHVINISTDKAVY*
NDmed CEDHPFEAIQTNLIGGQNVVEAALSHRVQHVINISTDKAVSPVNTMGATKLLSEKLFHQANRHVQNKGTLFCSVRFGNVLGSRGSVIPILFEQMMEGEPL 200

168
NDmed TITDKNMTRFFMSIDDAATLTLQSAAITKGGETFIFKMESLKLEELIHGFEEYASQHGLPRPAAVEVGKRPGEKLHEELTSPHEIESLYEWGNLYAILPE 300

168
NDmed PEKHPDFRKVNLPGYQSDQAPLITKERIAQIIEELHQEKKA*                                                           341
```

Sanchez Vizuete et al. Genome Announc. 2014 Sep 25;2(5).

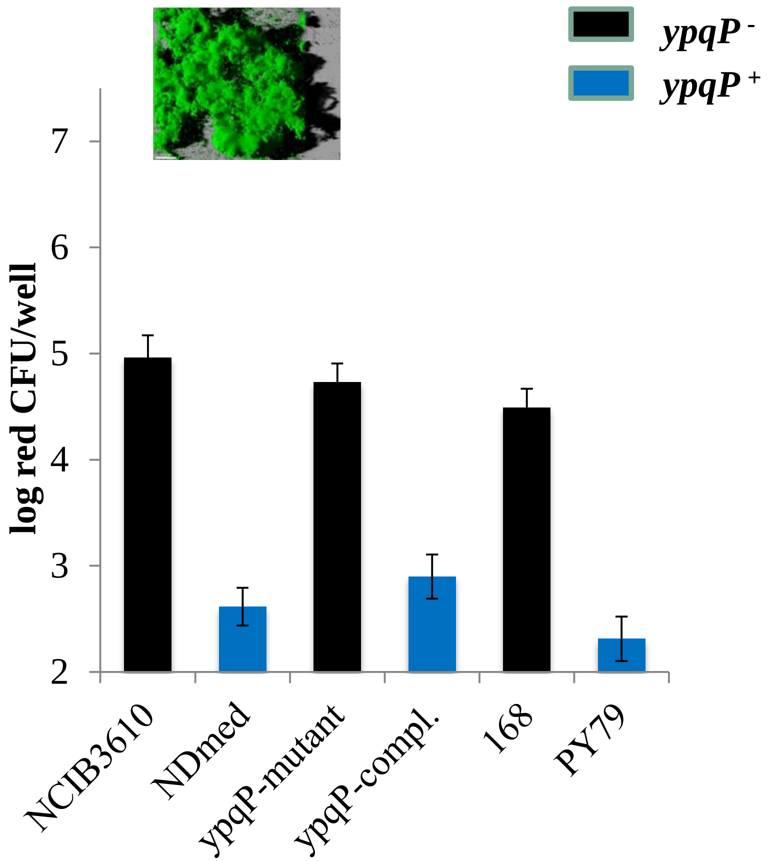
Effect of *ypqP* disruption in submerged biofilm structure and colony morphology in *B. subtilis* NDmed

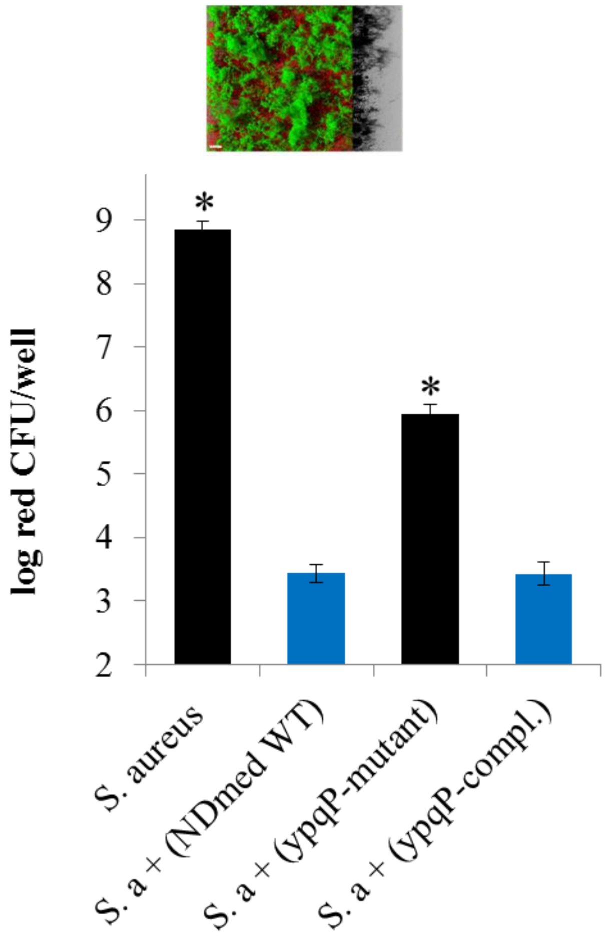


Sanchez Vizuete et al. AEM 2015

Antimicrobials tolerance of biofilms formed by *B. subtilis* *ypqP* disrupted or non-disrupted strains

OPA 10 g/L, 5min

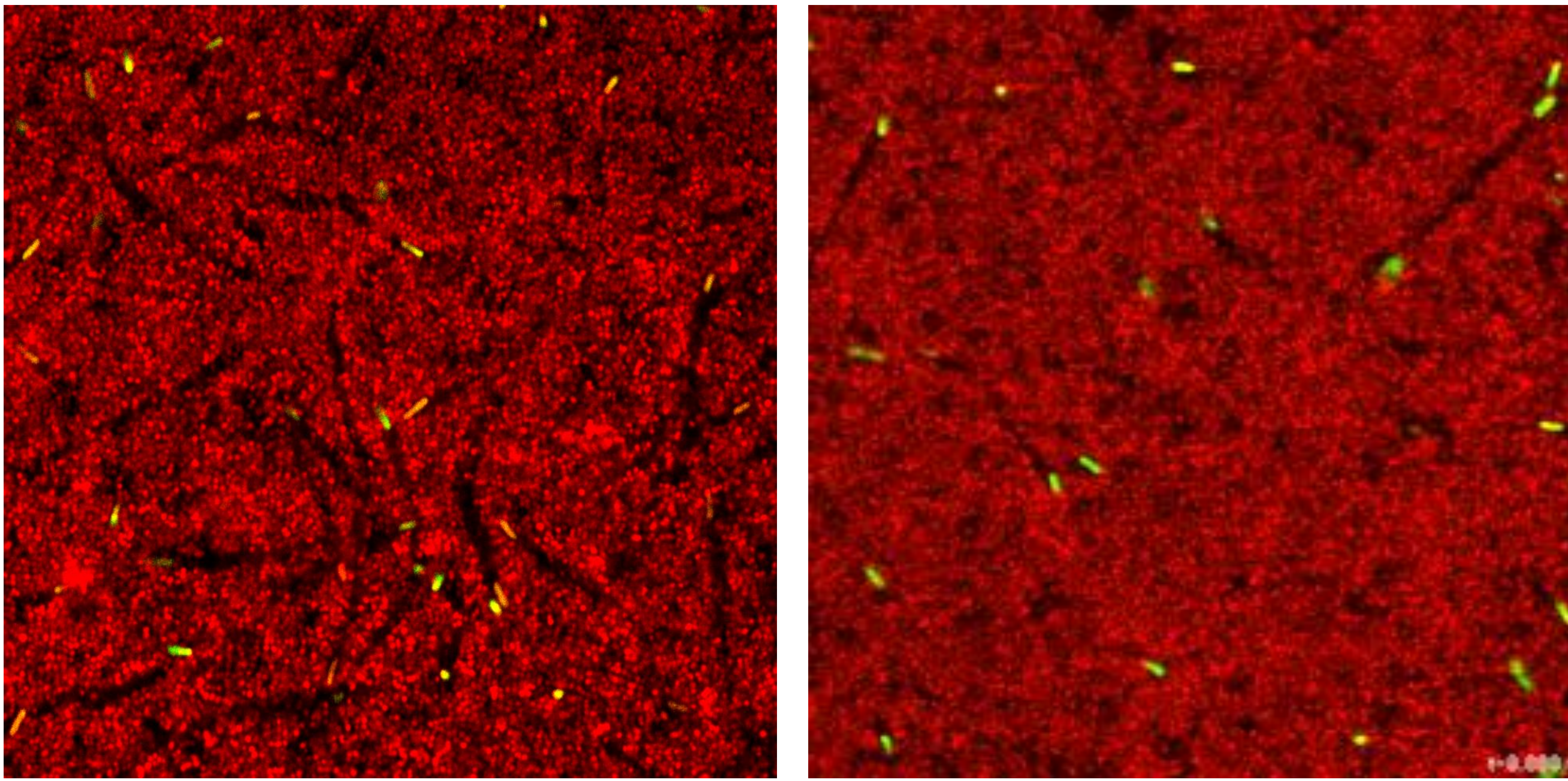




Sanchez Vizuete et al. AEM 2015

INFILTRATION OF THE BIOFILM MATRIX BY STEALTH SWIMMERS

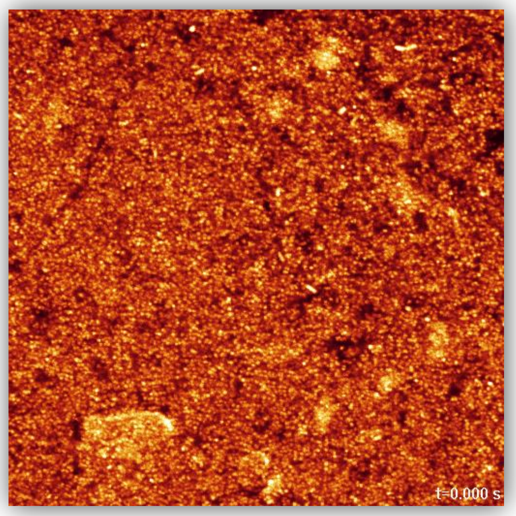
S.aureus biofilms (red) + *B. thuringiensis swimmers* (green)



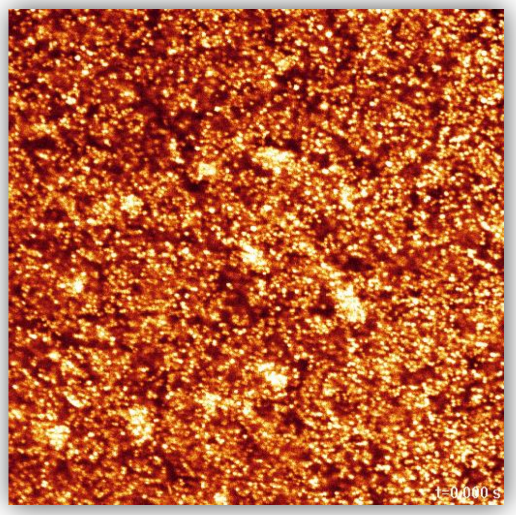
ANR GreenSwimmers

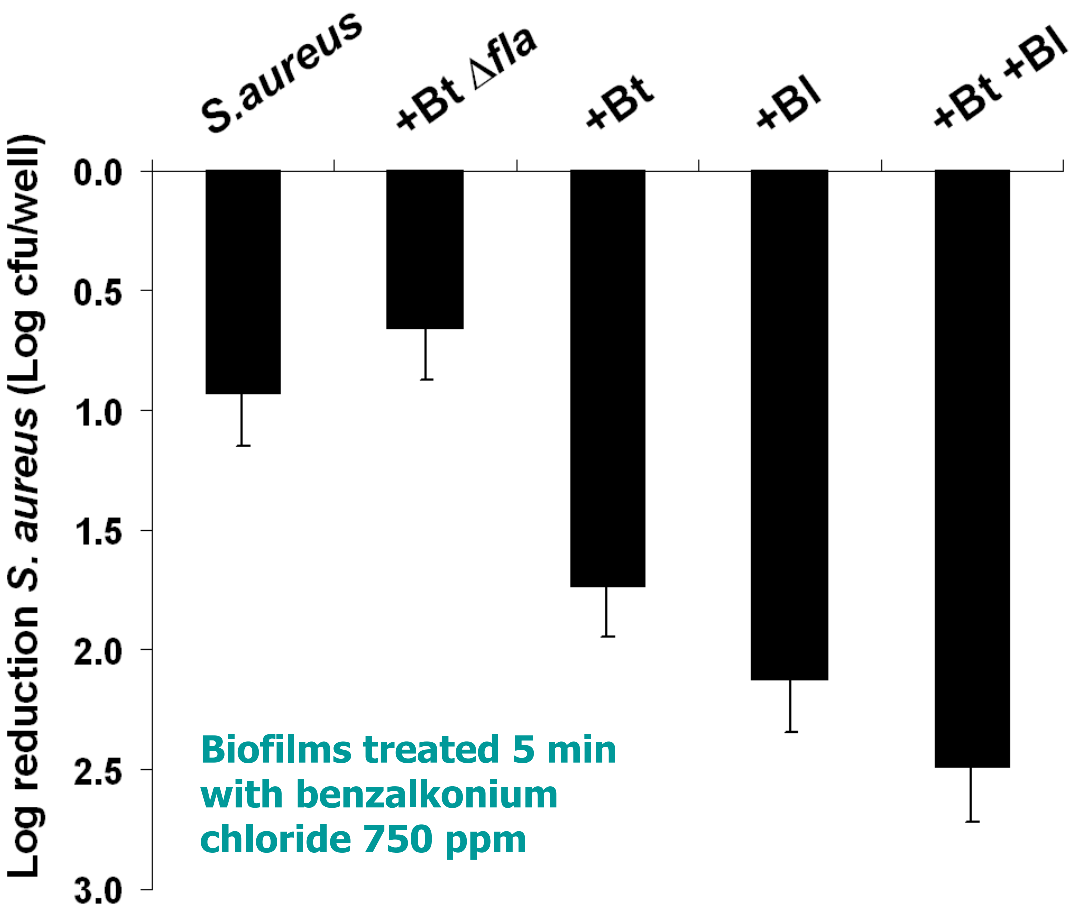
SWIMMERS INFILTRATION SENSITIZES *S. AUREUS* BIOFILMS TO CHEMICAL ANTIMICROBIALS

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



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

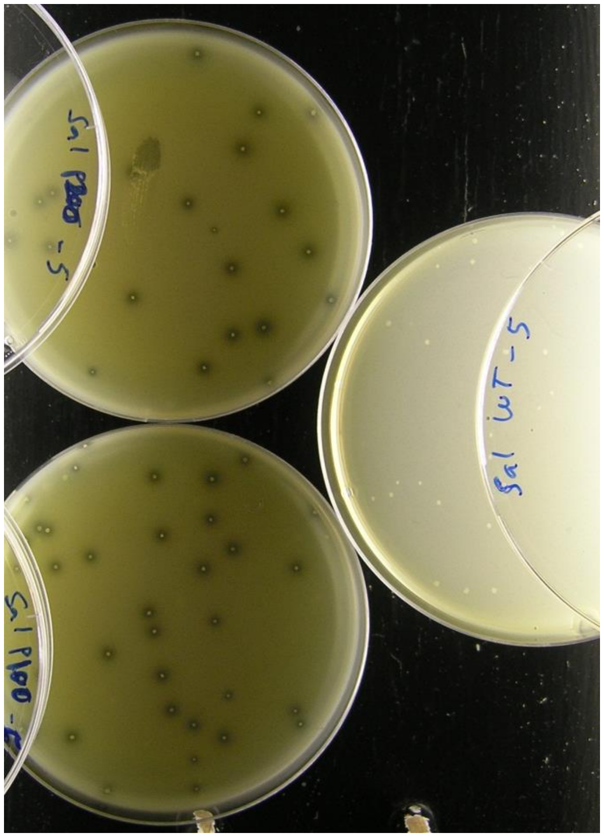




Houry *et al.* PNAS 2012

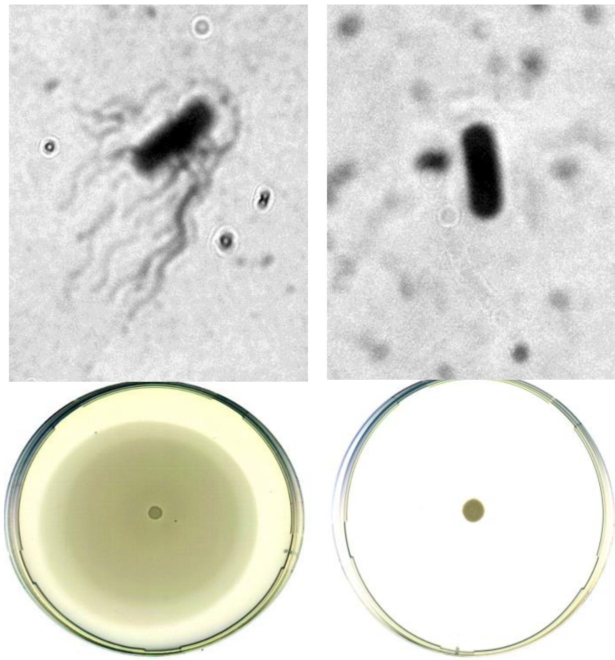
Can stealth swimmers deliver self-produced antimicrobial in the target biofilm ?

Lysostaphin (Lys+)



Bt WT

Bt *Δfla*

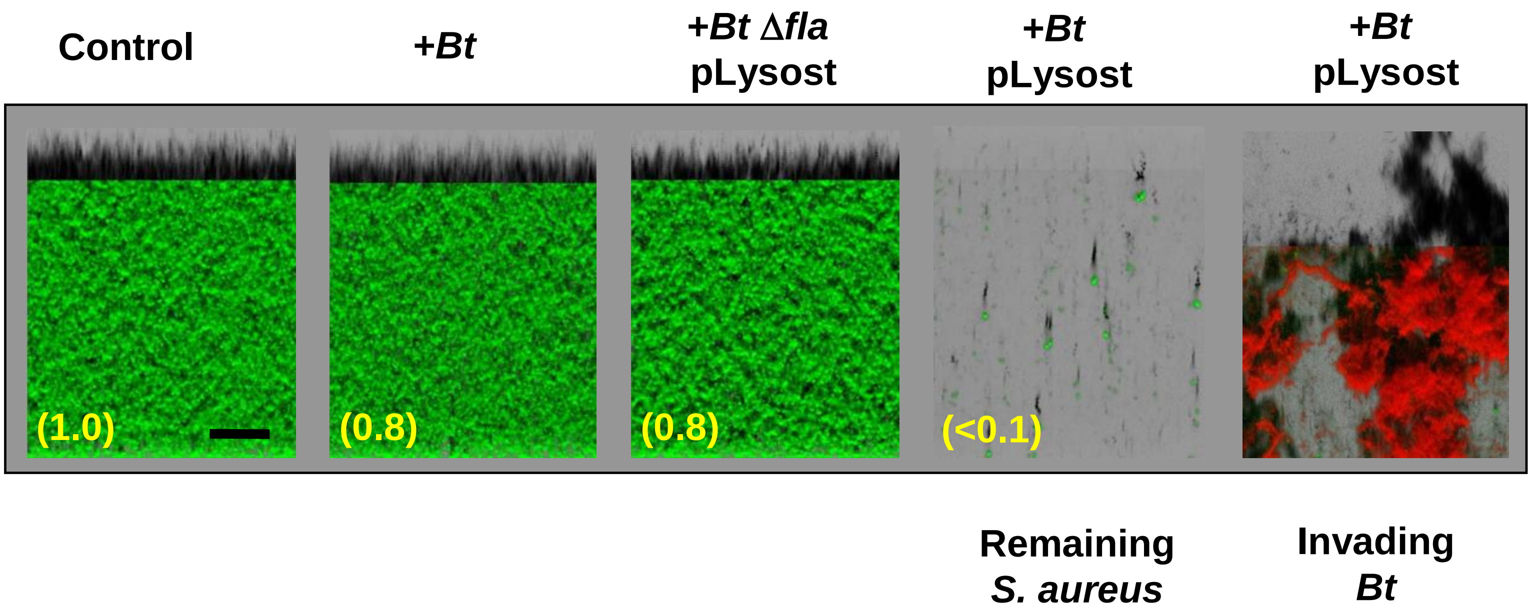


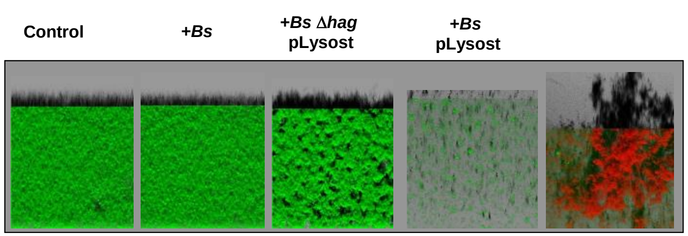
Motility +

Motility -

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

S.aureus **biofilms (normalized biovolumes)**





Similar results with *B. subtilis*

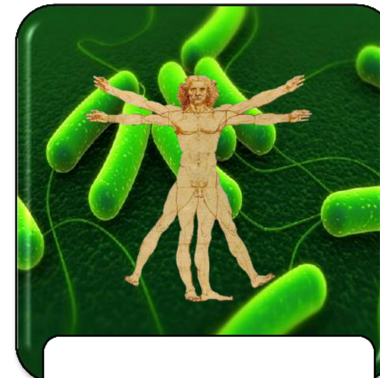
Houry *et al.* PNAS 2012

Toward chemical-free microbiological control in the food chain ?

Food production

Food processing

Food preservation

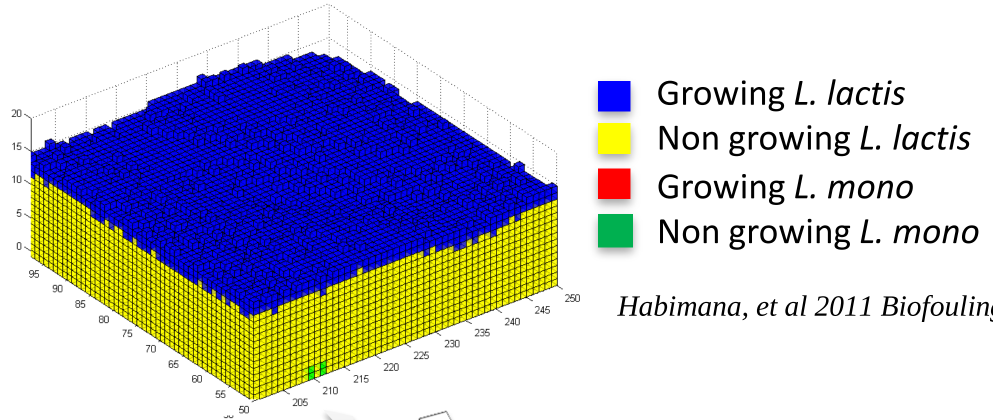


Foodborne infections

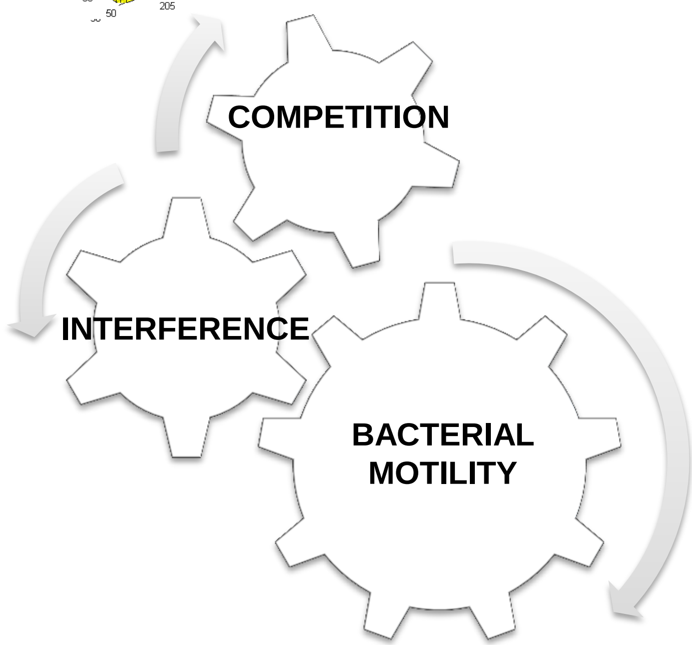
> fongicides, disinfectants, preservatives, antibiotics

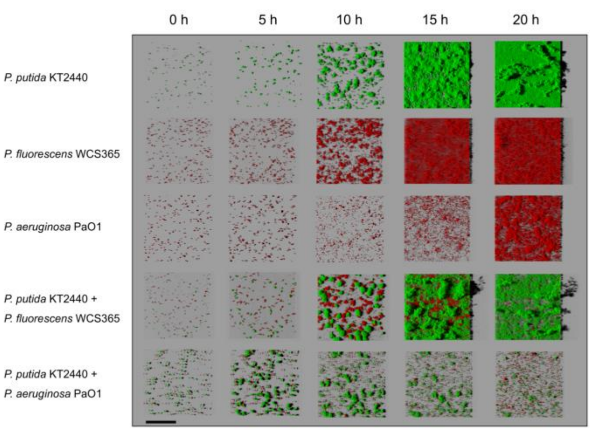
> biocontrol, protective biofilms, biopreservatives, probiotics

> Spatially-driven mechanisms of interactions

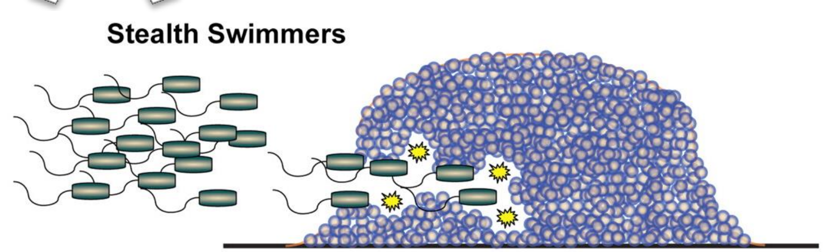


Habimana, et al 2011 Biofouling





Bridier et al, 2014 Biofouling



Houry et al., 2012 PNAS

Micalis Institute

INRA , AgroParisTech,

Université Paris-Saclay.

Team B2HM & MIMA2 Imaging center