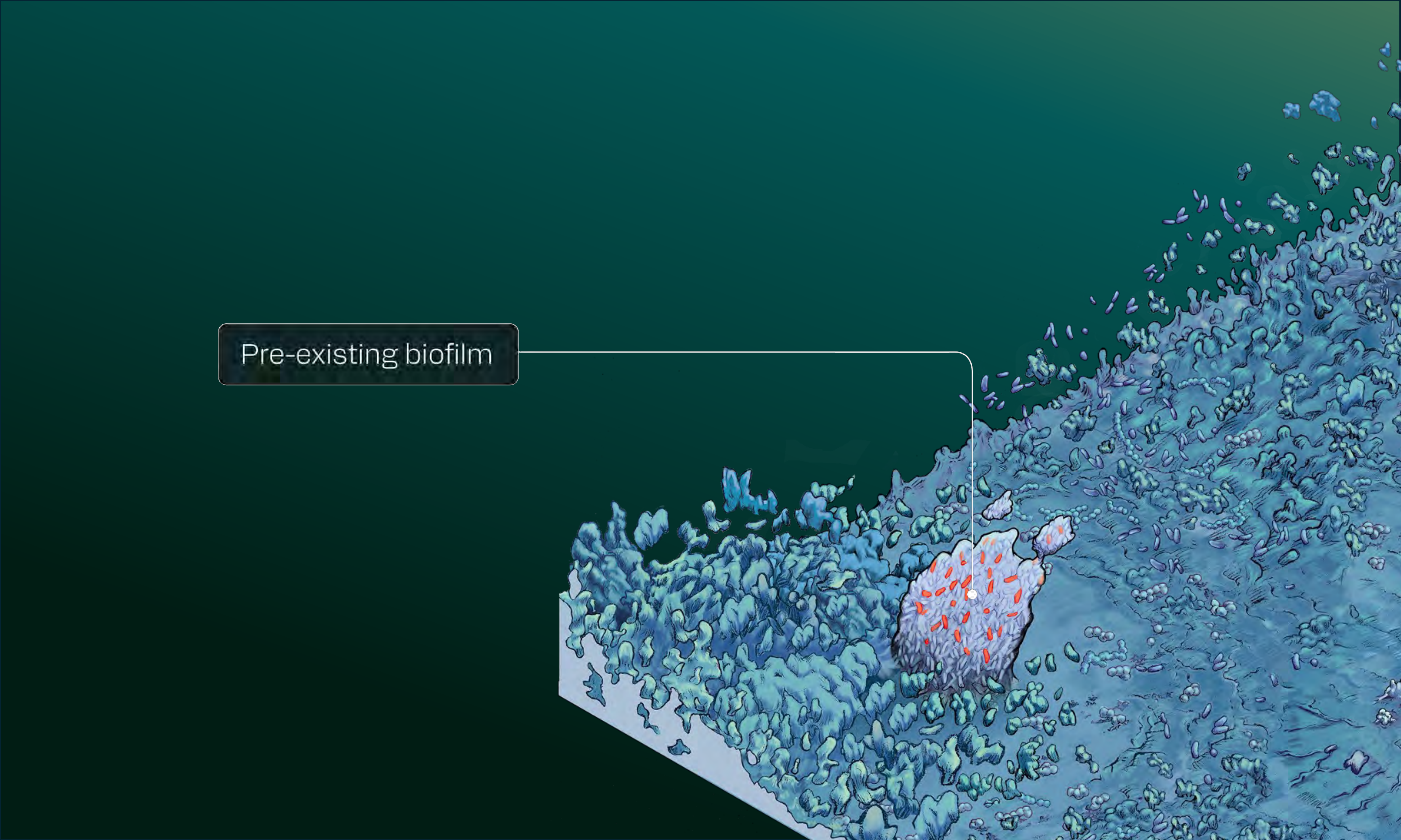
A1 RB-3 : « Biofilms bénéfiques : des communautés aux applications »

Beneficial biofilms

Unlocking the potential of biofilm properties in beneficial microbes for one health advancements

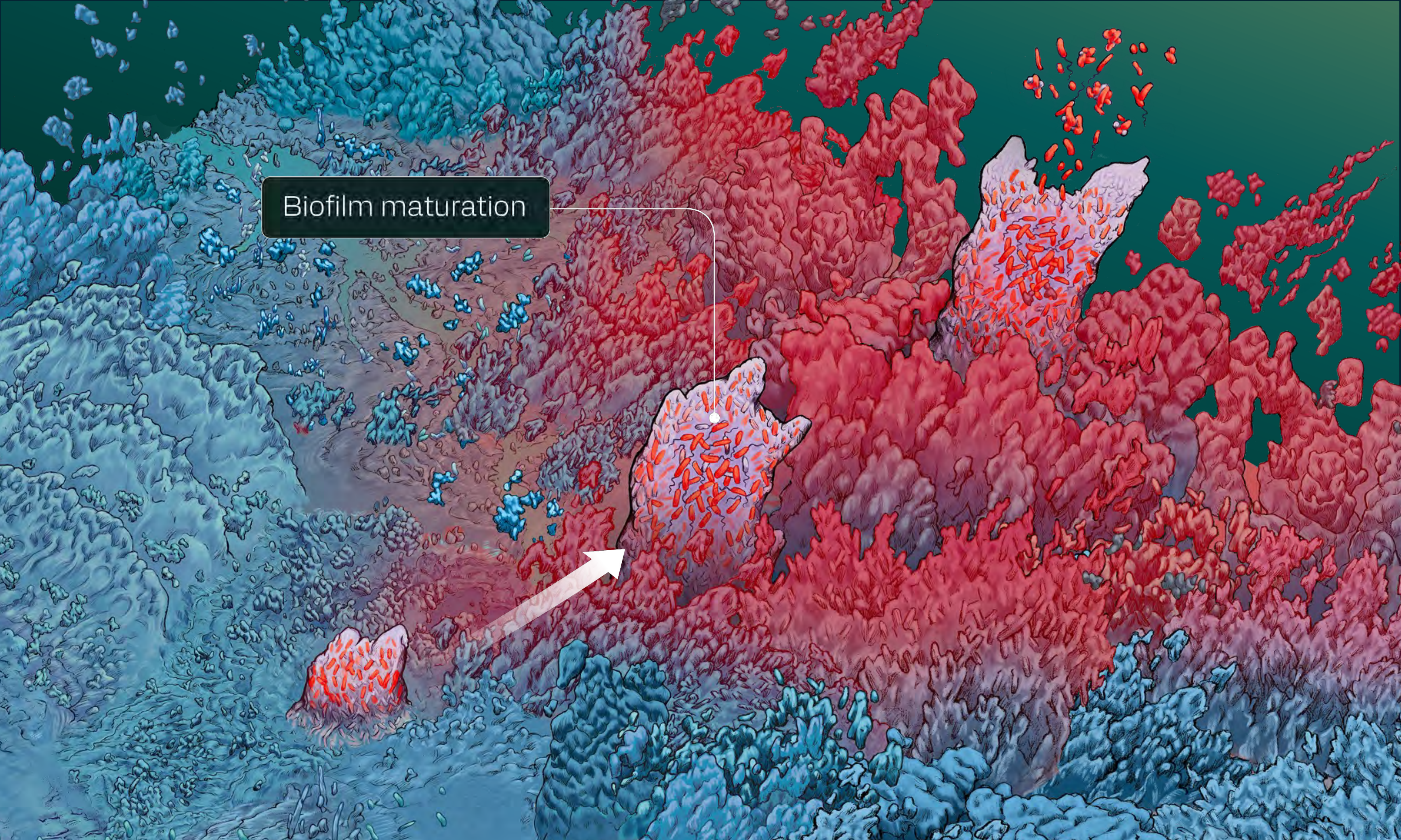

Pre-existing biofilm

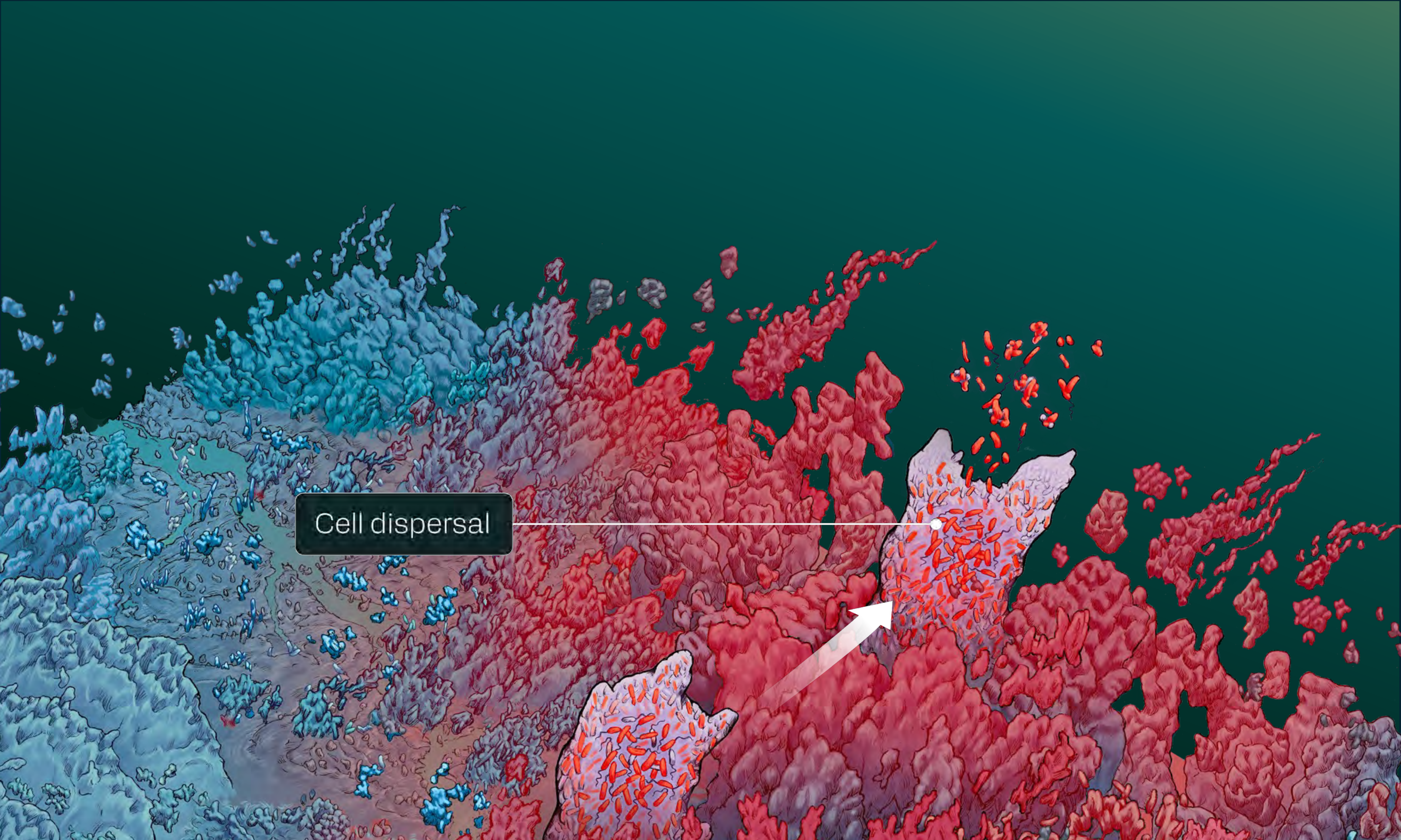
Cleaning and control intervention

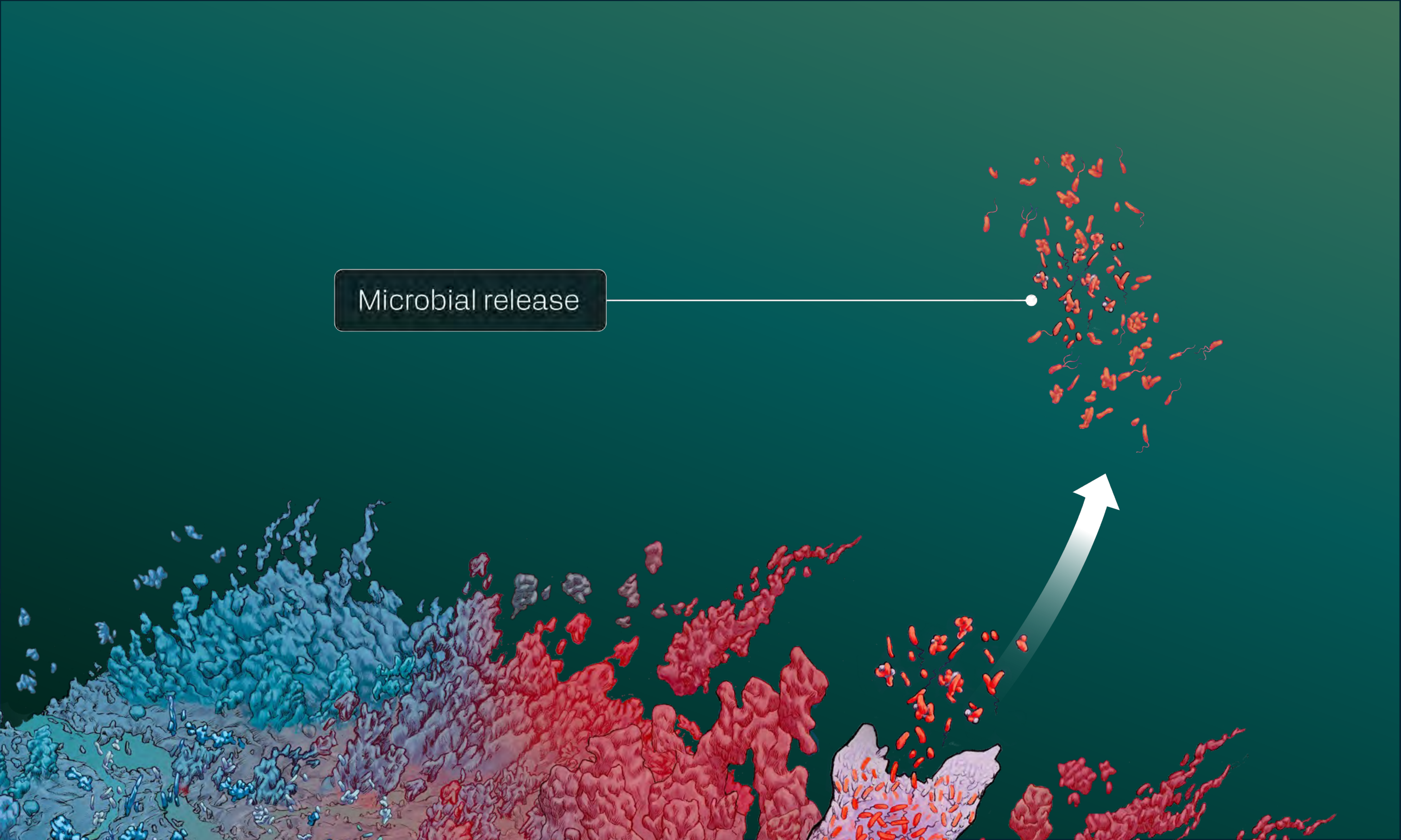
Schematic of a mature biofilm after surface treatment. The structure is only partially reduced due to biofilm resilience, driven by limited diffusion, metabolic specialization, and matrix-based protection.

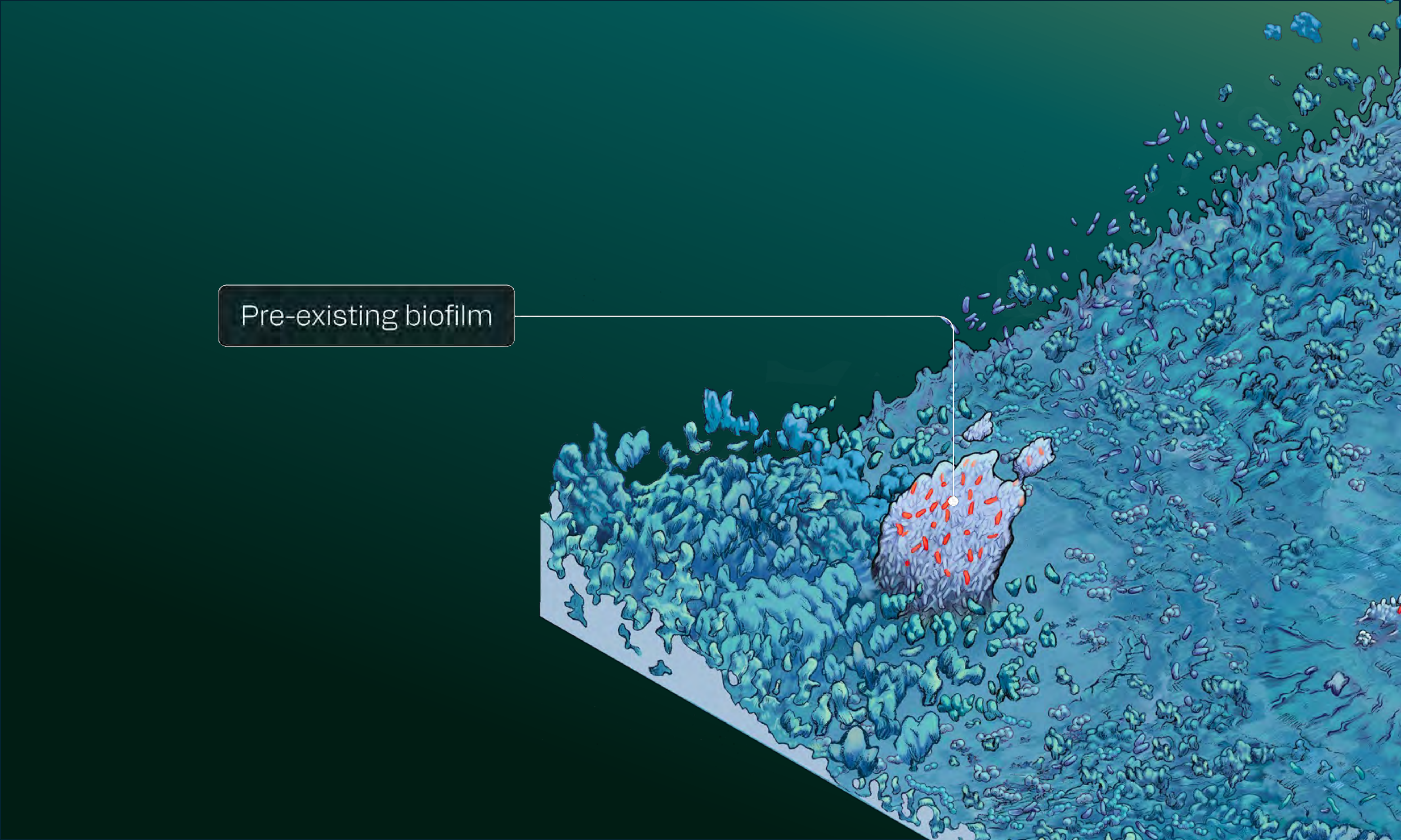

Residual biofilm survival

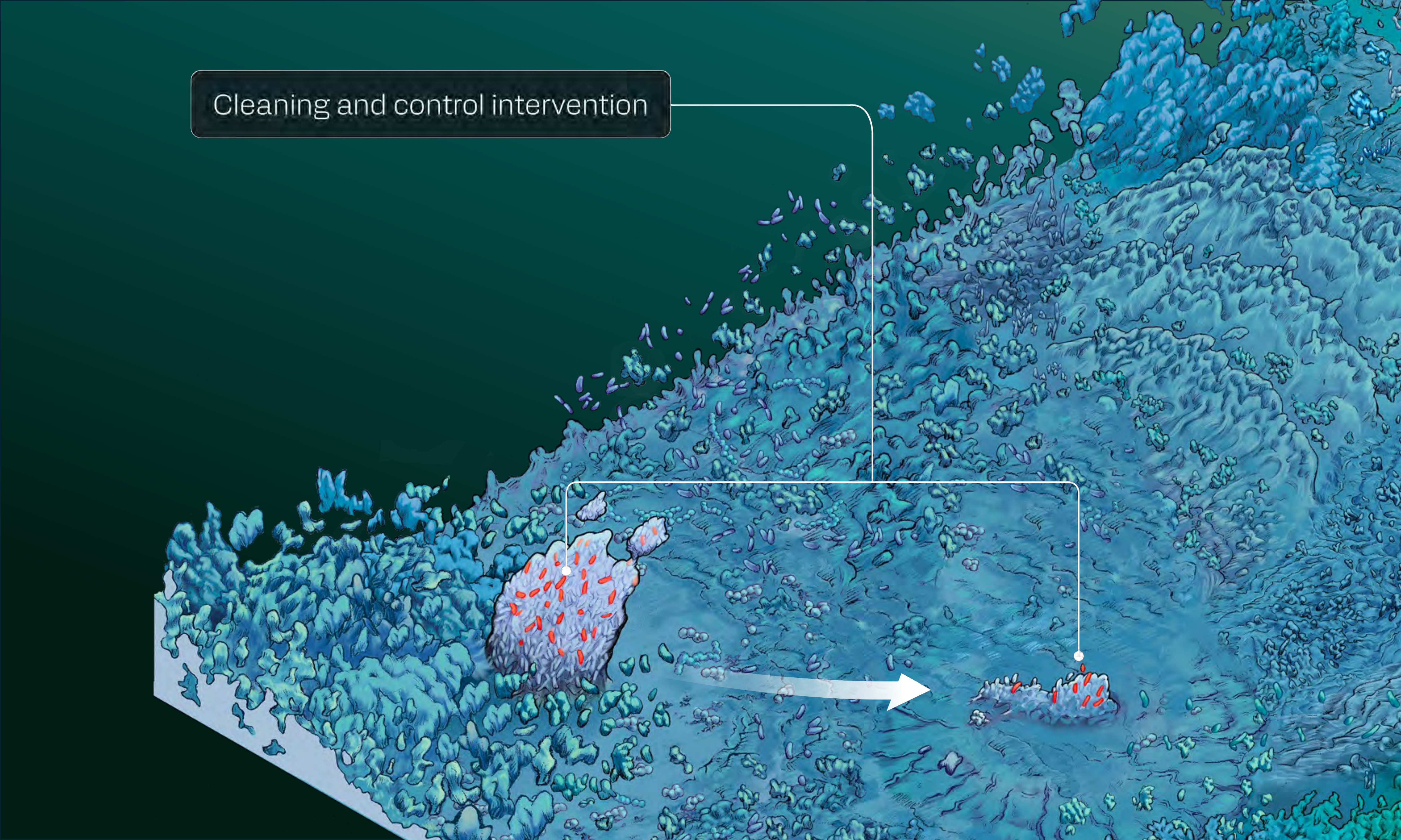

Post-treatment bloom


Biofilm maturation


Cell dispersal

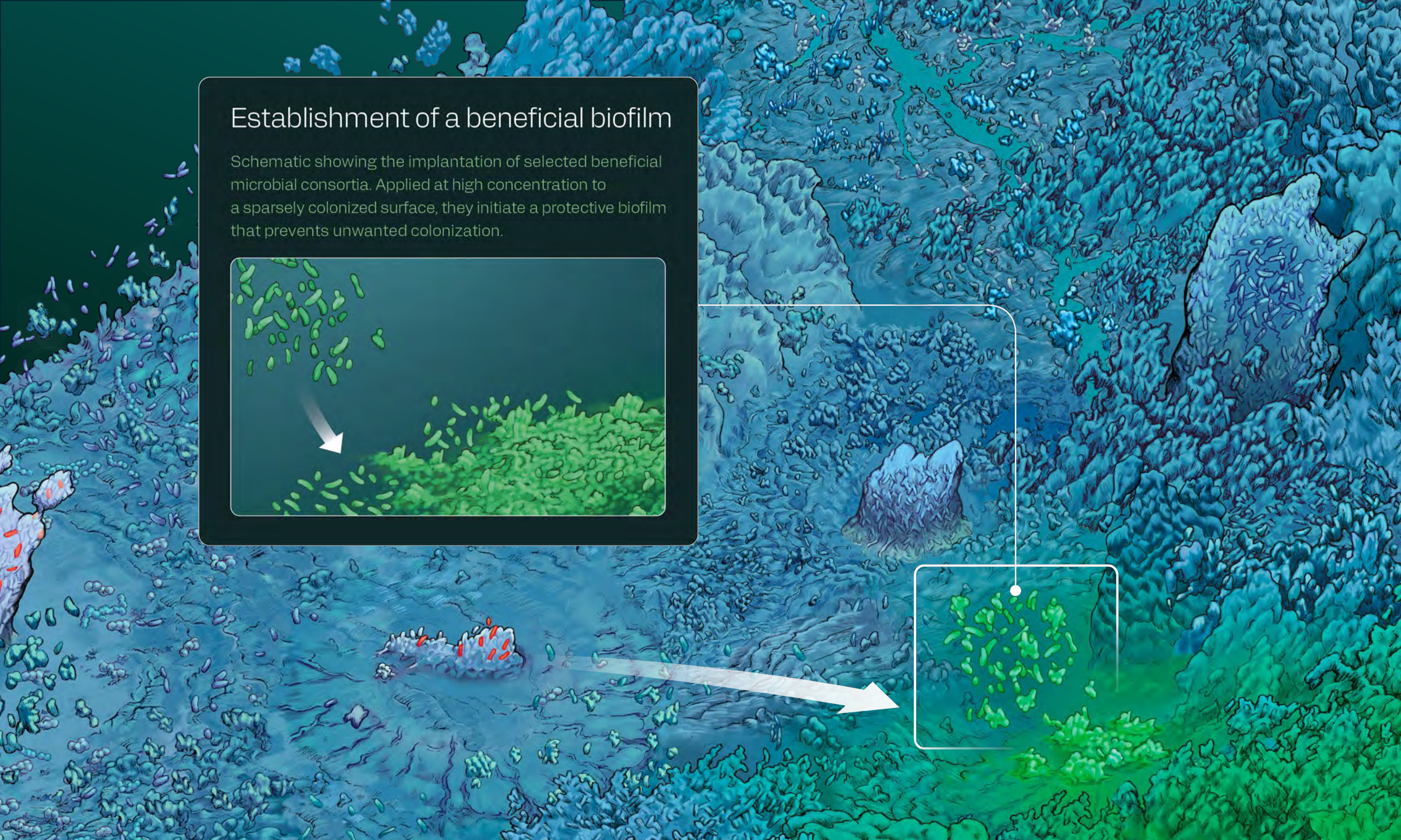

Microbial release

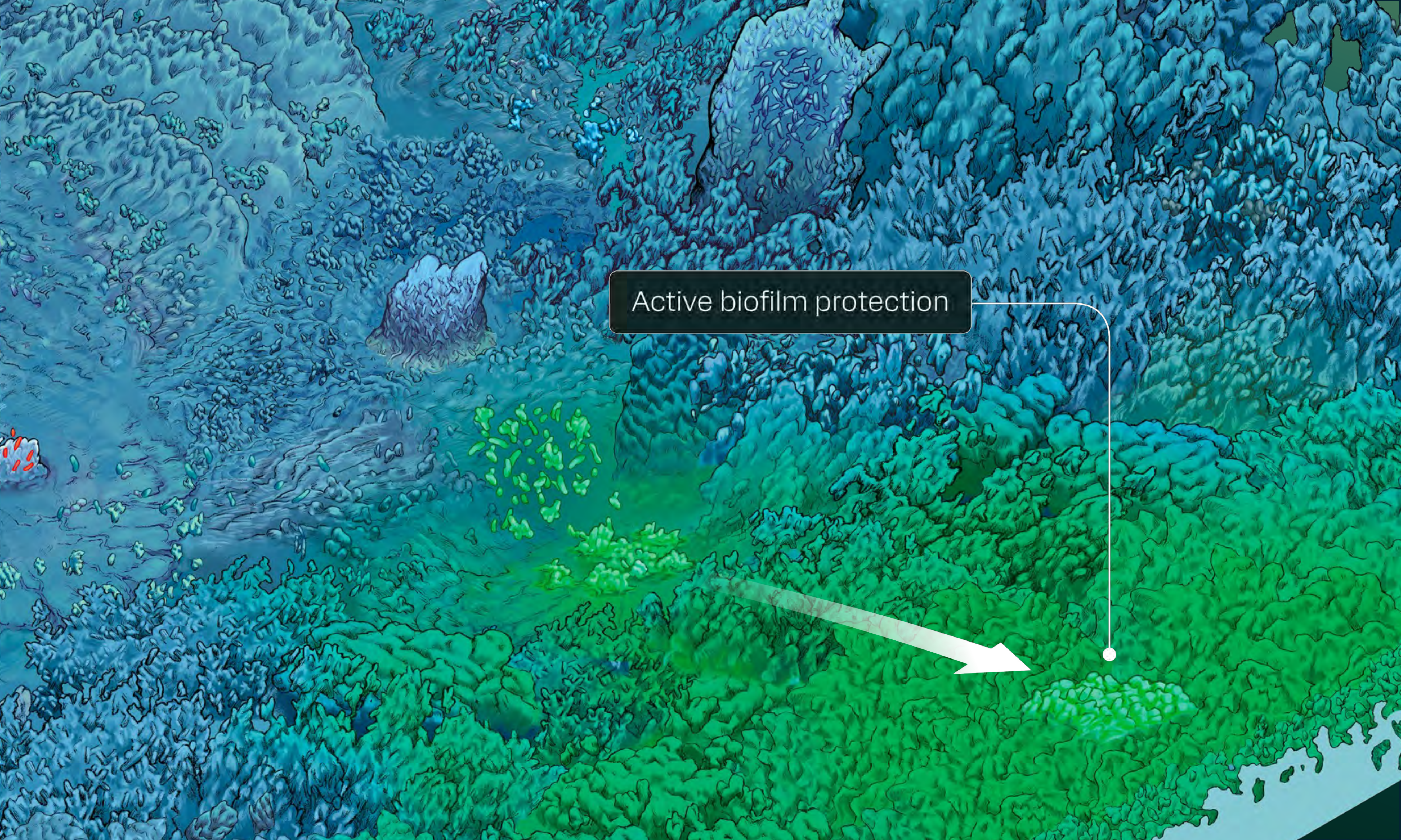

Pre-existing biofilm

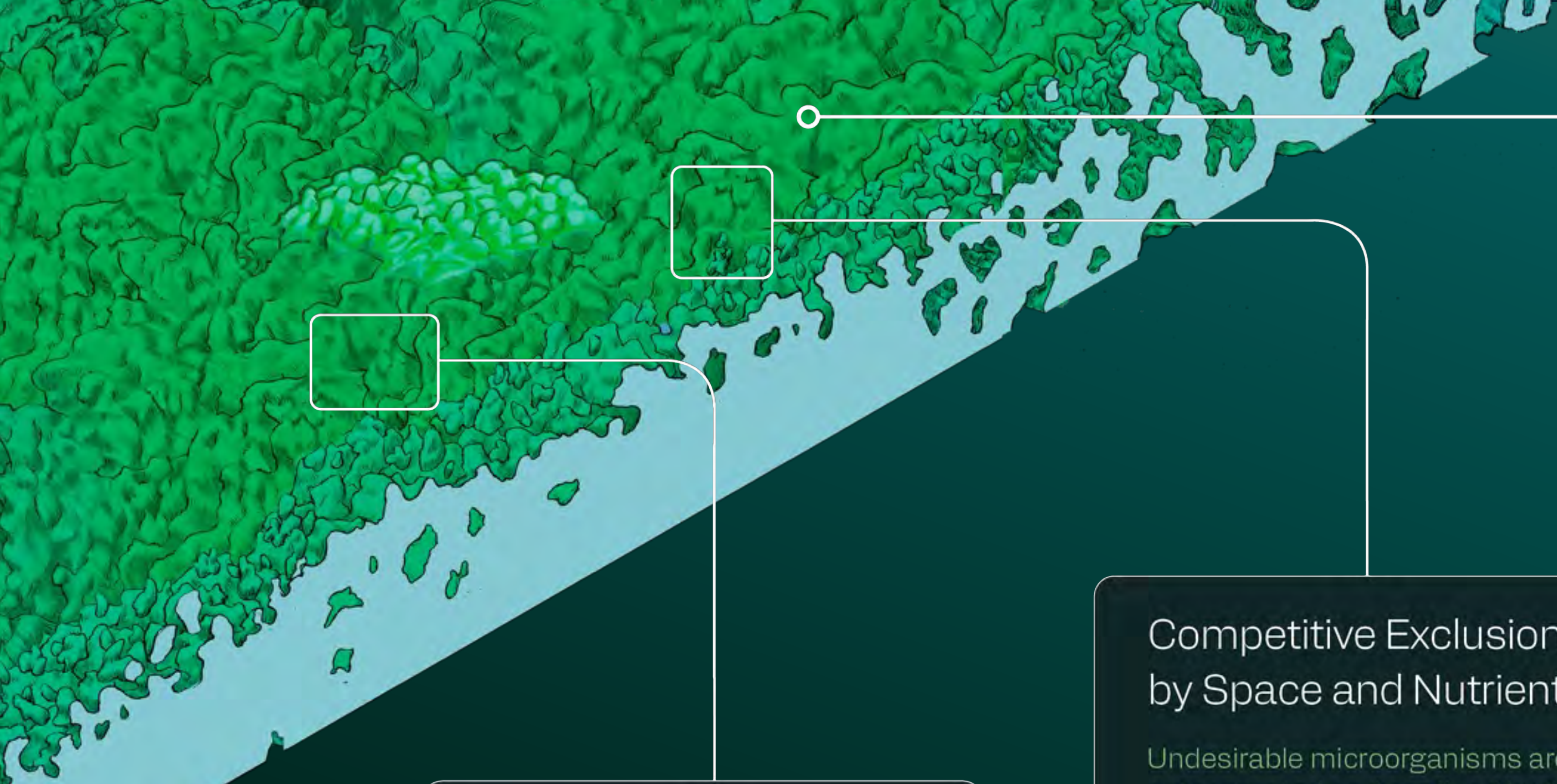

Cleaning and control intervention

Establishment of a beneficial biofilm

Schematic showing the implantation of selected beneficial microbial consortia. Applied at high concentration to a sparsely colonized surface, they initiate a protective biofilm that prevents unwanted colonization.




Active biofilm protection

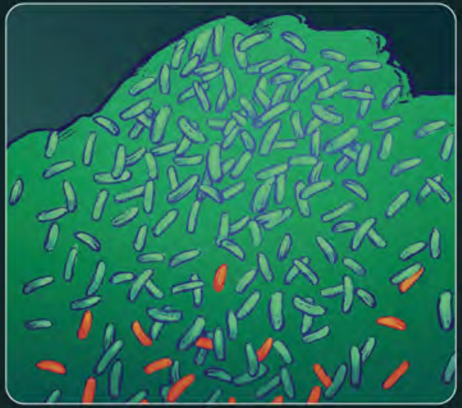


Adhesion inhibition

Approaching pathogenic bacteria are repelled by adhesion-inhibitory molecules secreted by the protective biofilm, preventing surface attachment.

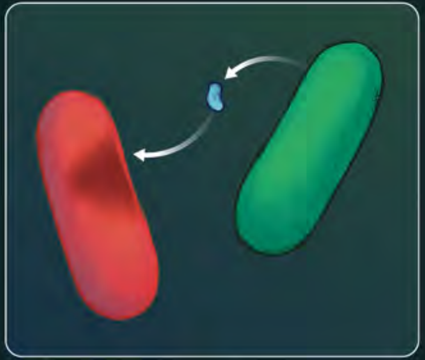
Competitive Exclusion by Space and Nutrients

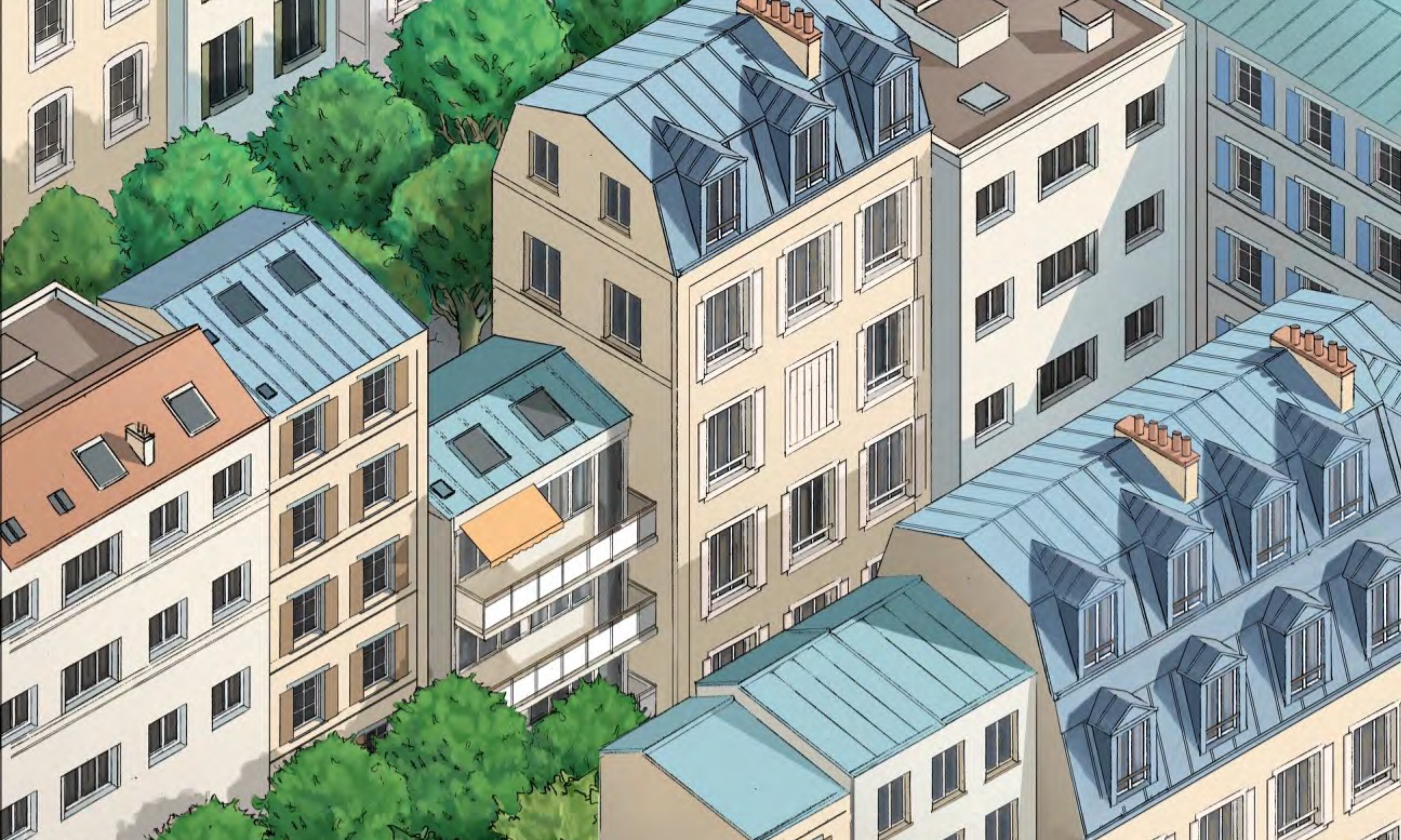
Undesirable microorganisms are outcompeted and confined by the dense beneficial population, which monopolizes both physical space and available nutrients within the biofilm.



Microbial interference

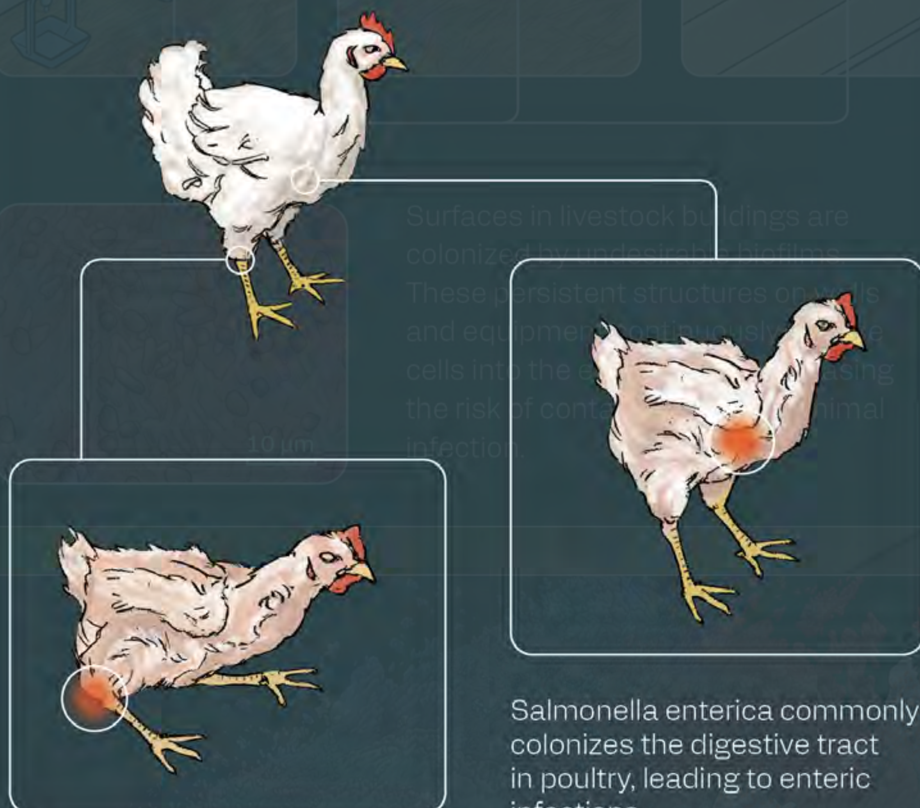
Beneficial bacteria interfere with unwanted populations by producing inhibitory metabolites that limit their growth or viability.





From contaminated surfaces to animal infections

Animals exposed to contaminated surfaces can develop localized infections, such as digestive tract colonization or joint infections, with potential propagation through cross-contamination between individuals.



Salmonella enterica commonly colonizes the digestive tract in poultry, leading to enteric infections.

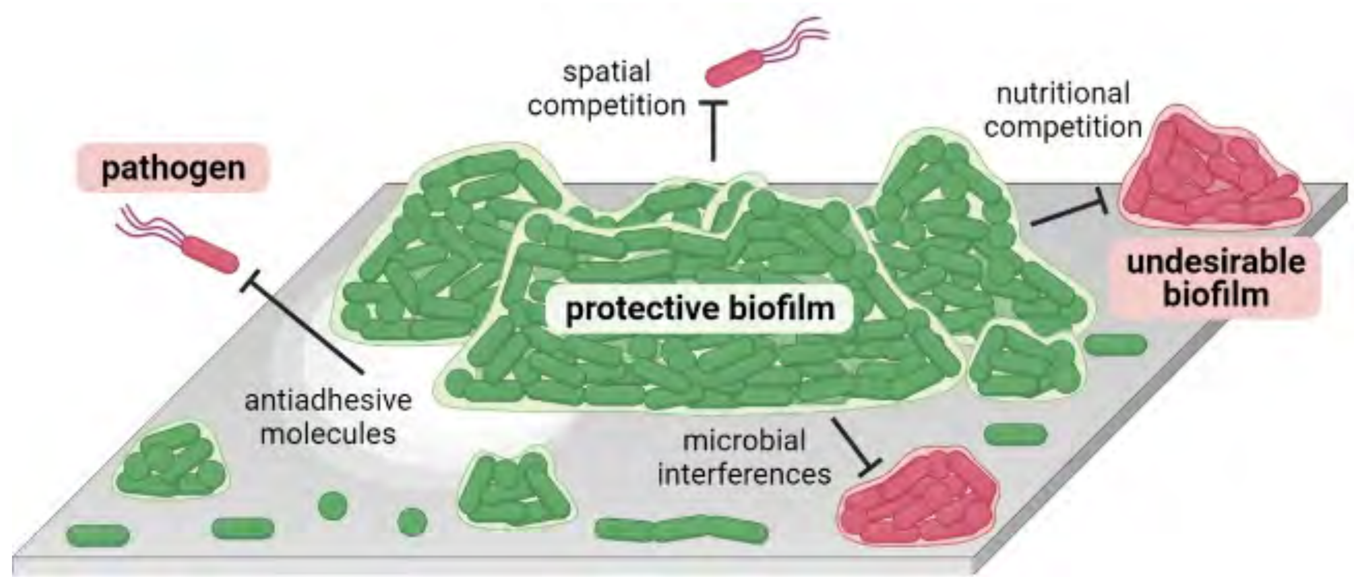
Enterococcus cecorum is frequently associated with joint infections in poultry, causing locomotor disorders.


Establishment of a protective beneficial biofilm
5 µm
A selected consortium of beneficial microorganisms is sprayed onto surfaces to establish a protective biofilm.
This active barrier competitively excludes undesirable microorganisms, preventing their attachment and proliferation.

É—e ÉCOLE ESTIENNE PARIS INRAE

Graphic Design: ©Antoine Carlioz

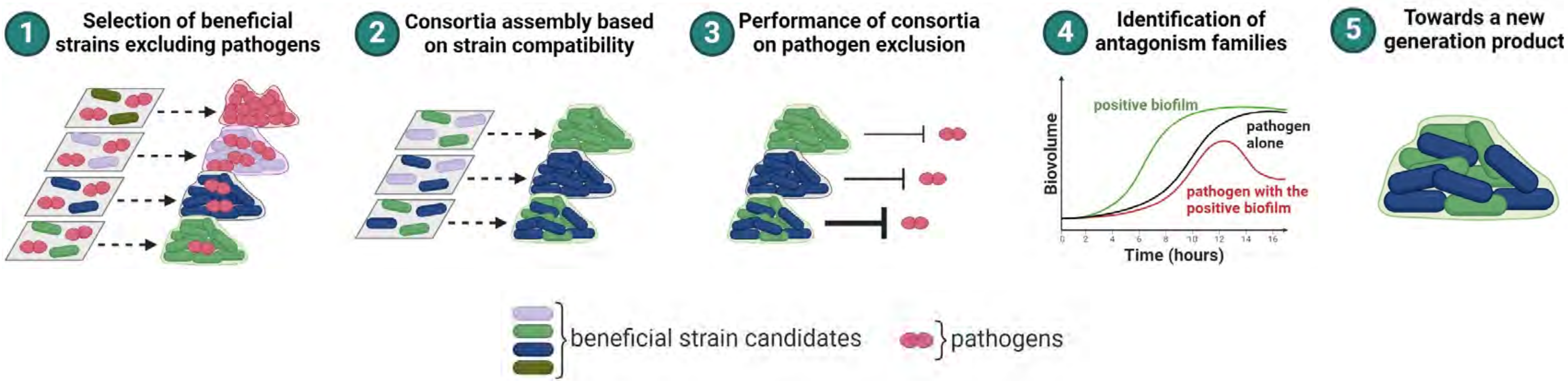
Exclusion of undesirable microorganisms by the settlement protective biofilms



Virgile Guéneau, PhD

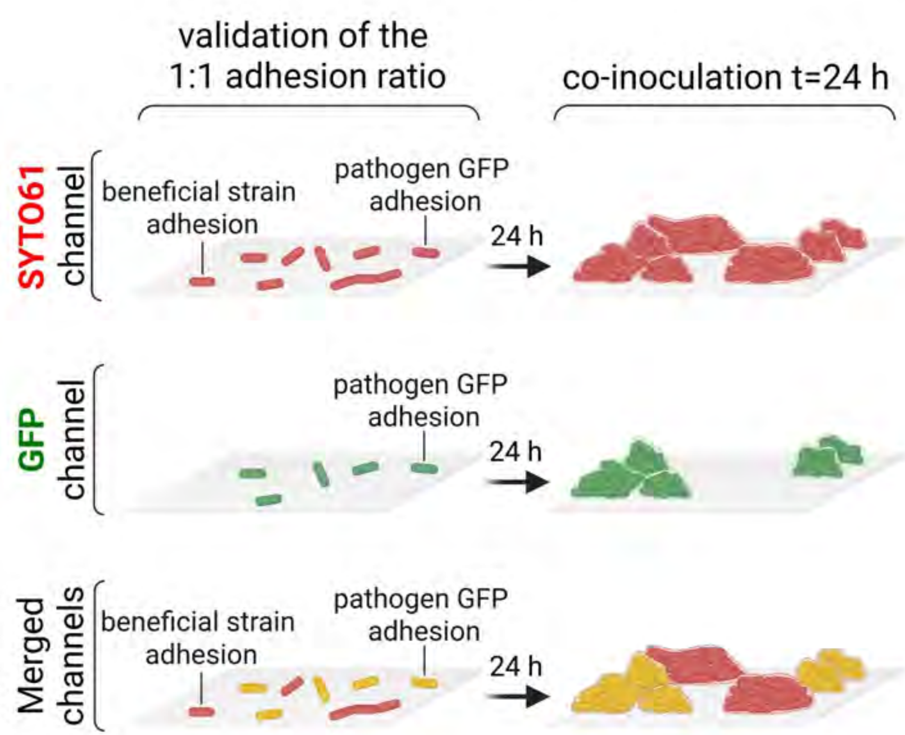
3D imaging-driven assembly of beneficial multi-species biofilms targeting undesirable bacteria

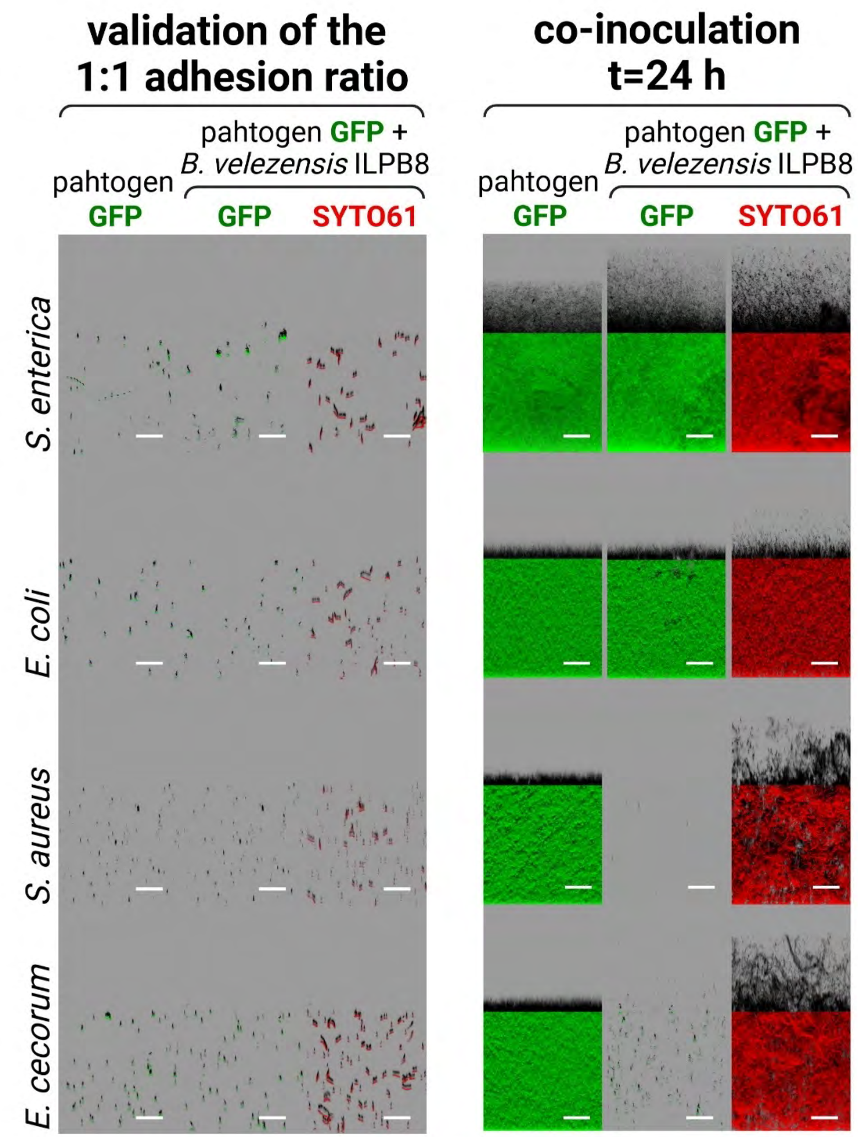
Virgile Guéneau, Laurent Guillier, Cécile Berdous, Marie-Françoise Noirot-Gros, Guillermo Jiménez, Julia Plateau-Gonthier, Pascale Serror, Mathieu Castex, Romain Briandet



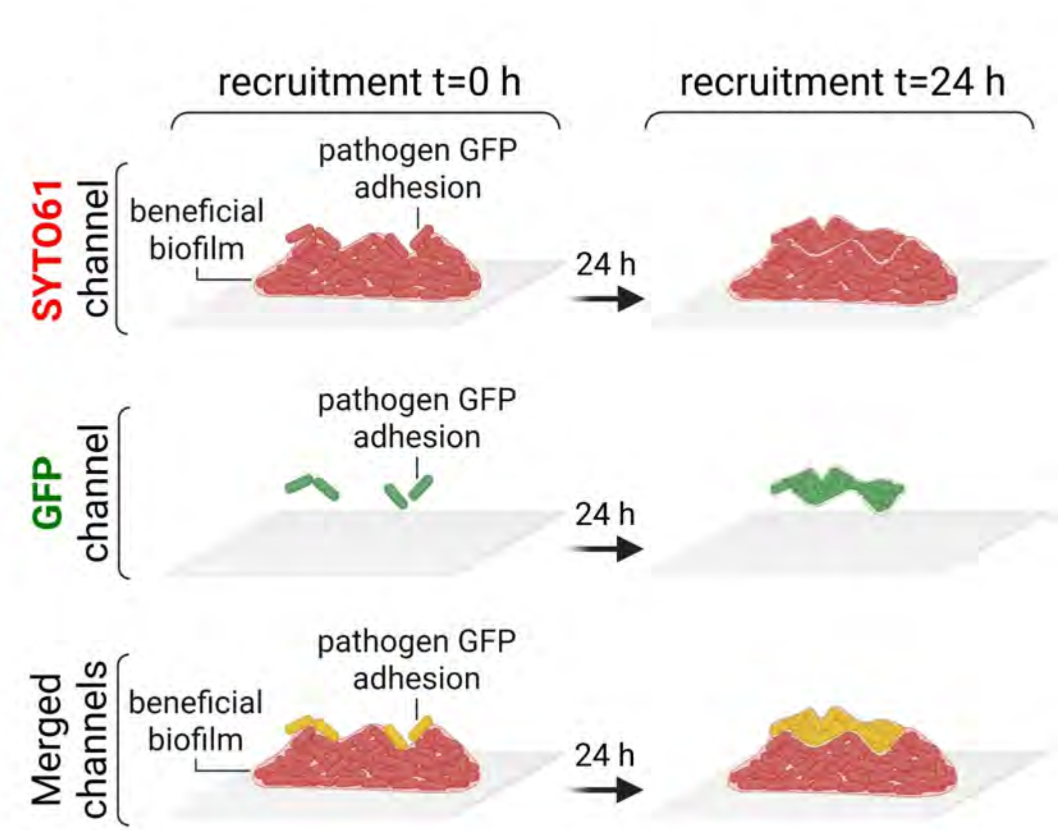
doi: https://doi.org/10.1101/2024.10.16.618781

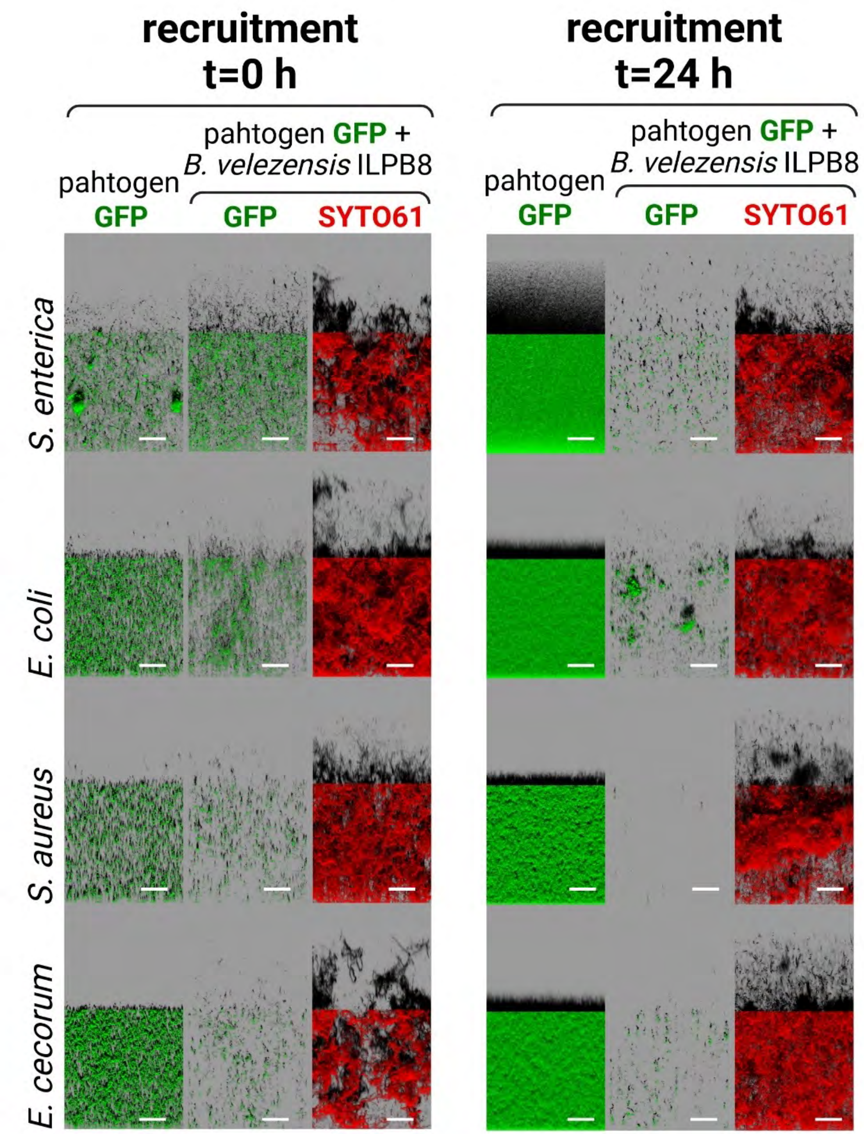
3D Imaging of co-culture models to investigate microbial interactions



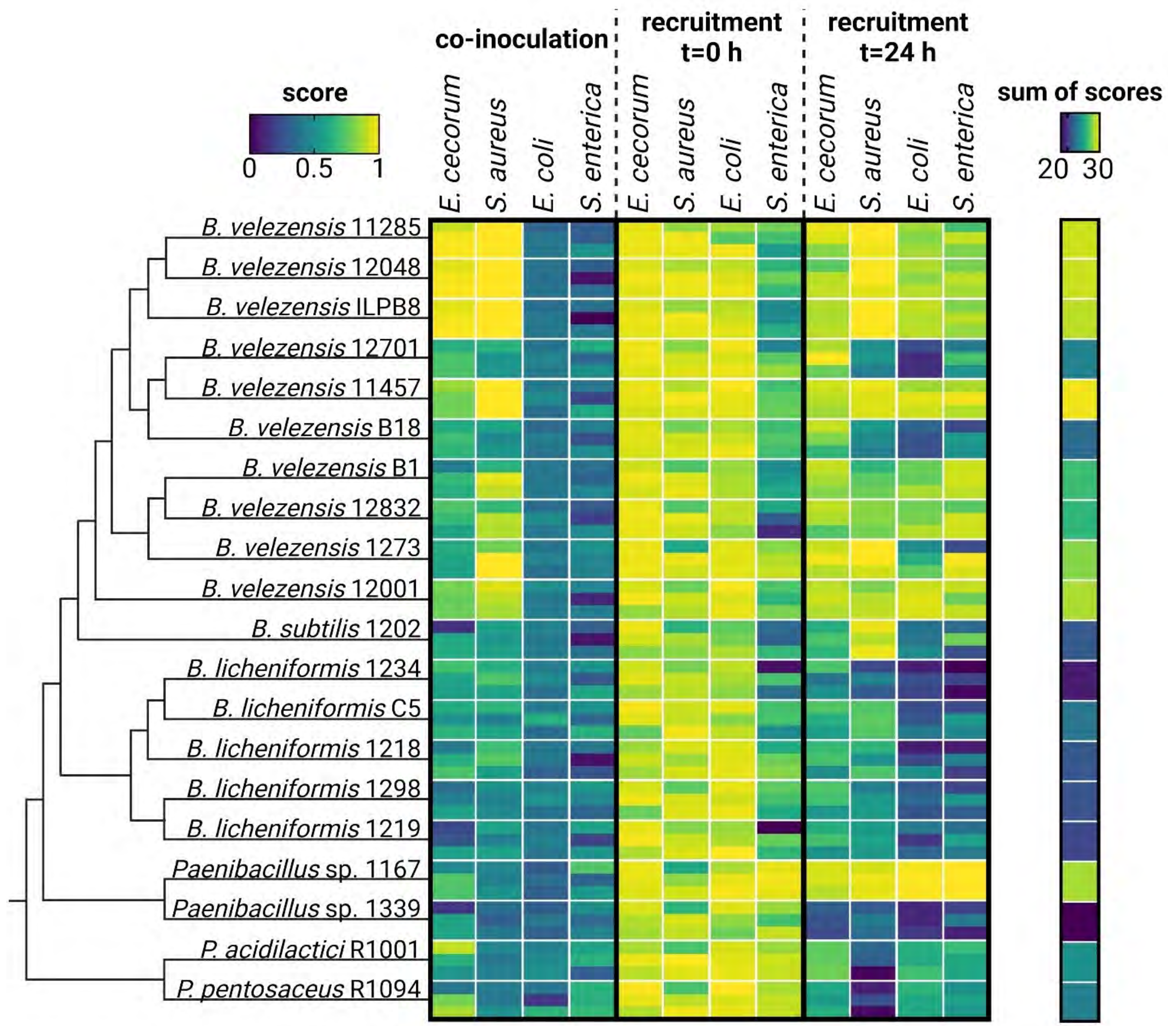


3D Imaging of co-culture models to investigate microbial interactions



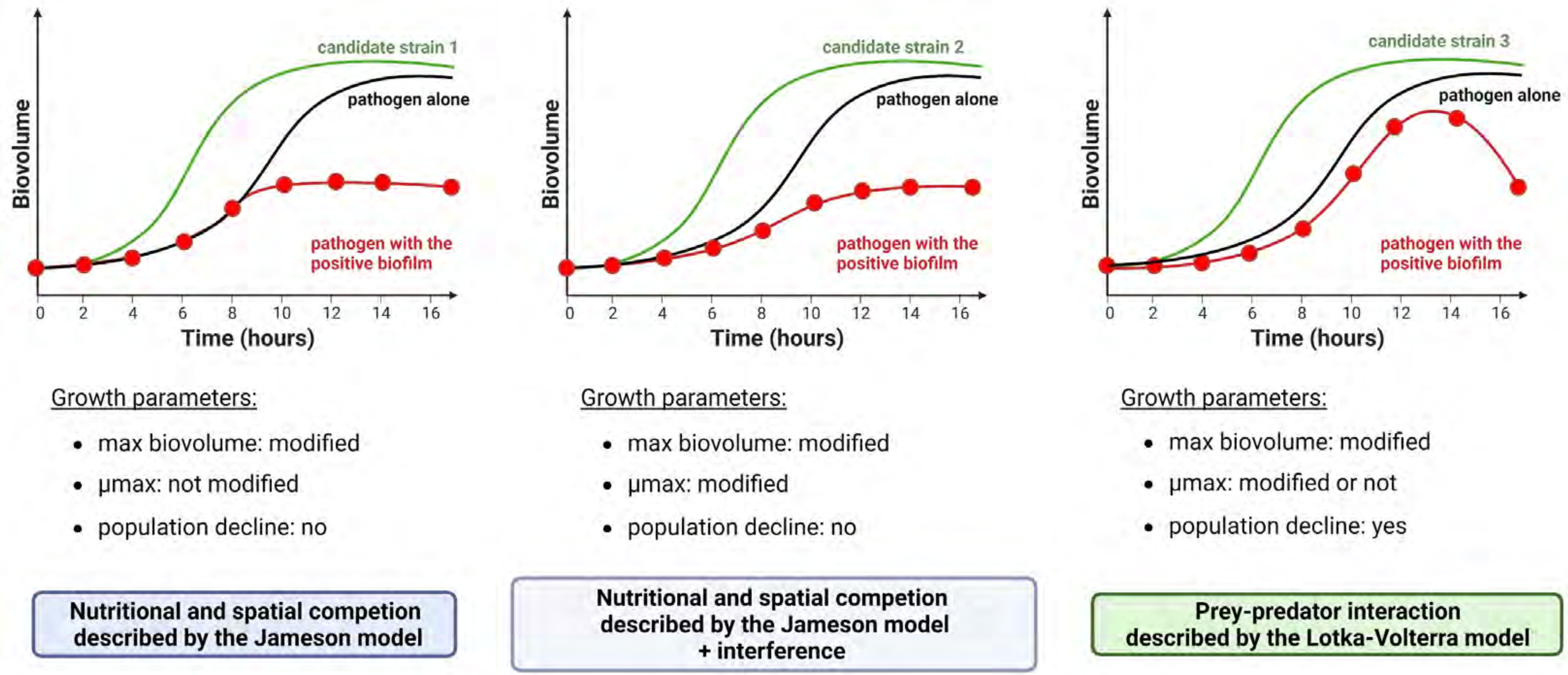


Assessing antagonistic activity against undesirable bacteria



- Diversity of responses
- Link between phylogenetic distance and exclusion
- Exclusion potential revealed when the biofilm is already formed
- Selection of best candidate strains

Time-resolved analysis of antagonistic mechanisms using 3D Imaging

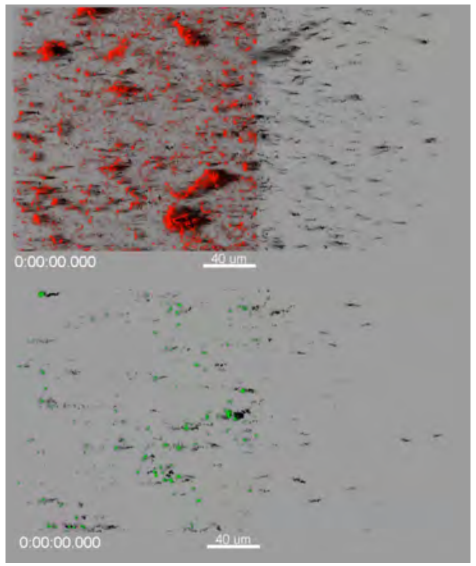


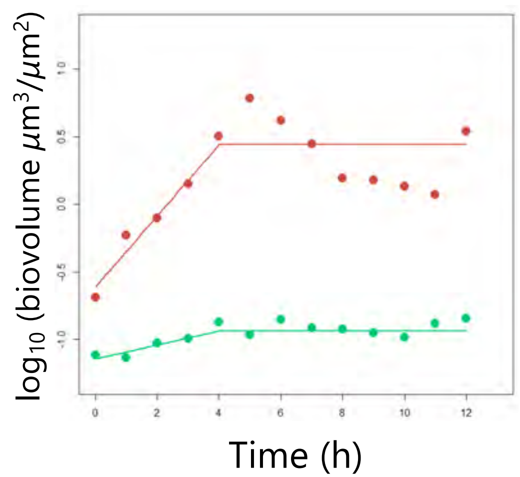
Case studies of pathogen exclusion

E. cecorum exclusion by *B. velezensis*

Initial ratio in the co-inoculation and recruitment model:

E. cecorum* < *B. velezensis

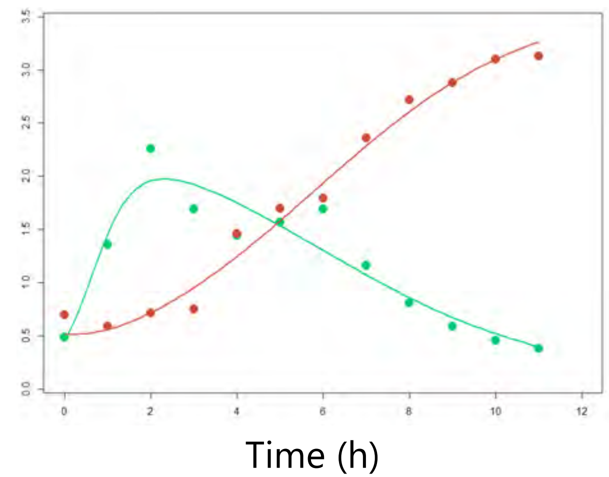


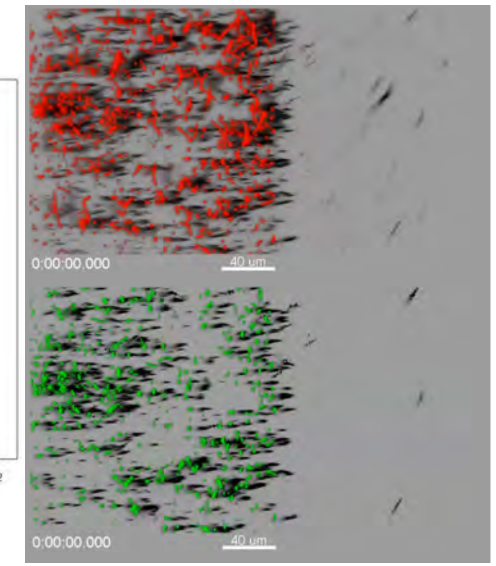


FM4-64 to label all the biofilm
GFP to label the pathogen

Initial ratio in the co-inoculation model:

E. cecorum* > *B. velezensis





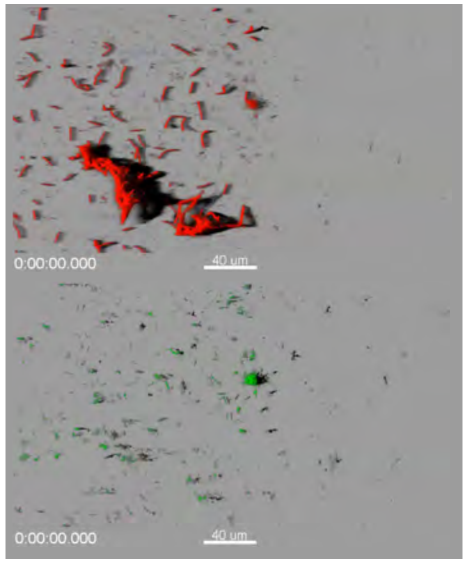
Nutritional and spatial competition described by the Jameson model + interference

Predation described by the Lotka-Volterra model

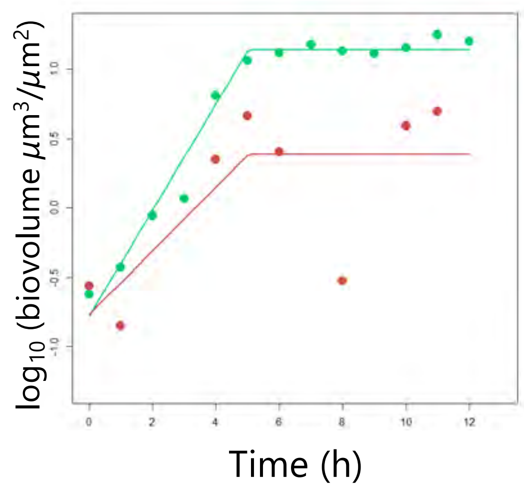
Case studies of pathogen exclusion

Pre-established B. velezensis biofilms inhibit S. enterica colonization

Co-inoculation model with all initial ratios:

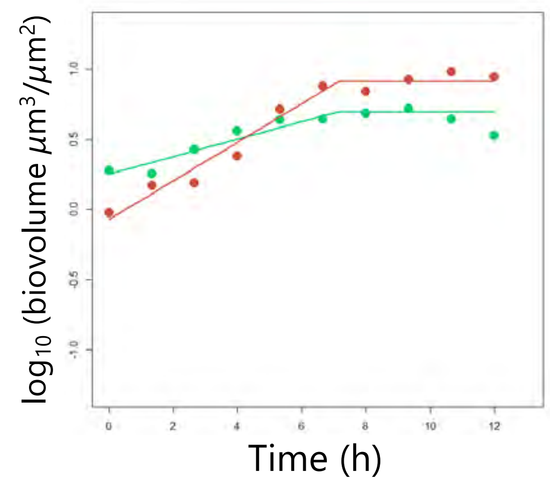


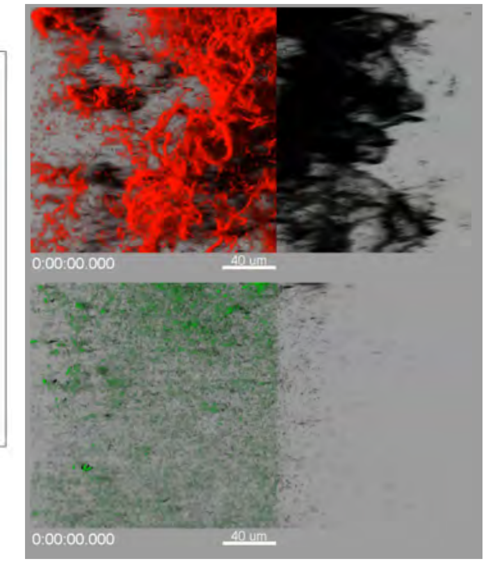
FM4-64 to label all the biofilm
GFP to label the pathogen



S. *enterica* growth is not affected

Recruitment model:



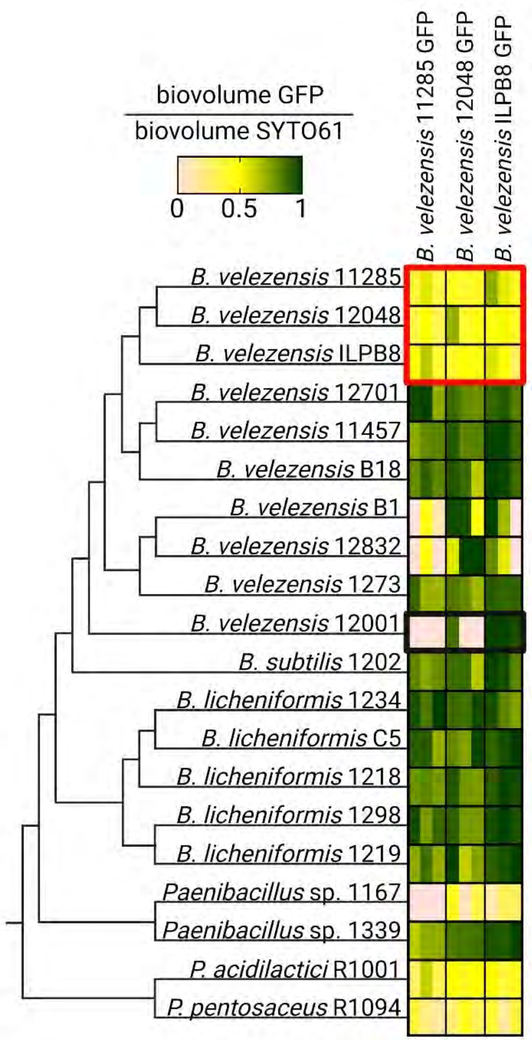


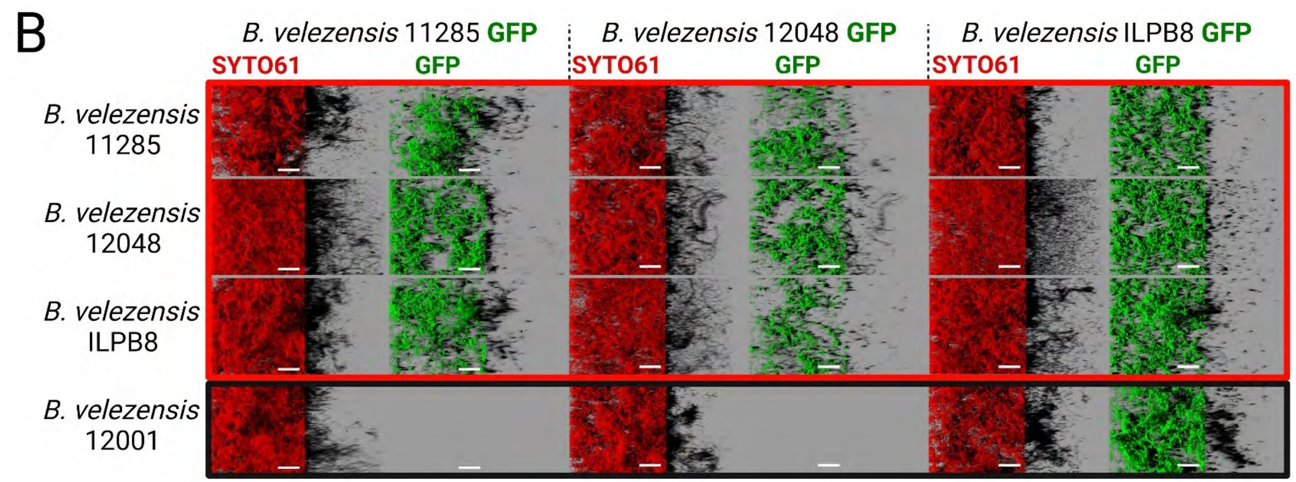
Nutritional and spatial competition described by the Jameson model + interference

→ Generation time: **40 min to 5h**

Design & compatibility of beneficial consortia

Evaluating compatibility in beneficial multispecies consortia

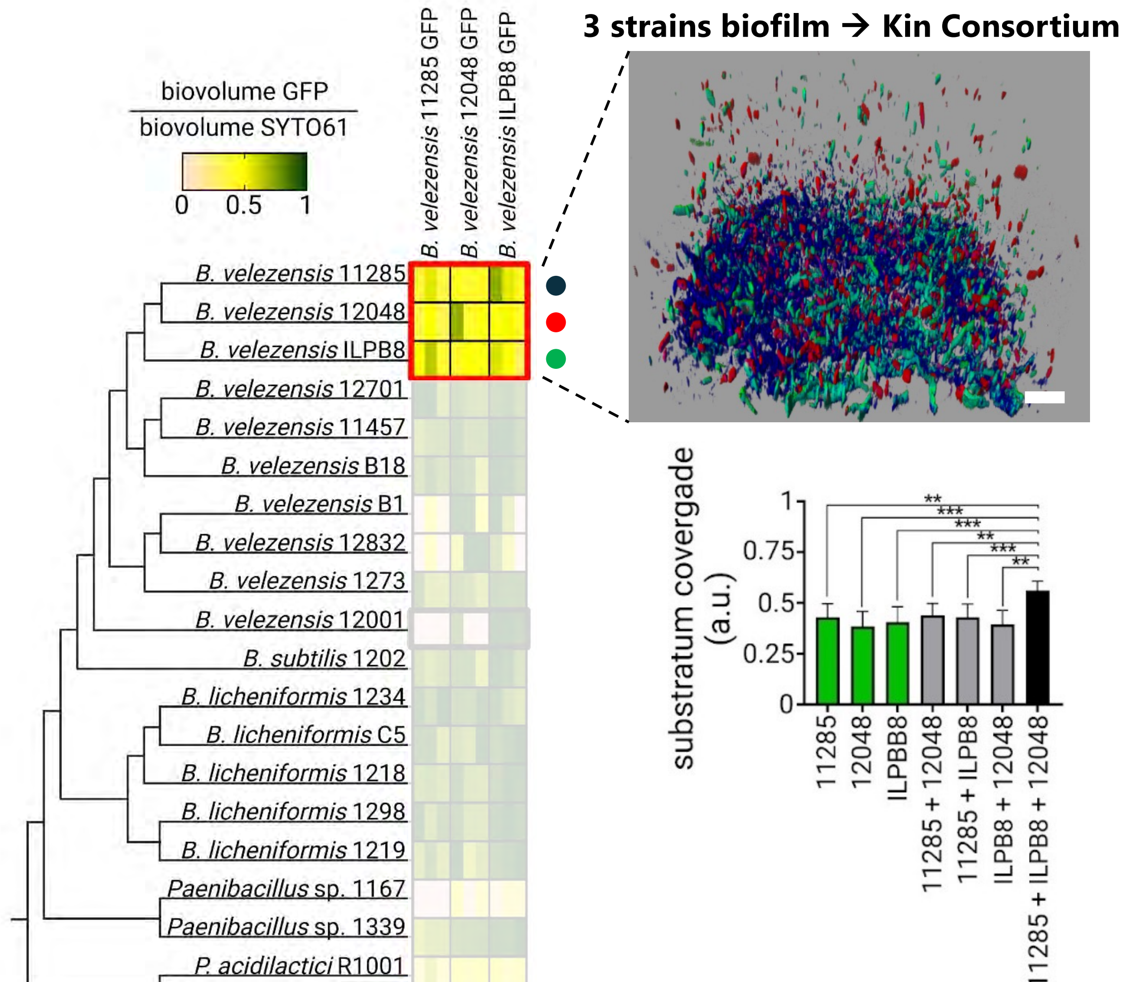




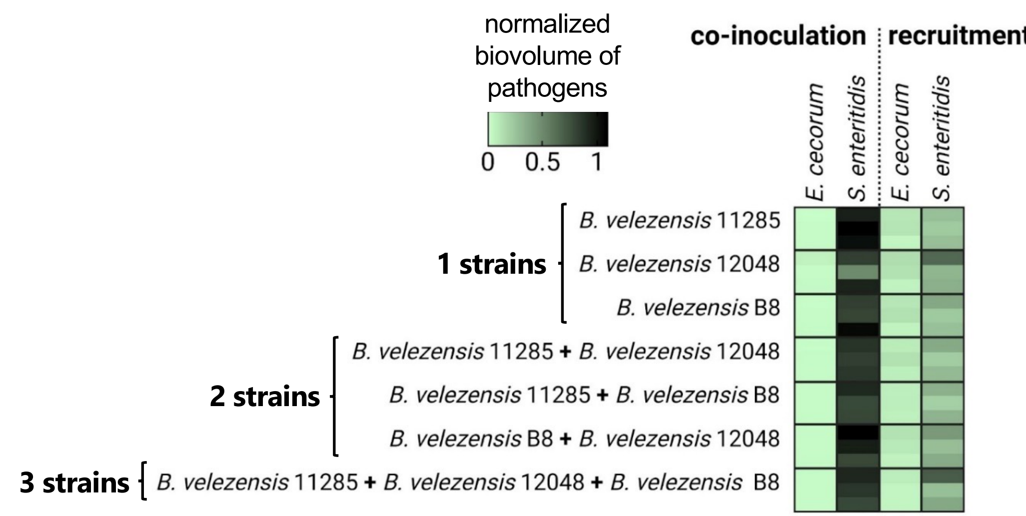
- **Very limited compatible consortia**
- Compatible strains are very close or very distant phylogenetically

Design & compatibility of beneficial consortia

Multi-strains Bacillus velezensis kin-consortium



Better substrate coverage

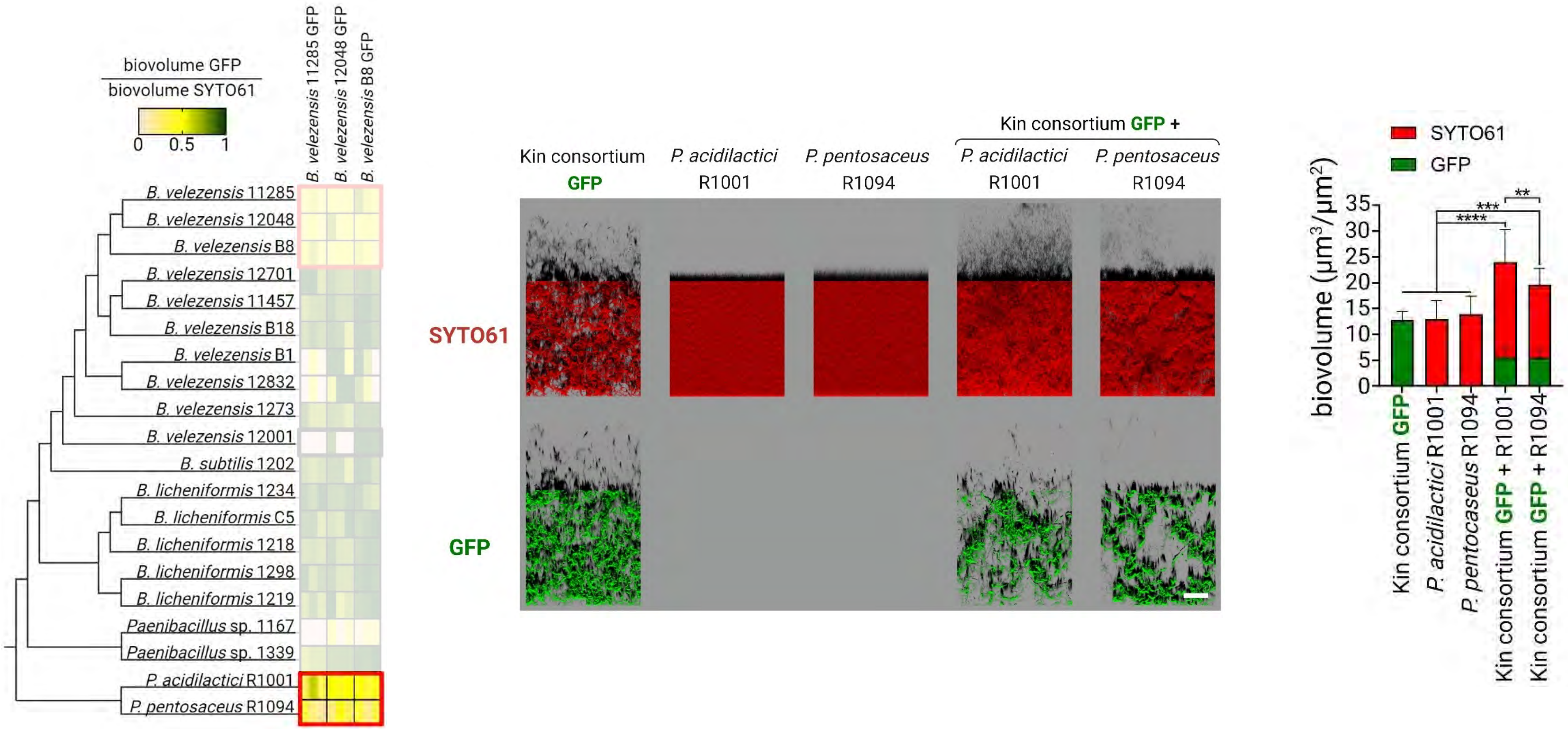


No additive effect of compatible SynComs on antagonism

→ Same antagonistic mechanisms?

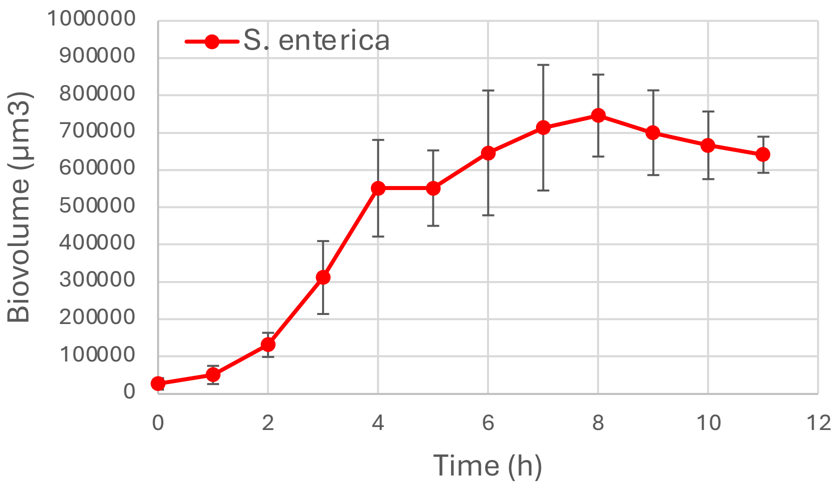
Design & compatibility of beneficial consortia

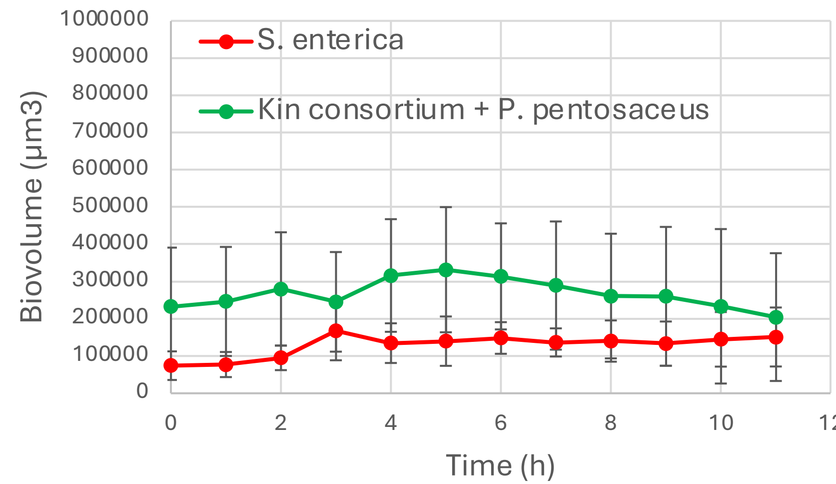
Multi-genus biofilms: enhancing protective effects through synergy?



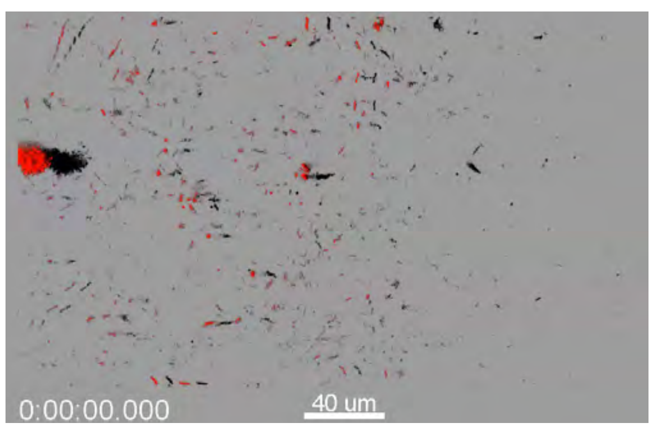
Design & compatibility of beneficial consortia

Multi-Genus biofilms: enhancing protective effects through synergy?

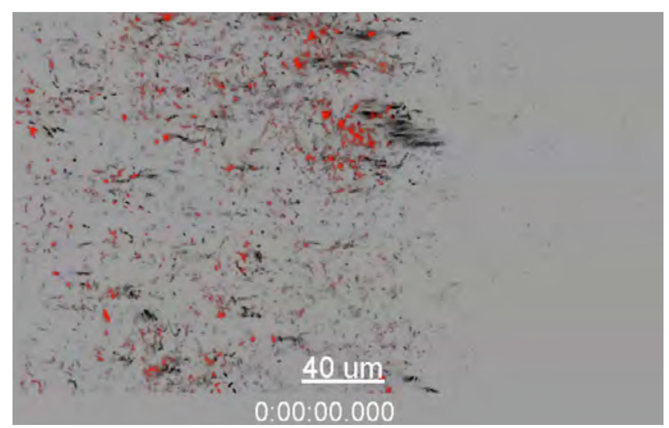




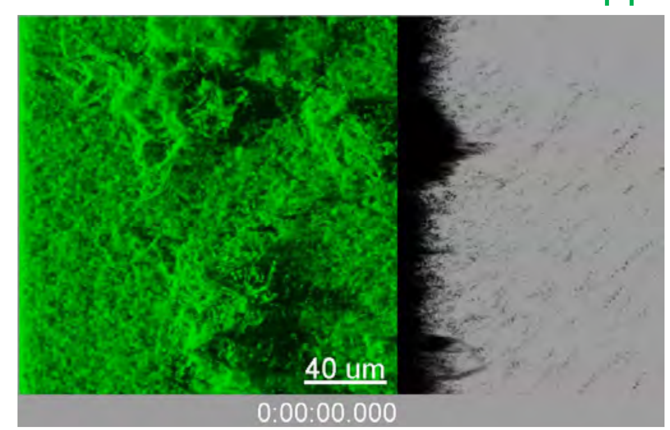
S. enterica



S. enterica



B. velezensis + *Pediococcus* spp.

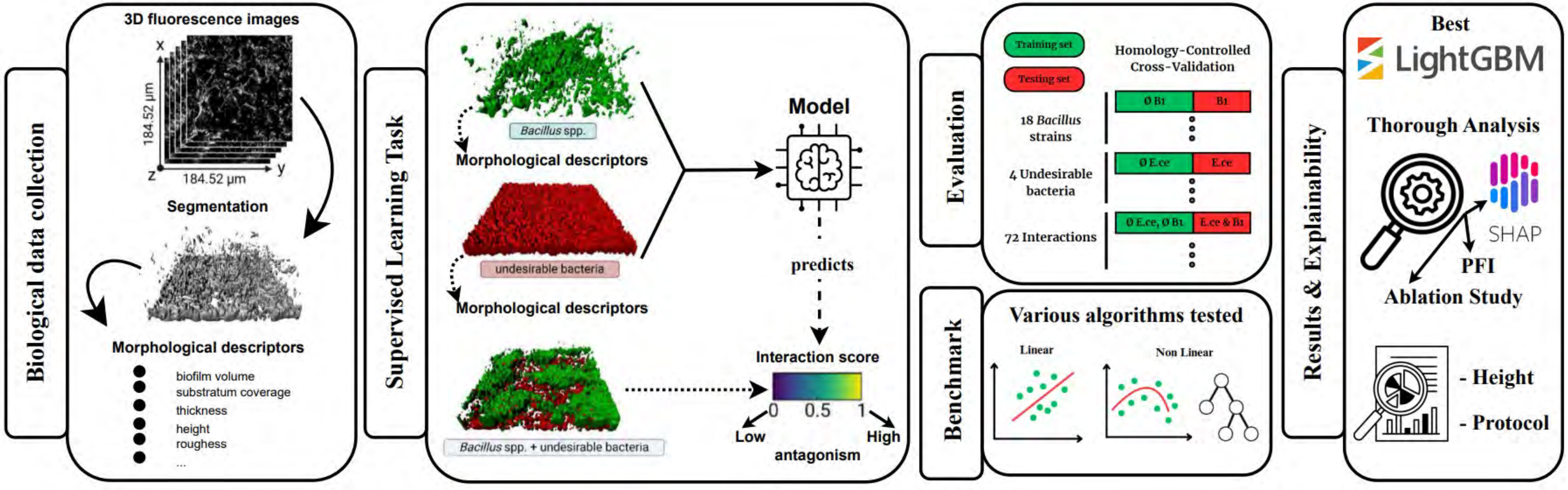


AI-guided strain selection

AI-assisted selection of beneficial strains for pathogen exclusion

Strategy: use morphological descriptors of mono-strain biofilms to predict the interaction with pathogens

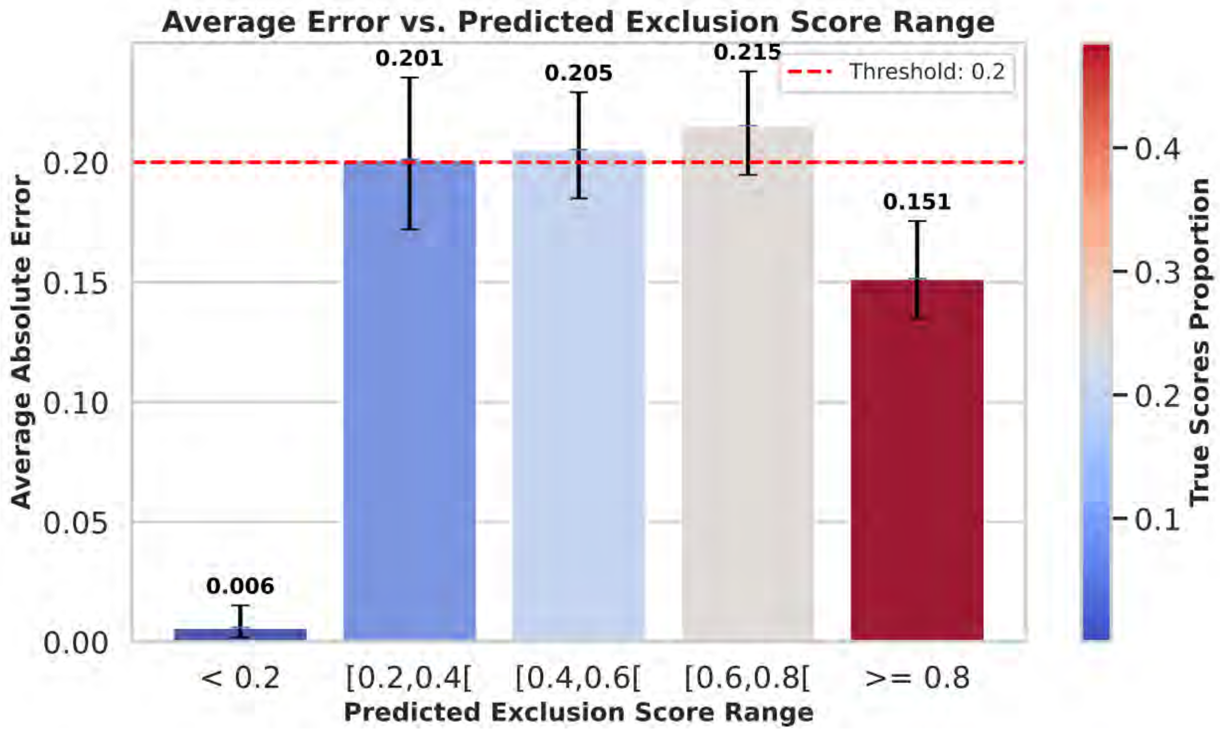
Objective: machine learning to reduce experiments for candidate strains selection



Raphaël Rubrice et al. 2025, Artificial Intelligence In Life Scienc

AI-guided strain selection

Accurate prediction of high exclusion potential using machine learning



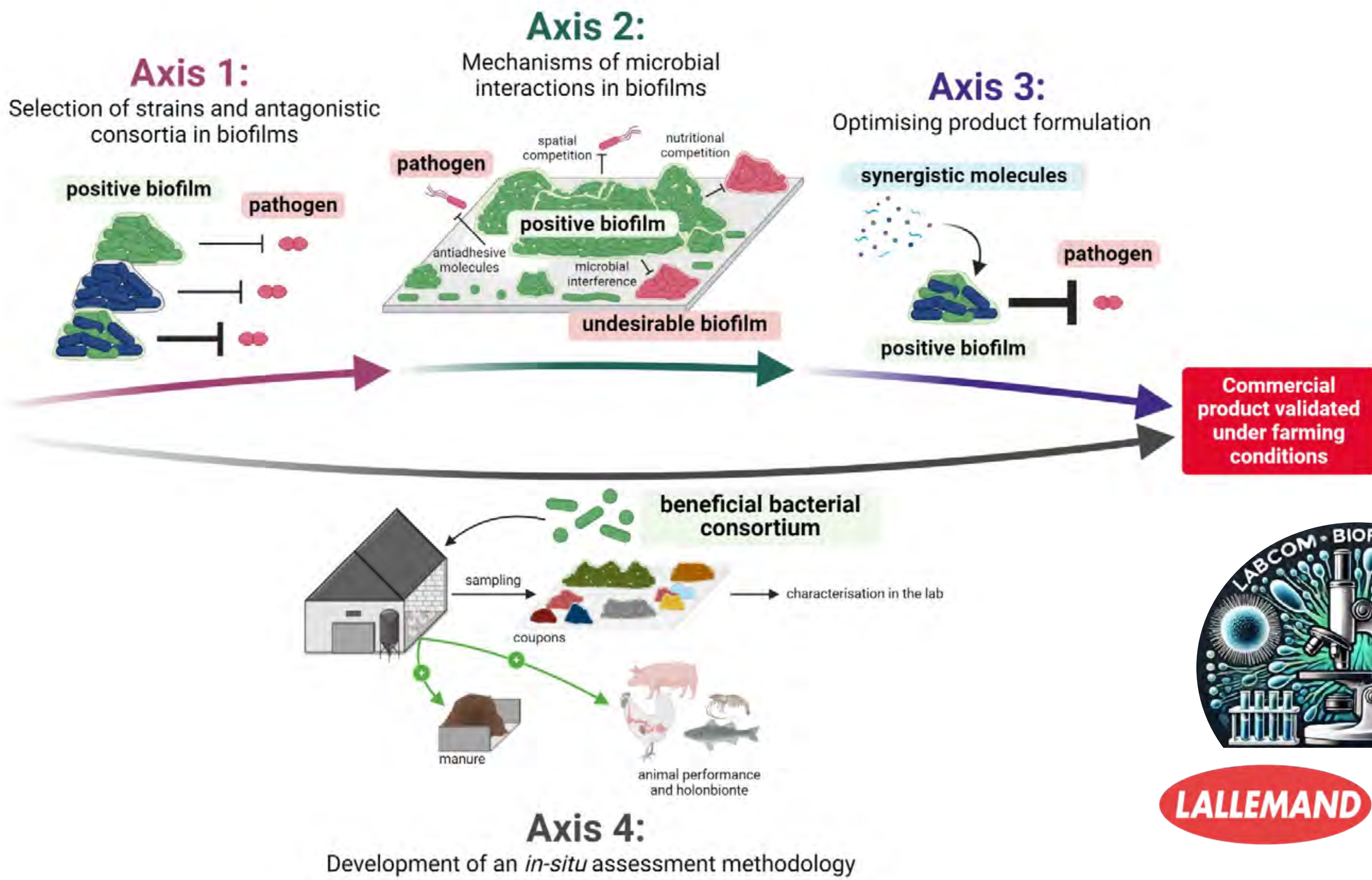
Many perspectives

- add genomes
- deep-learning on raw images
- other biofilm models
- add more data

Raphaël Rubrice et al., submitted

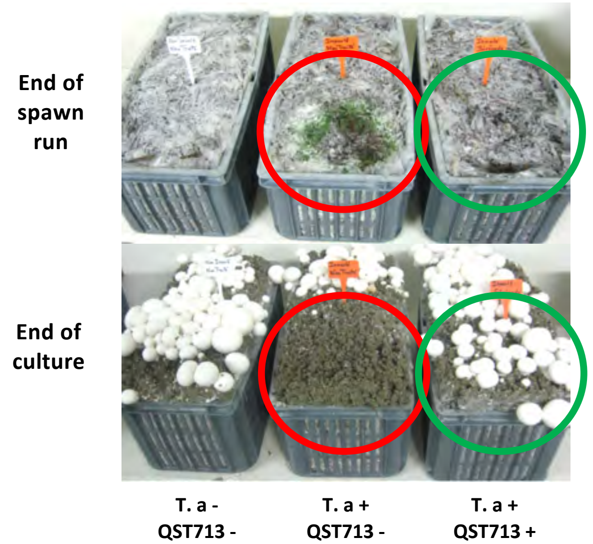
Project & Perspective: LabCom **Biofilm**1**Health**

Unlocking the potential of biofilm properties in beneficial microbes for One Health advancements

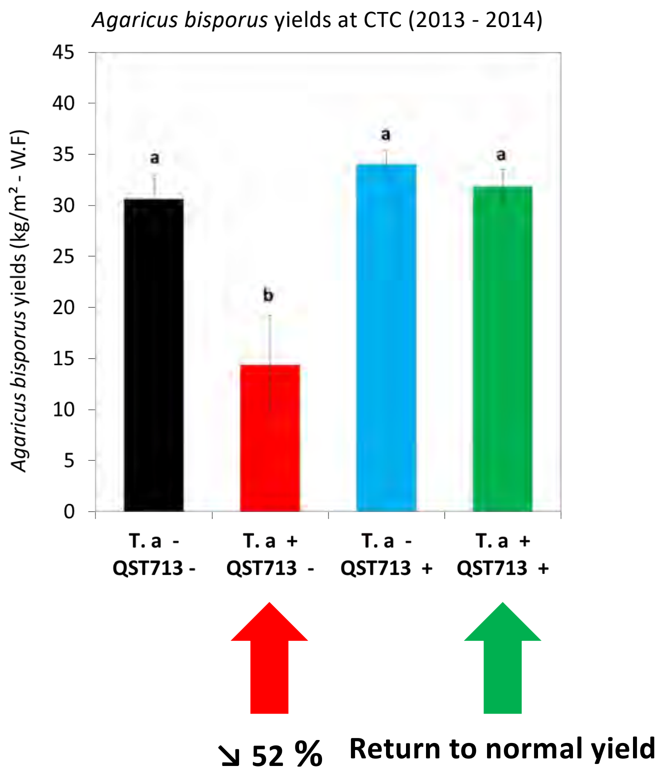


Protective biofilm in the French button mushroom sector

> 25 % of annual losses of *Agaricus bisporus* → *Trichoderma aggressivum* (green mould)
> Biocontrol used for 10 years in France → *Bacillus ~~subtilis~~ velezensis* QST713

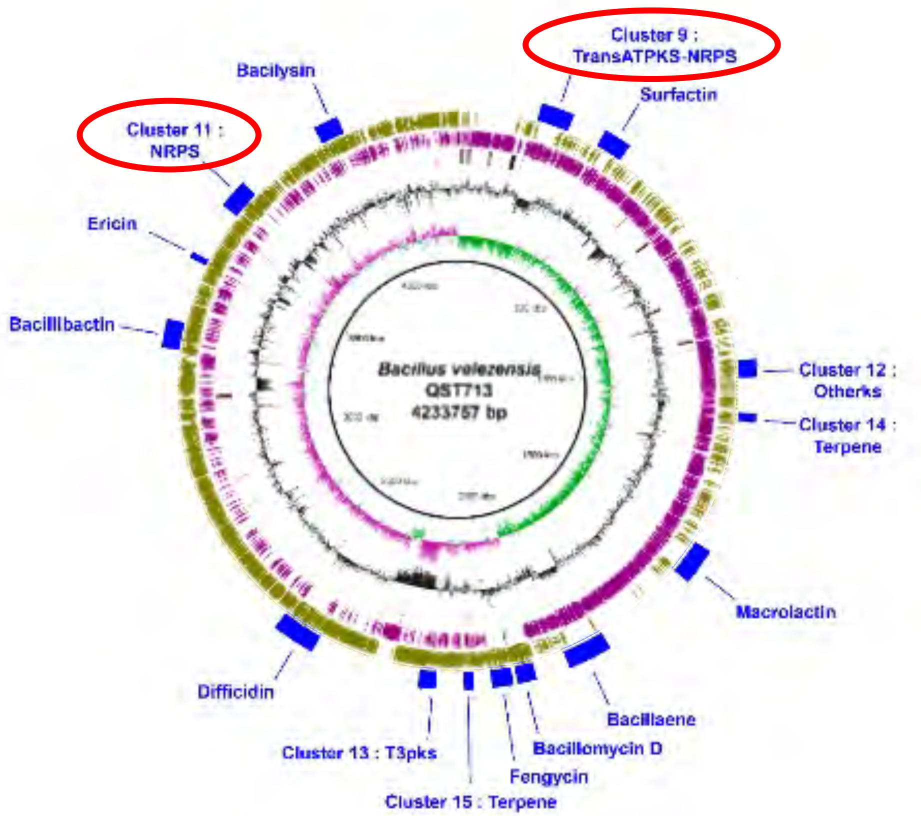


Centre technique du champignon (CTC) - BI N°21 - June 2008



Pandin C, et al. J Biotechnol. 2018 Jul 20;278:10-19

Bacillus velezensis QST713



***Bacillus velezensis* strain QST713**	
Genome size (bp)	**4233757**
G + C content (%)	45,9
Total number of genes	4263
Coding sequences (CDSs)	4056
rRNAs genes	25
tRNAs genes	79
ncRNAs genes	5
Pseudo genes (total)	98

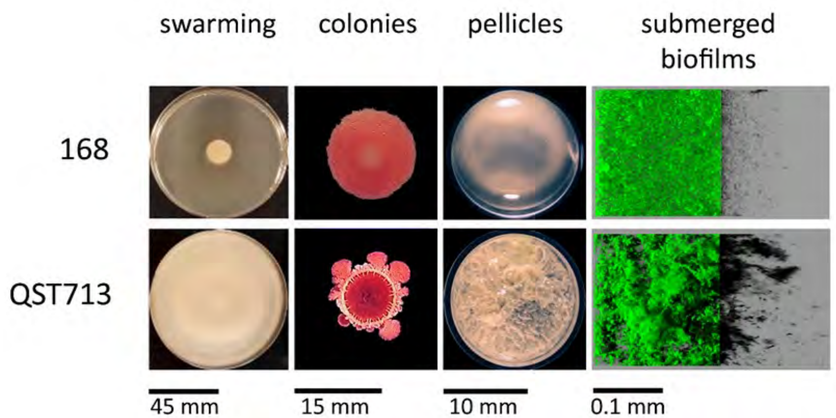
Secondary metabolites: antiSMASH webserver

Cluster number	Synthetase	Metabolites	bp	Bioactivity
1	NRPS	Surfactin	65414	Antimicrobial
2	TransATPKS	Macrolactin	85893	Antibacterial
3	TransATPKS-NRPS	Bacillaene	102672	Antimicrobial
4	NRPS	Bacillomycin D	44669	Antimicrobial
5	TransATPKS-NRPS	Fengycin	49543	Antimicrobial
6	TransATPKS	Difficidin	100457	Antibacterial
7	Bacteriocin-NRPS	Bacillibactin	66790	Antibacterial
8	NRPS	Bacilysin	58252	Antibacterial
9	TransATPKS-NRPS	-	77736	Not determined
10	Lantipeptide	Subtilin-like / Ericin	15123	Antibacterial
11	NRPS	-	68430	Not determined
12	OtherKS	-	41245	Not determined
13	T3PKS	-	41101	Not determined
14	Terpene	-	20741	Not determined
15	Terpene	-	21884	Not determined

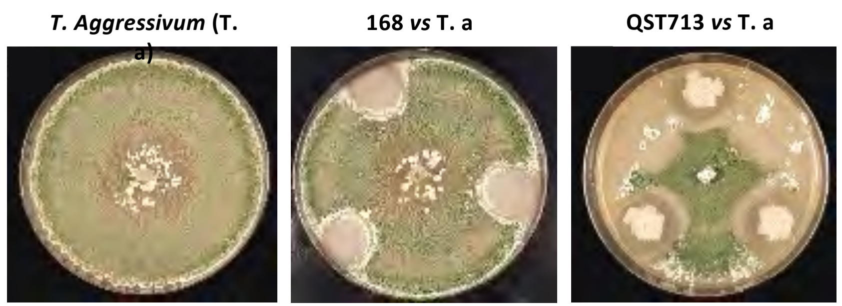
- *B. velezensis QST713* = **12%** of the genome allocated to the biosynthesis, regulation and transport of antimicrobials
- *B. subtilis* = **4-5%** (Stein 2005; PMID:15853875)
- *B. velezensis* FZB42 = **8.5%** (Chen *et al.* 2009; PMID: 19041913)

- **2 potential new antimicrobials**

QST713 Abilities: biofilm formation, swarming motility & antibiosis



Phenotypic comparisons of *B. velezensis* QST713 with *Bacillus subtilis* 168

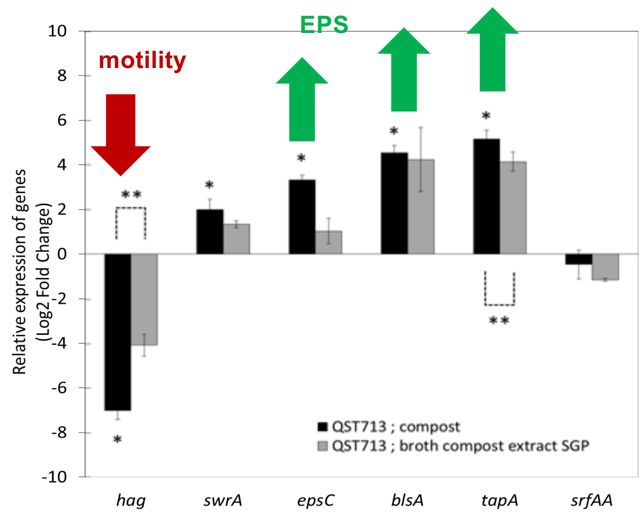


Interaction tests on agar plates with *B. velezensis* QST713 or *B. subtilis* 168 with *T. aggressivum* at 25°C

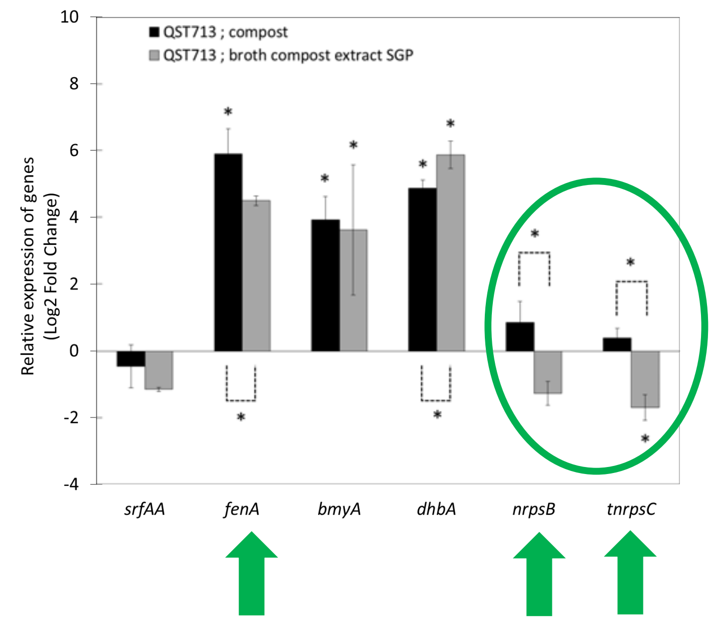
- ***B. velezensis* QST713 :**
  - Swarming motility
  - Complex macrocolony structure which is mucoid under a thick and dry surface layer
  - Thick & solid pellicle
  - Spatially organised submerged-biofilm

- **Pathogen inhibition test:**
  - No inhibition of *T. aggressivum* by *B. subtilis* 168
  - Antibiosis of *T. aggressivum* by *B. velezensis* QST713 : growth inhibition and sporulation inhibition

In situ gene expression of the biocontrol agent



Control group: QST713 in broth compost extract (planktonic), exponential growth phase (EGP)



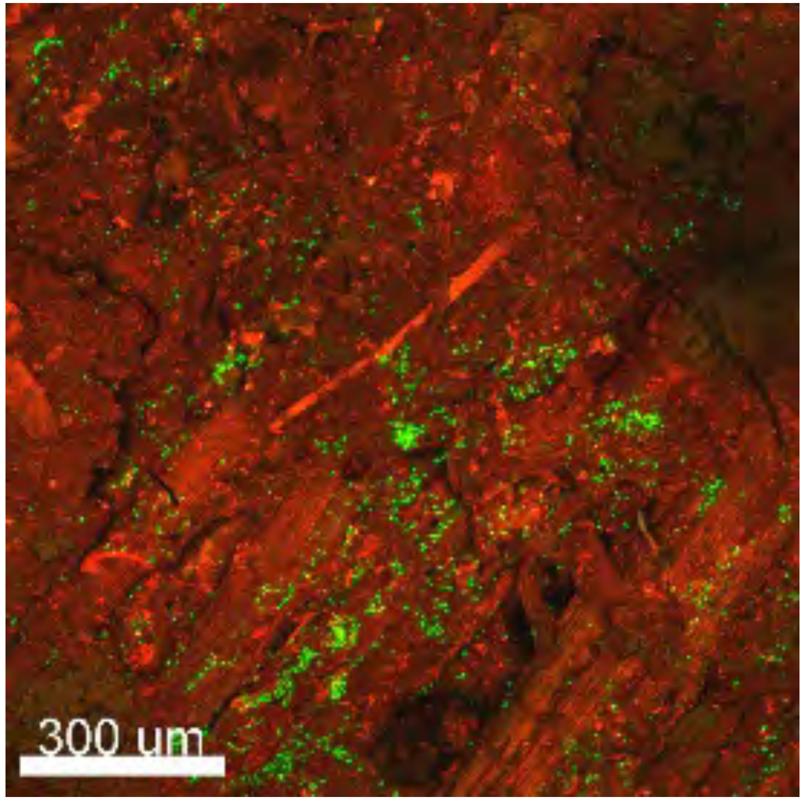
> biofilm is the preferential mode of life of the biocontrol agent in compost micromodels

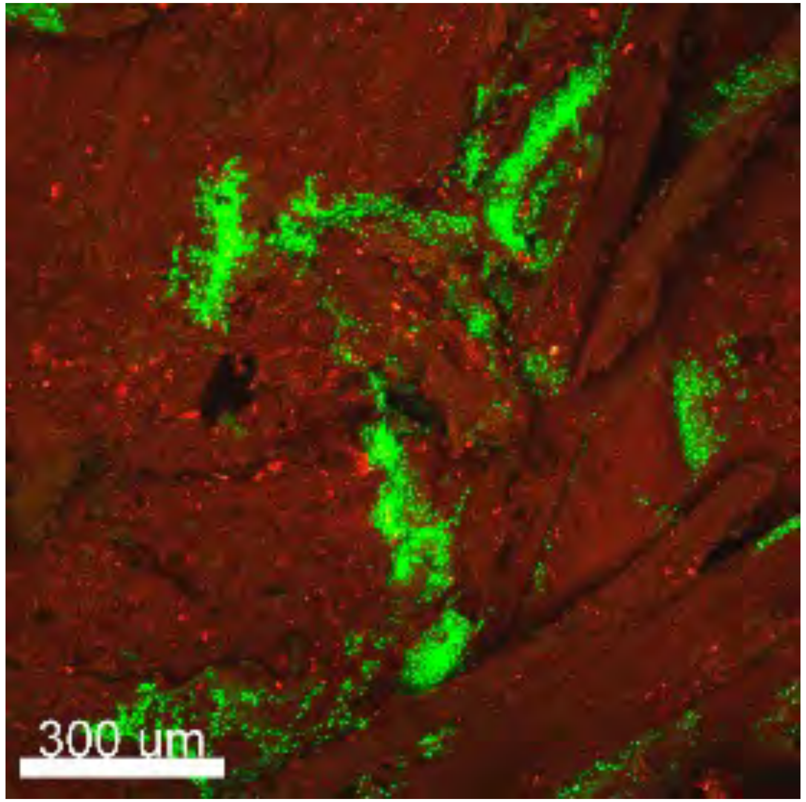
> Several antimicrobials upregulated in the biofilm form

Pandin C et al. Appl Environ Microbiol. 2019 May 30;85(12)

Direct visualisation of biofilm formation in the compost by fluorescent *B. velezensis* QST713 at 6 days of culture

Confocal laser scanning microscopy visualisation



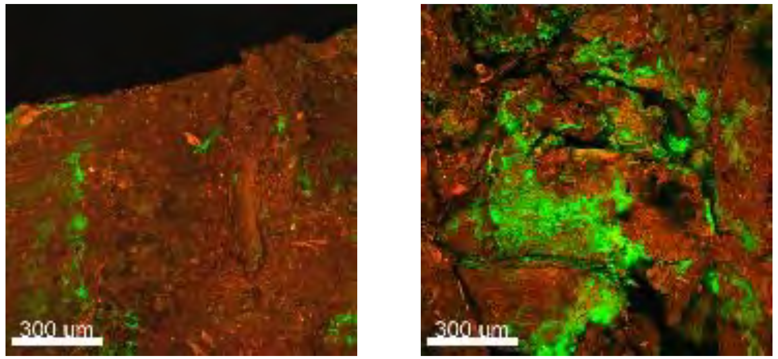


Pandin C et al. Appl Environ Microbiol. 2019 May 30;85(12)

Biofilm formation by fluorescent *B. velezensis* in the compost

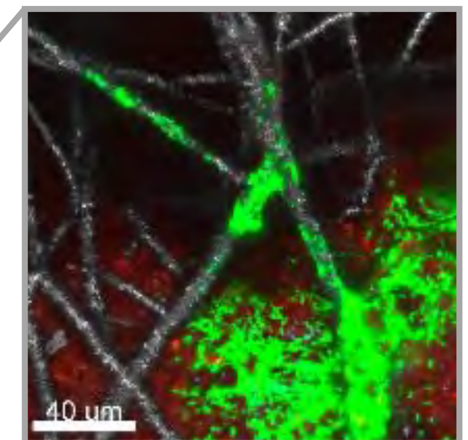
➢ Biocontrol strain *B. velezensis* FZB42 authorized in button mushroom field

FZB42



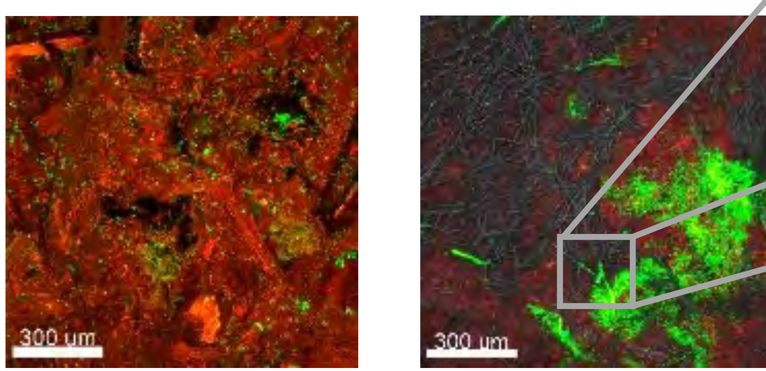
Use of available biofilm and antimicrobial gene mutants of strain FZB42

Proximity with *Agaricus* mycelium



➢ Other *B. velezensis* strain with biocontrol effect against fungi

SQR9



micro-aggregates were most frequently observed

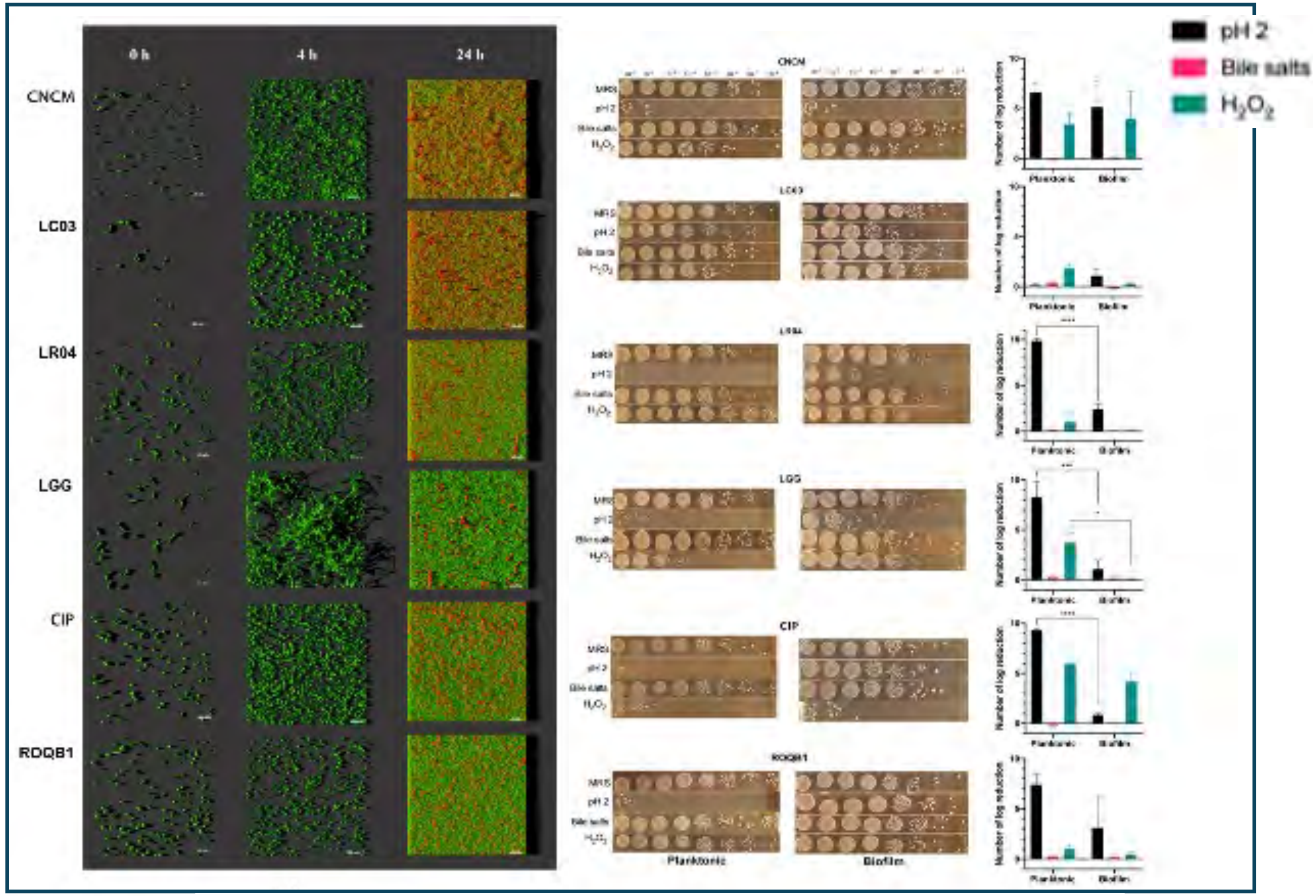
massive spatially organized biofilms were punctually observed

Confocal laser scanning microscopy visualisation

Bacteria use fungal « highways » to disperse?

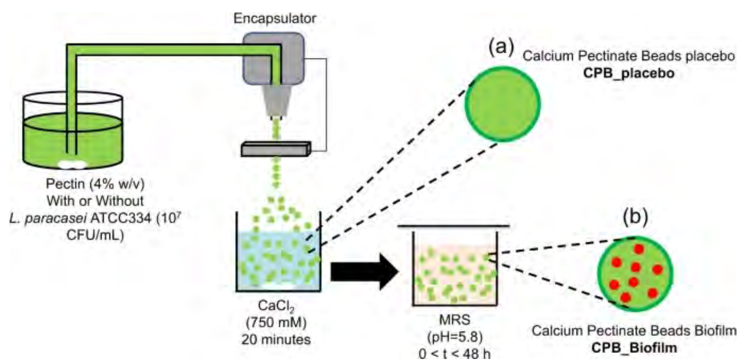
Simon et al. 2015 (PMID:26432804)

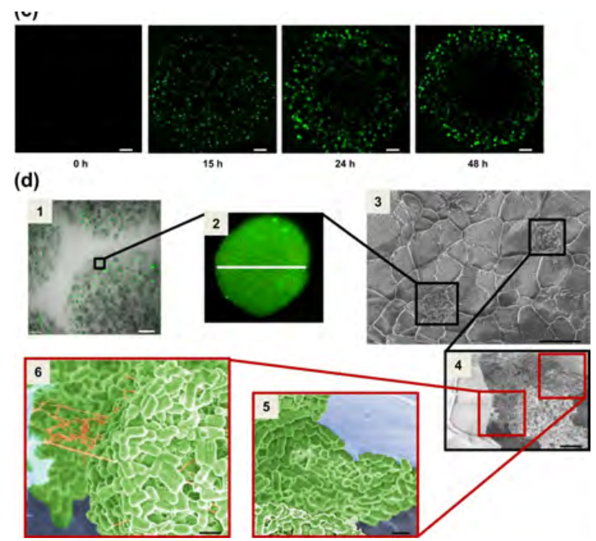
Potential probiotic strains tolerance to digestive stresses

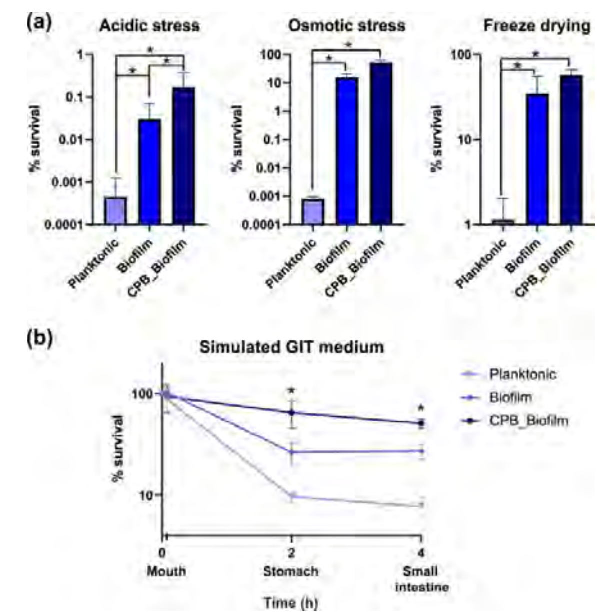


Chamignon et al. 2020. Microorganisms

Biofilm-like microcolonies encased in calcium-pectinate beads increases probiotic properties of *Lacticaseibacillus paracasei*

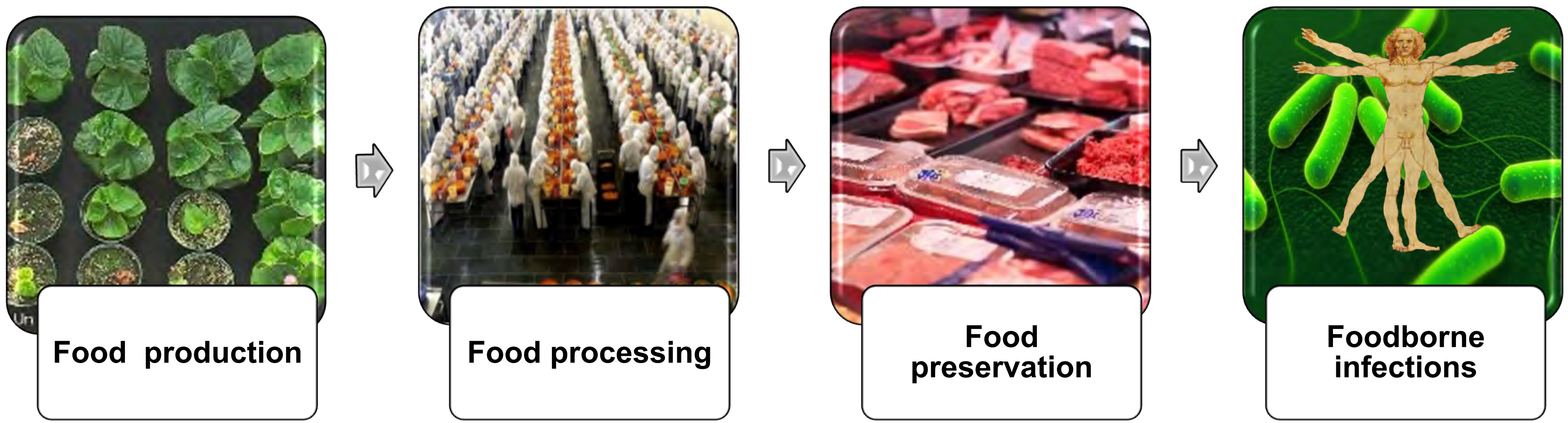






Heuman et al., NPJ Biofilm & Microbiome 2020

Toward microbial-based solution for biofilm control?



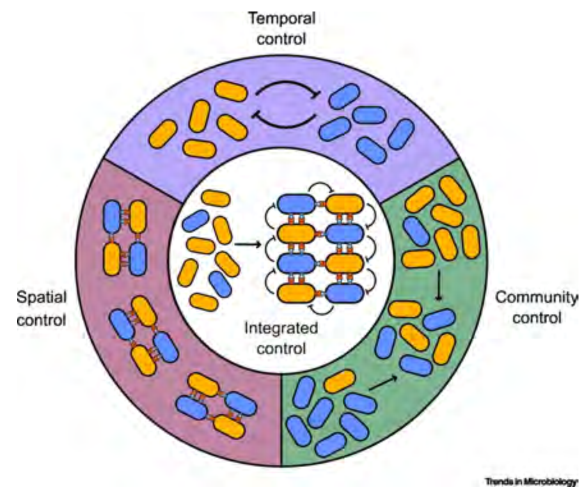
> pesticides, disinfectants, preservatives, antibiotics

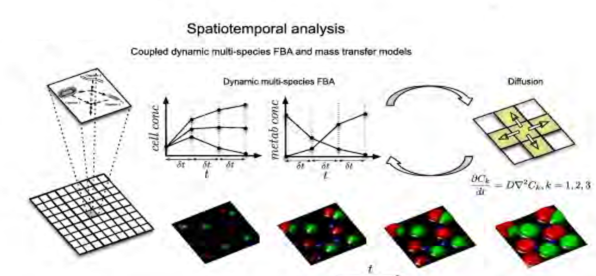
> biocontrol, bioprotection, biopreservation, probiotics

Future perspectives and challenges

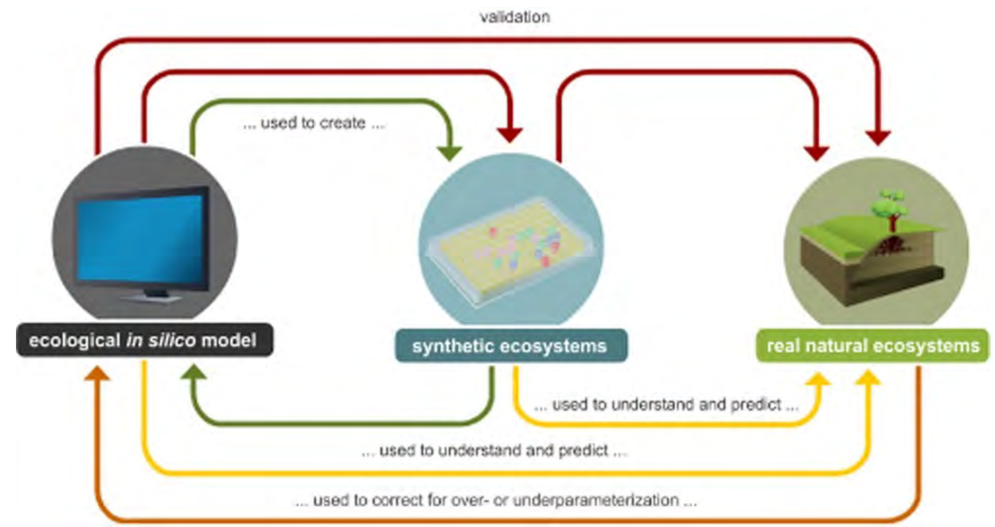
> synthetic microbial ecology, artificial and programmable biofilms

SYNTHETIC
MICROBIAL
ECOLOGY





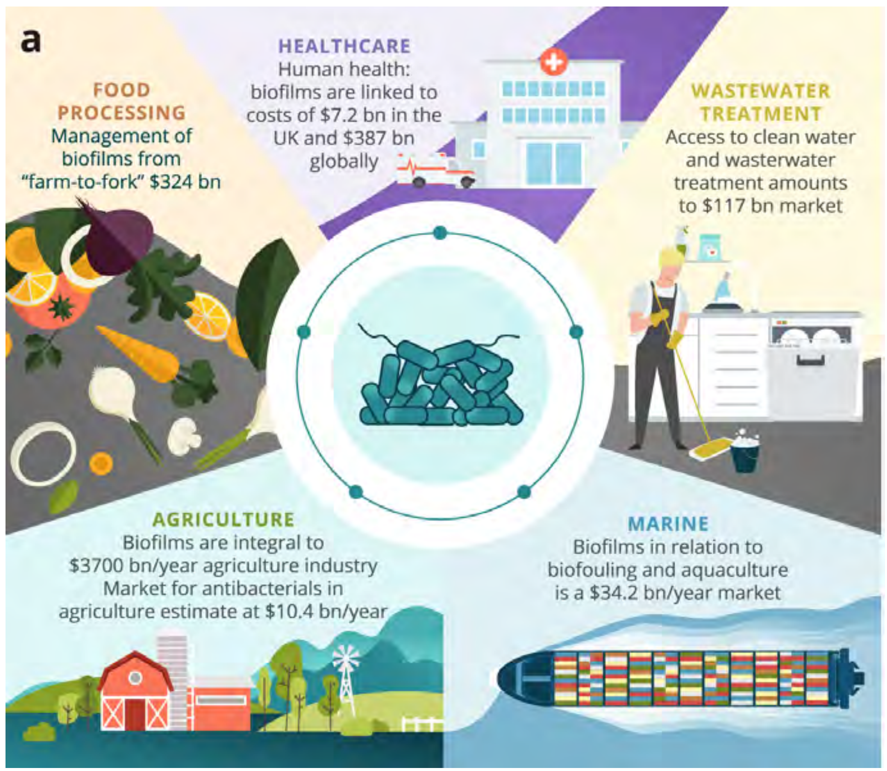
https://doi.org/10.1016/j.jmb.2015.10.019



https://enviromicro-journals.onlinelibrary.wiley.com/doi/10.1111/1462-2920.12343

Future perspectives and challenges

>Translational challenges and opportunities in biofilm science



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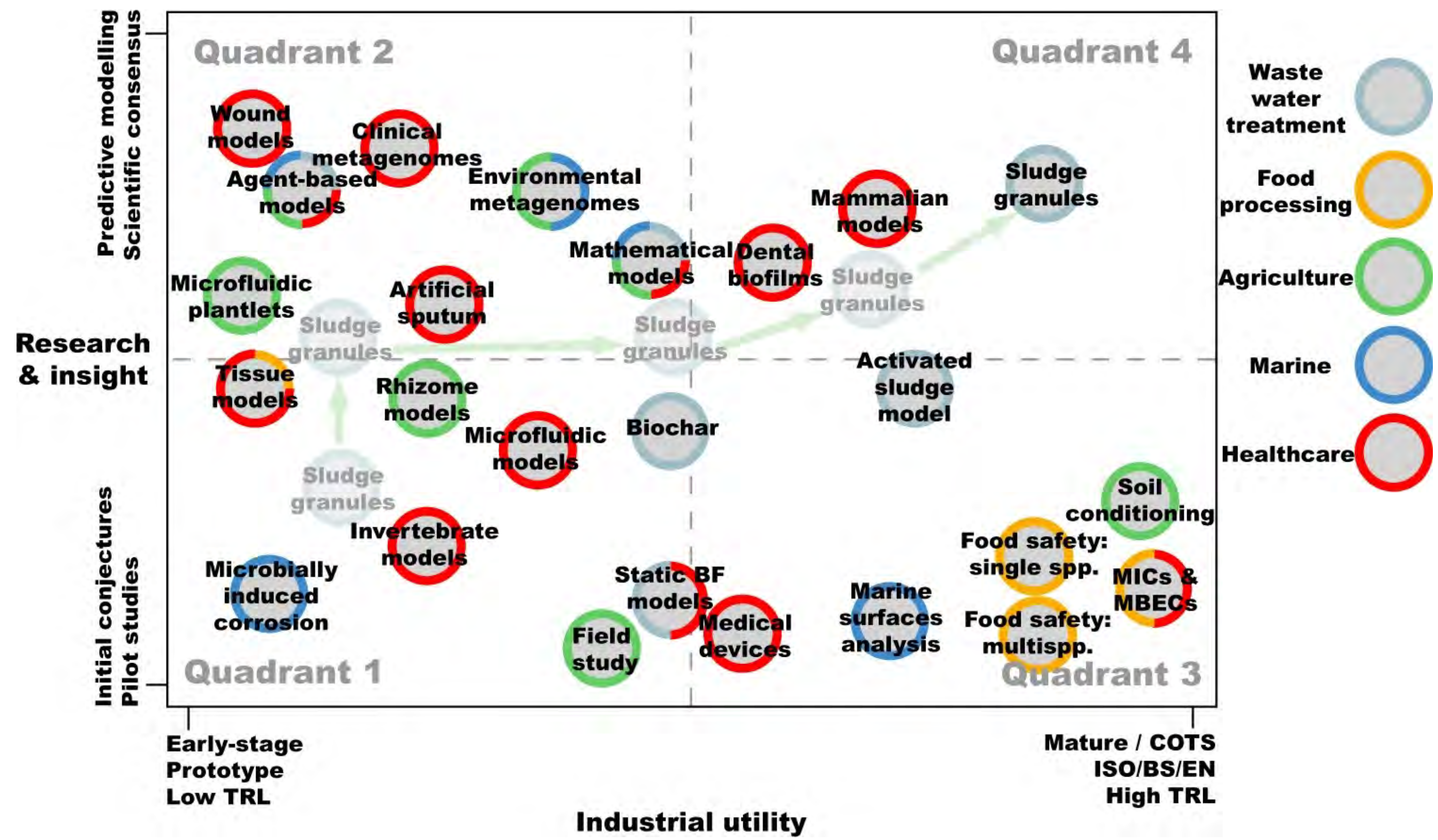

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ISC Infrastructure Scientifique Collective MIMA2 MICROSCOPY AND IMAGING PLATFORM

FERMENTS DU FUTUR

Thank you !