



ÉCOLE PRATIQUE
des HAUTES ÉTUDES

PSL 



UNIVERSITÉ
DE LORRAINE

Interactions Biofilms – Matériaux Enjeux du Futur

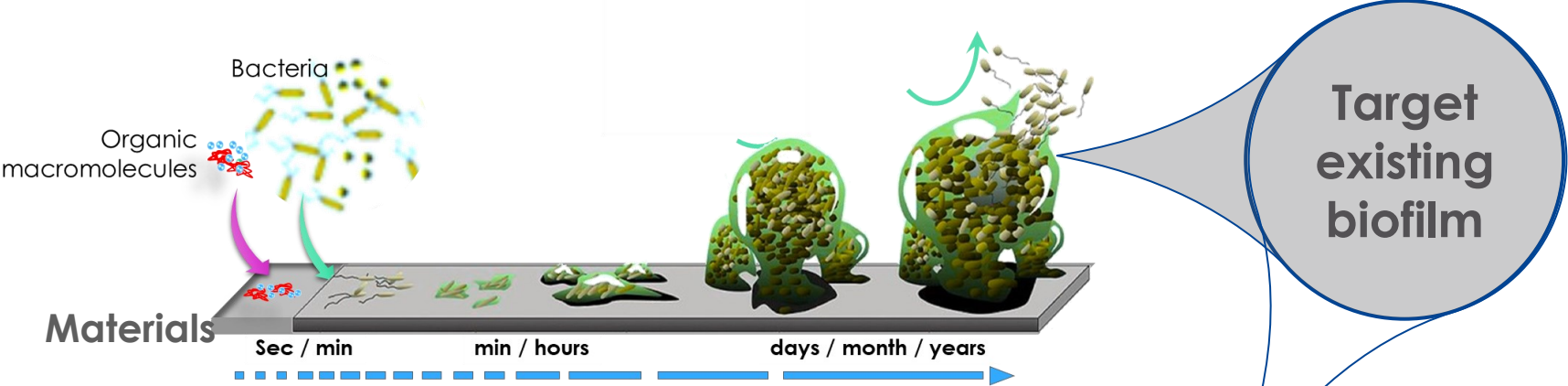
Stratégies Anti-Biofilms

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Antibiofilm strategies



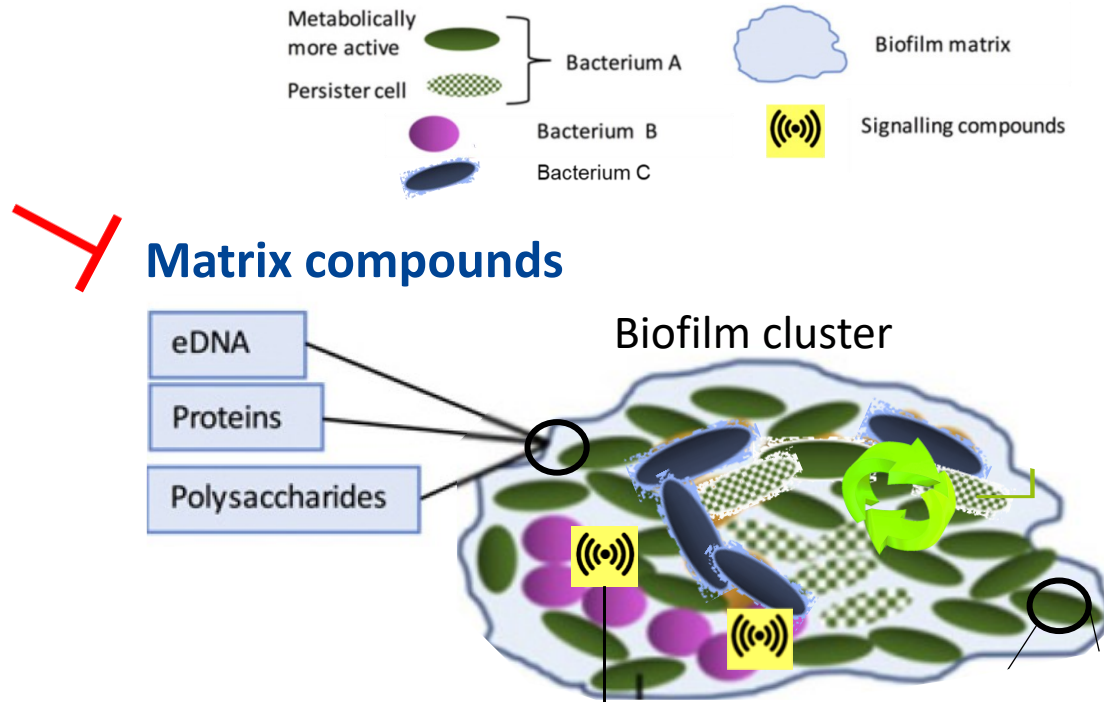
Antimicrobial agents

Modes of action:

- Weakening
- Disrupting
- Killing

Des molécules empruntées à la nature, De multiples cibles connues...

Dnases
Hydrolases (DispersinB)
Endopeptidase
(Lysostaphine)
Biosurfactants
Phages
...



Bacteriophage-based technology

Advantages

- Specificity
- Self-replication
- Cell lysis, EPS lysis
- Low perturbation of the ecosystem (prey/phage equilibrium)

Phagotherapy

- Virus of bacteria
- Lysogenic action



30- 300 nm

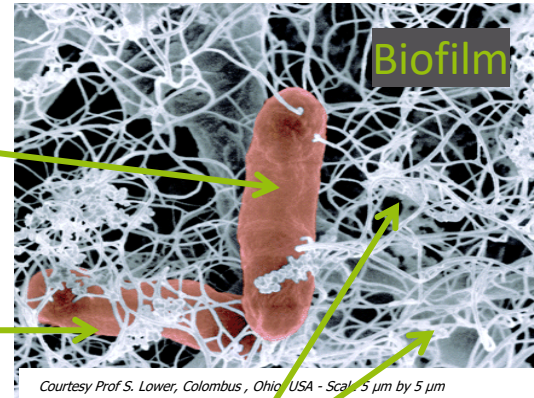
Industrial systems

Legionella genus-specific bacteriophages

(Lammertyn *et al.* Microb. Ecol. 2008)

Pseudomonas genus-specific bacteriophages

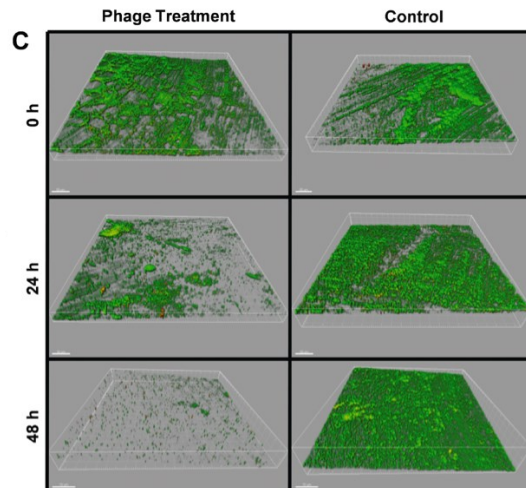
(Alveset *et al.* Microb. Biotechnol. 2016)



Courtesy Prof S. Lower, Columbus, Ohio USA - Scale 5 μm by 5 μm

Phage-borne polysaccharide depolymerases

(Hughes *et al.* Microbiology 1998)



Bacteriophage-based technology

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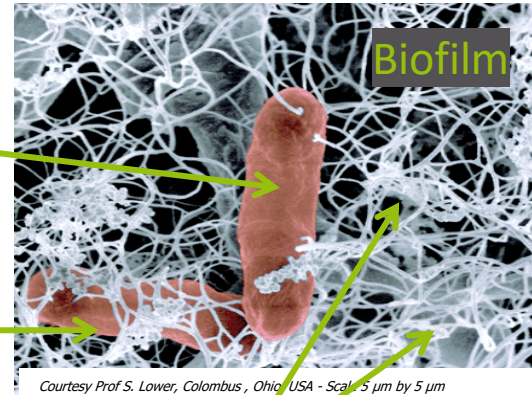
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Phage-borne polysaccharide depolymerases

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Limitations

Diffusion limited by EPS

(Briandet *et al.* 2008; Vidakovic *et al.* 2018)



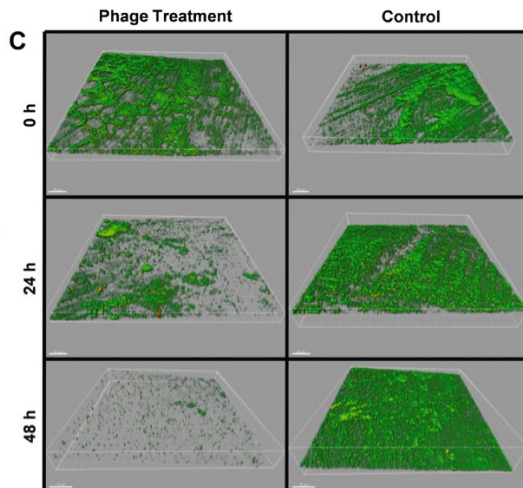
→ Important but **incomplete cleaning**
Due to bacterial resistance and low ratio prey/predator,

Major issue

→ complex bacterial consortia
→ Need phage cocktails

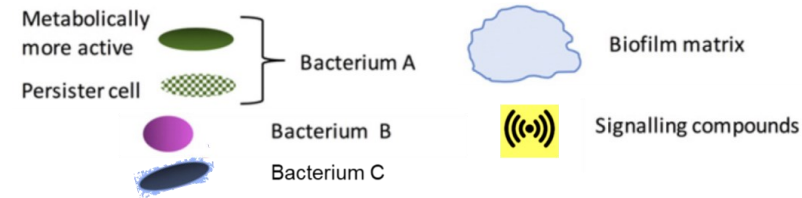
In situ studies

- Membrane fouling (Goldman *et al.* 2009)
- GAC filters (Zhang *et al.* 2013)

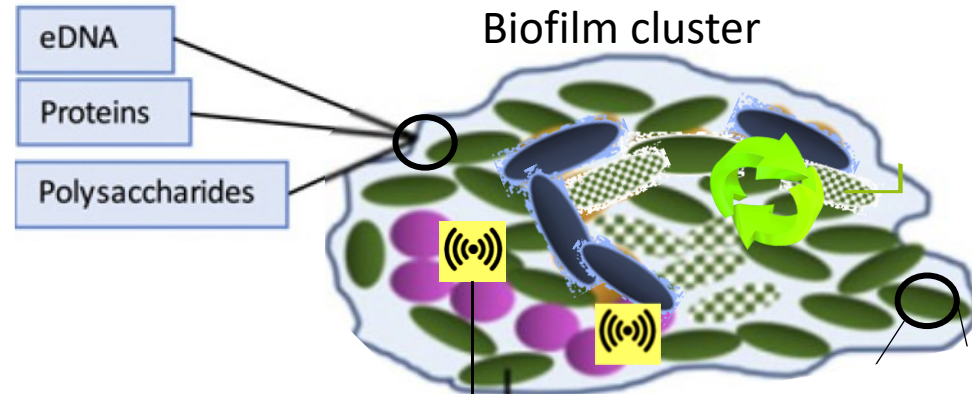


Des molécules empruntées à la nature, De multiples cibles connues...

- Dnases**
- Hydrolases** (DispersinB)
- Endopeptidase** (Lysostaphine)
- Biosurfactants**
- Phages**
- ...



Matrix compounds

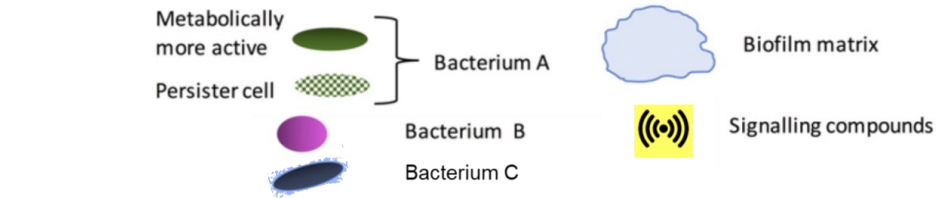


Signalling compounds

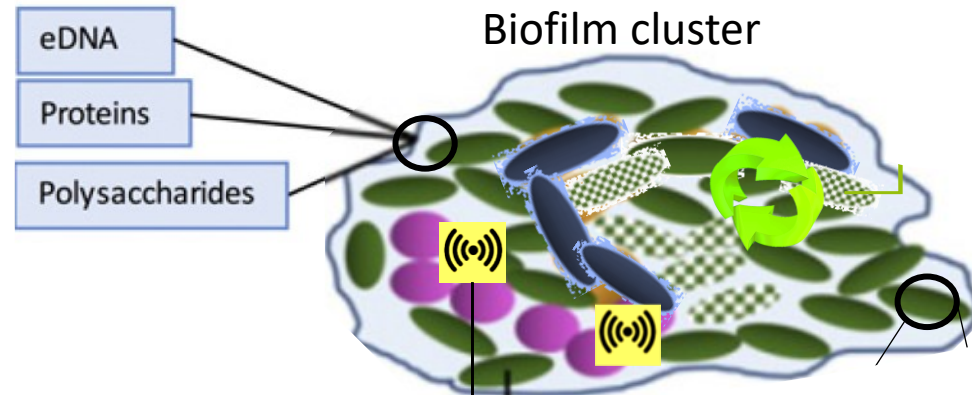
- Quorum sensing inducer AHL-mediated
- Cyclic-di-GMP
- Alarmone (p)ppGpp

Des molécules empruntées à la nature, De multiples cibles connues...

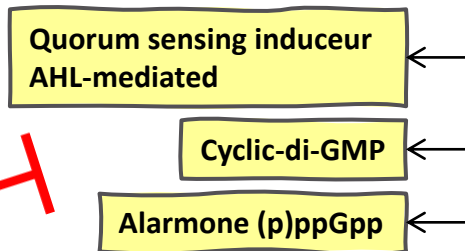
Dnases
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Matrix compounds



Signalling compounds

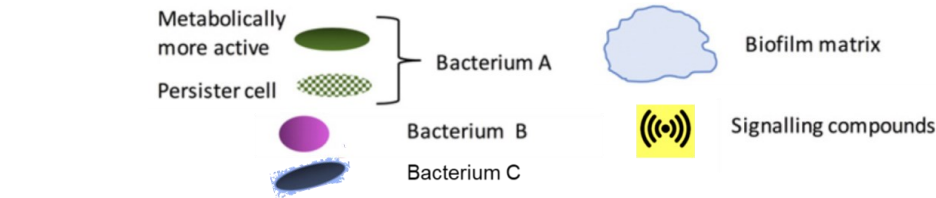


Antimicrobial Peptides
(AMP) (LPP4010, LP3145, LL-37, P-1038, 1037, 1038...)
QS inhibitors
 as furanone, polyphénols
 (catechine, acide tannique),
Flavonoids (quercitine)
 ...

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- QS inhibitors**
as furanone, polyphénols
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- Flavonoids** (quercetine)
- ...



Matrix compounds

- eDNA
- Proteins
- Polysaccharides

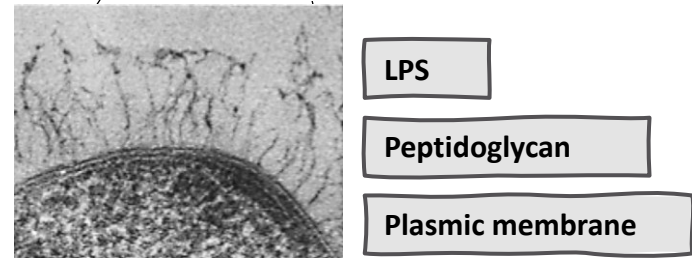
Bacterial physiology

- Cell division and survival
- Macromolecule synthesis

Signalling compounds

- Quorum sensing inducer
AHL-mediated
- Cyclic-di-GMP
- Alarmone (p)ppGpp

Bacterial components



- AMP**
- Lantibiotics** (nisin, subtilin,...)
- Bacteriocines** (bovicin,,,,)
- ...

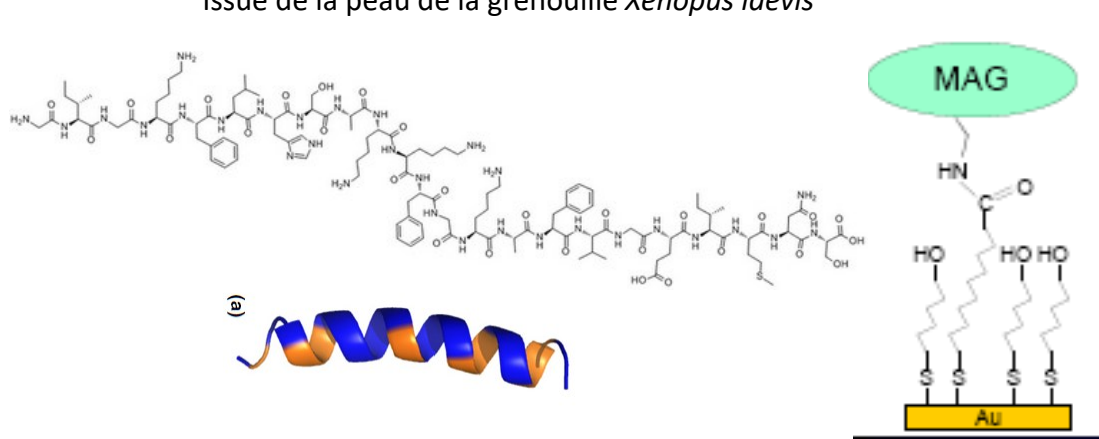
- AMP** (tannic acid, phenols, usinic acid, gramicidine, microcin, buforin I, PR-39, ...)
- Sophorolipids**
- Chitosan**
- ...



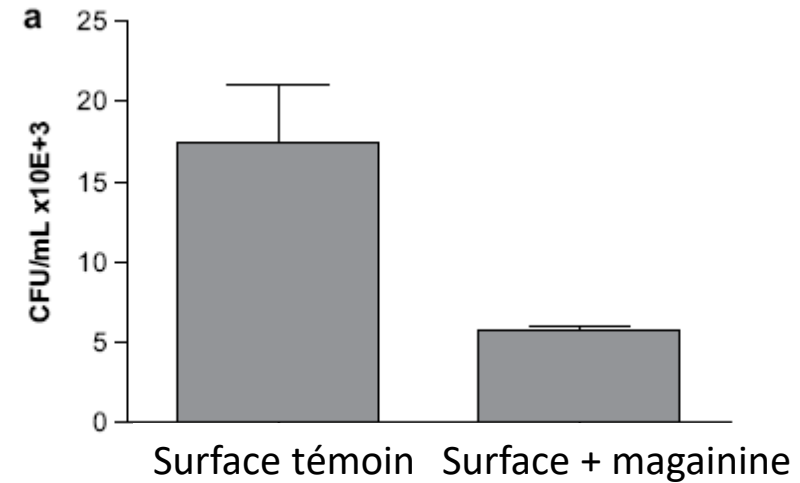
AMP-base surfaces: the magainin example

Magainine et son greffage sur une surface

Issue de la peau de la grenouille *Xenopus laevis*

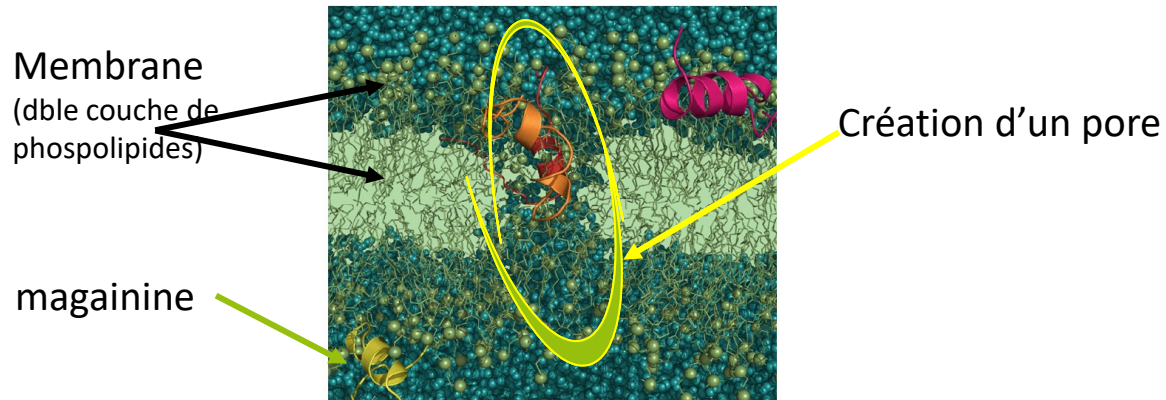


Activité antimicrobienne sur *Listeria ivanovii*

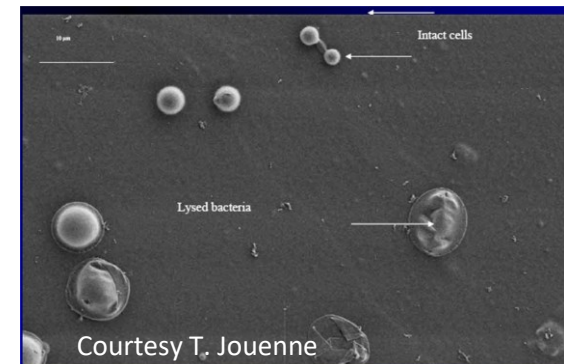


Interaction peptide – membrane plasmique

Simulation numérique



Bactéries altérées sur la surface



...mais (les limites...)

- domaines agroalimentaire et médical
- applicabilité dans des circuits d'eau !?

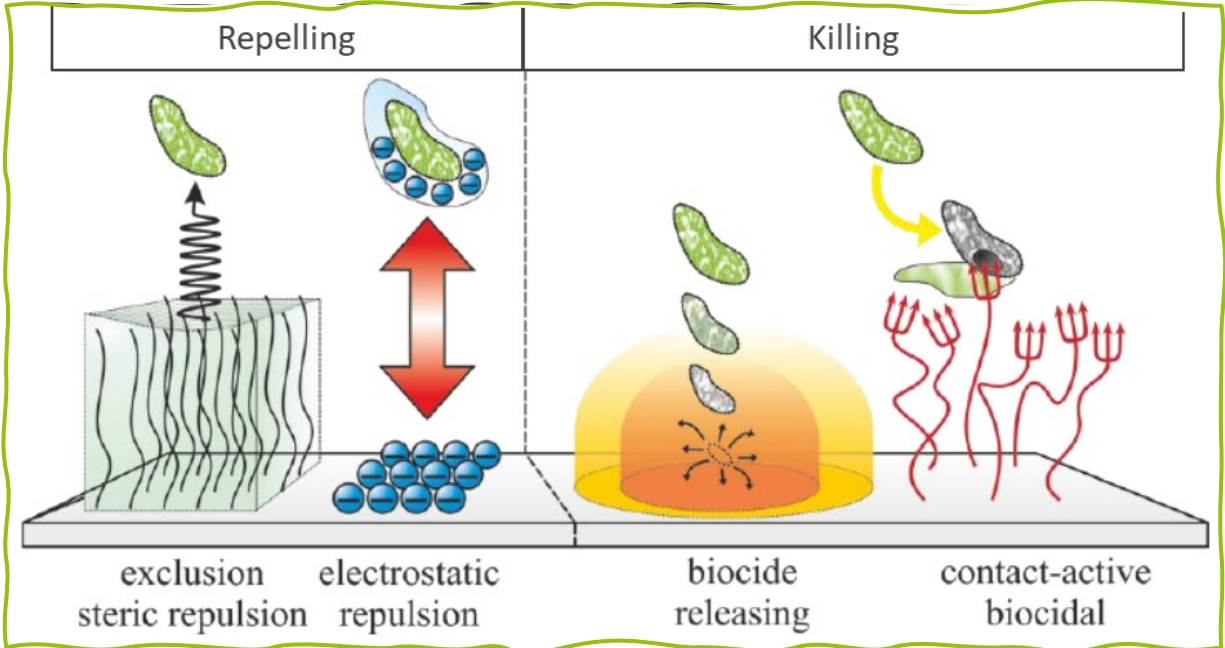
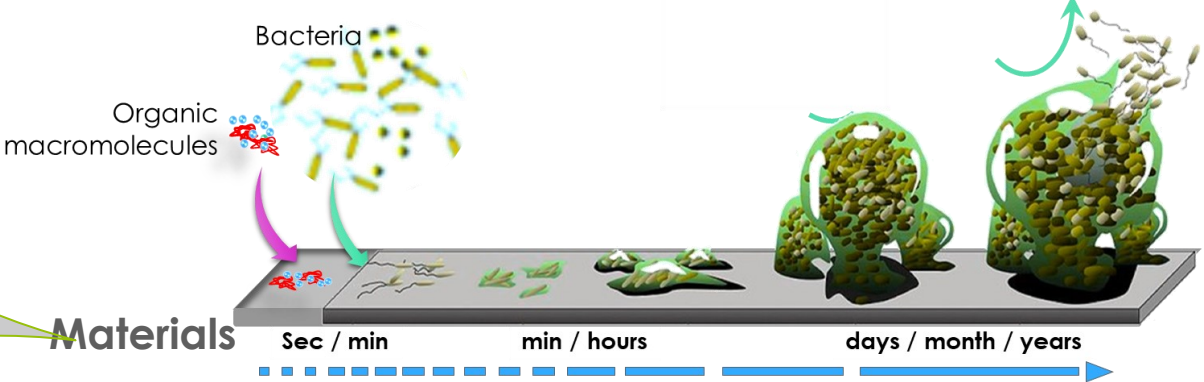
- démonstrations *in vitro*
- biofilms modèles (pathogènes)
- biofilms jeunes
- conditions statiques

- de faibles quantités de molécules anti-microbiennes disponibles pour une forte quantité de matières premières nécessaire.



Antibiofilm strategies

Prevent biofilm formation



→ compréhension des interactions bactérie-surface = Anti-ADHESION



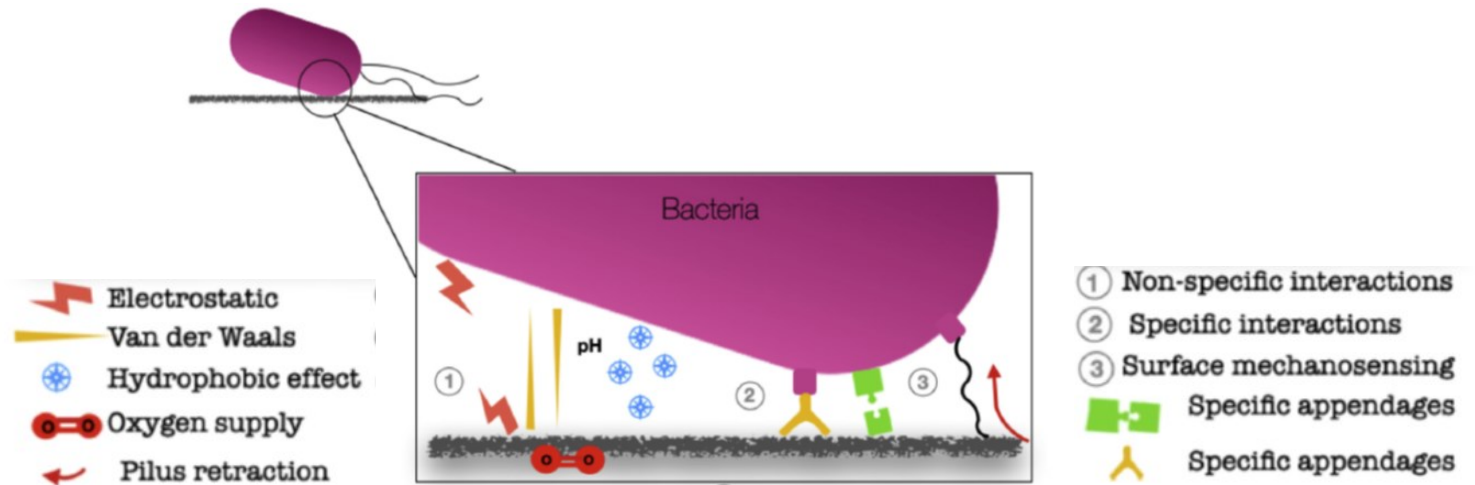
Adhesion, multiparametric parameters

Facteurs liés au microorganisme

Caractéristiques physico-chimiques de la surface microbienne (hydrophobie, charge)

Phase de croissance (état physiologique)

Adaptation phénotypique

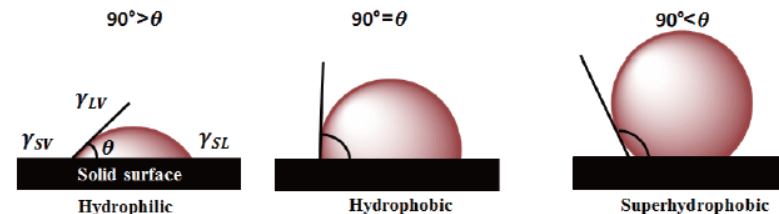


Facteurs liés au support

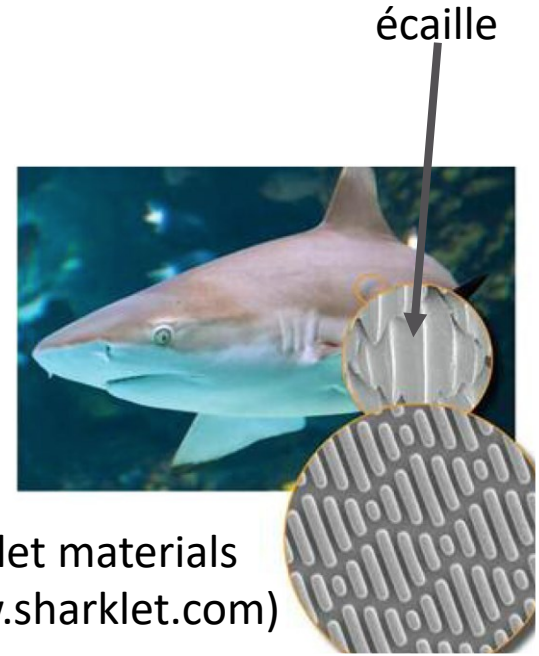
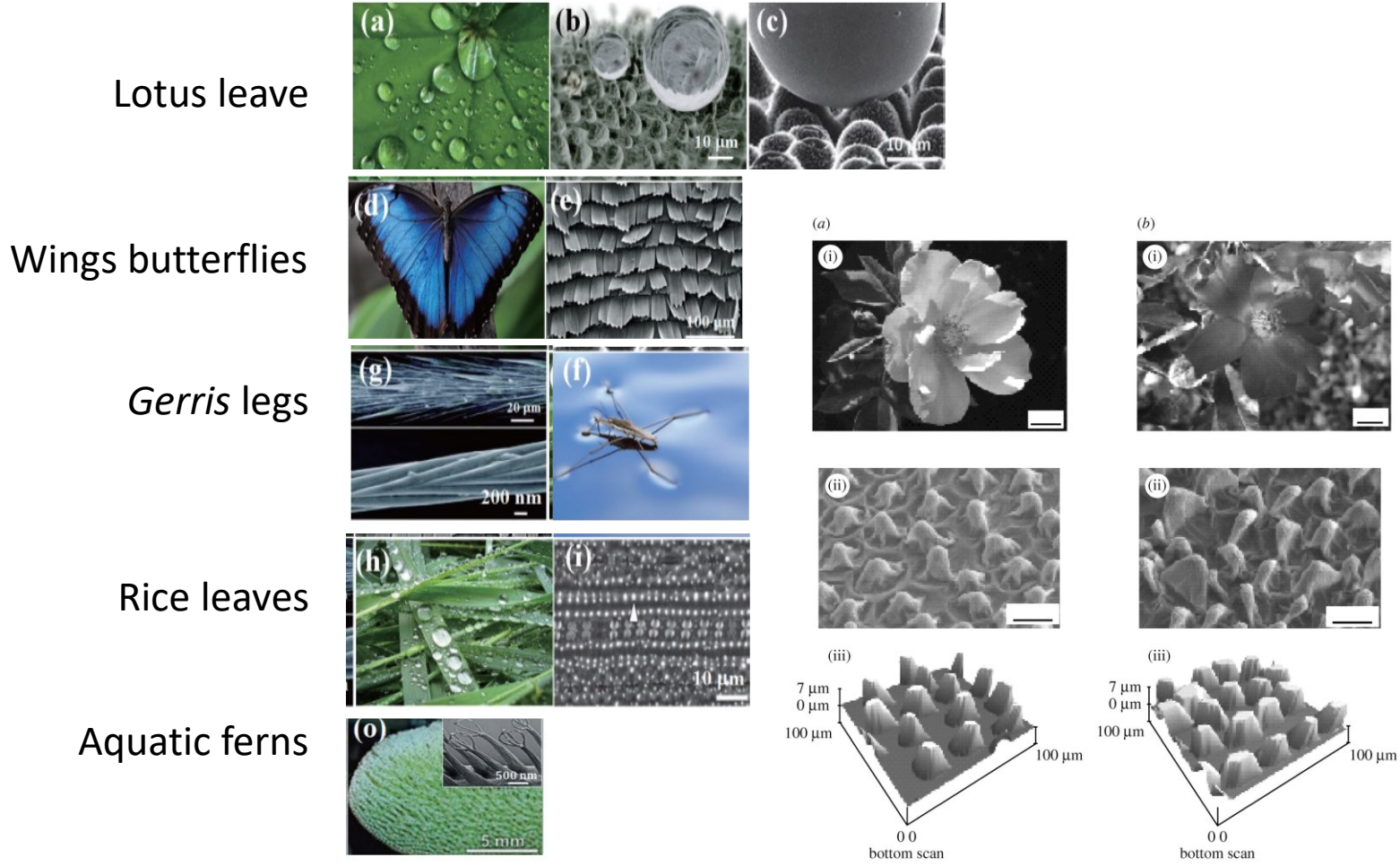
Rugosité et micro-topographie »

Charge de surface

Hydrophobie



Nature, an inspiration for microtopography and hydrophobic biomimetic materials



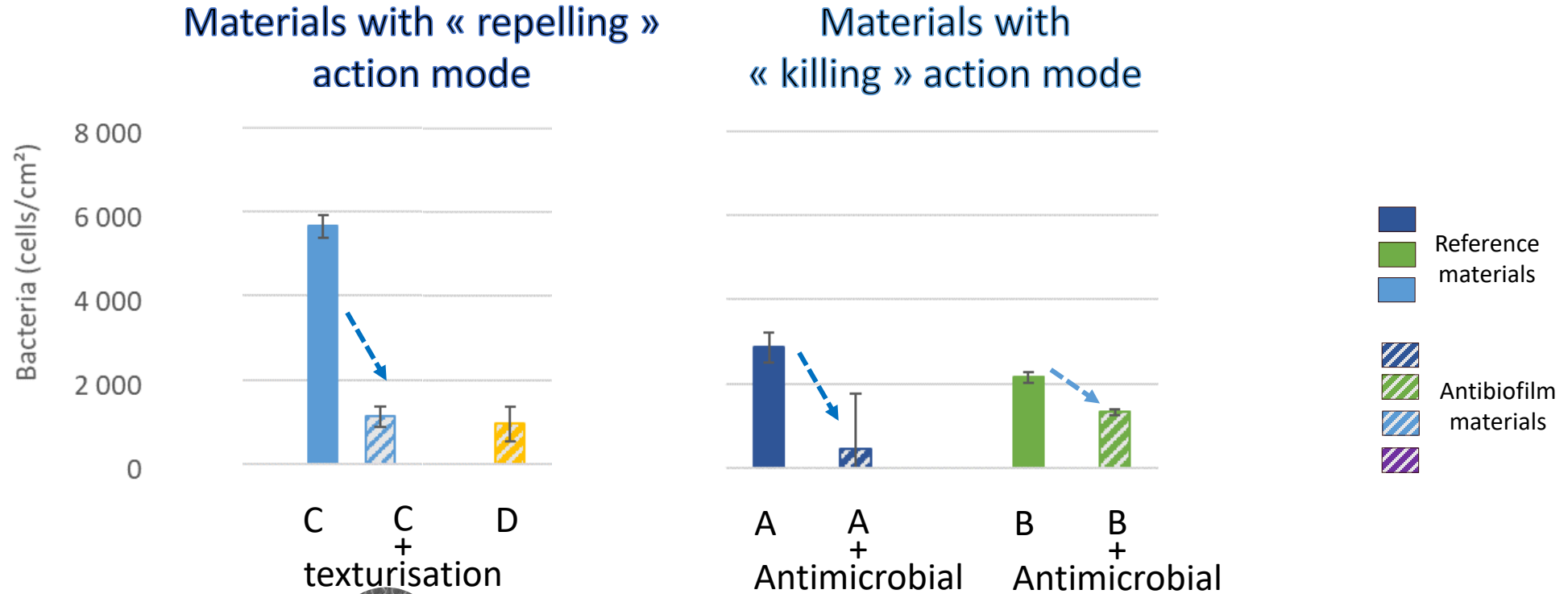
Sharklet materials
(www.sharklet.com)

Matériau biomimétique

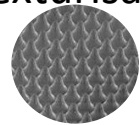




Surface modification, an efficient strategy on early colonization (potable water)

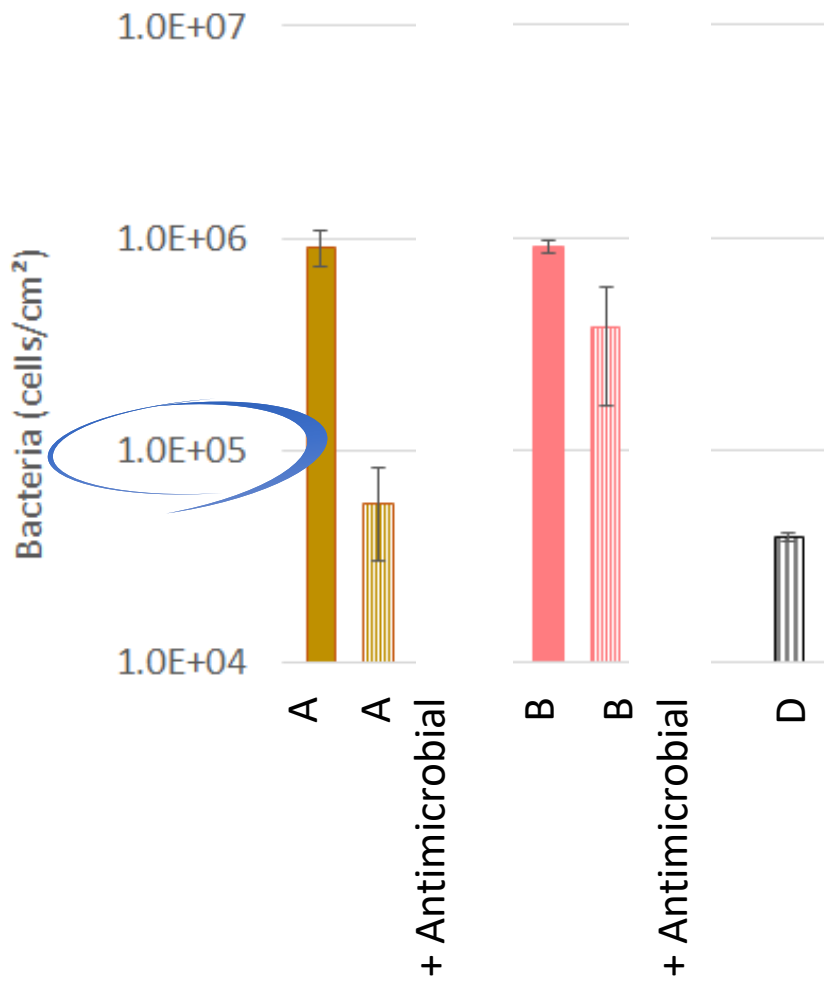


All are hydrophobic
→ **Ho adhesion forces (AFM) = 6 to 10 nN**





Tested materials allow the formation of a mature biofilm (6 weeks) ... but some more than others...

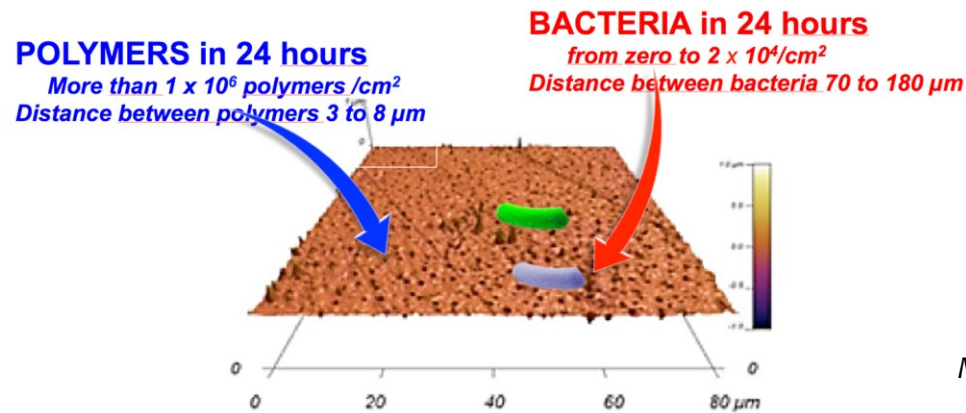


Rational for mature biofilm formation, even on previous so-called anti-biofilm surfaces ?



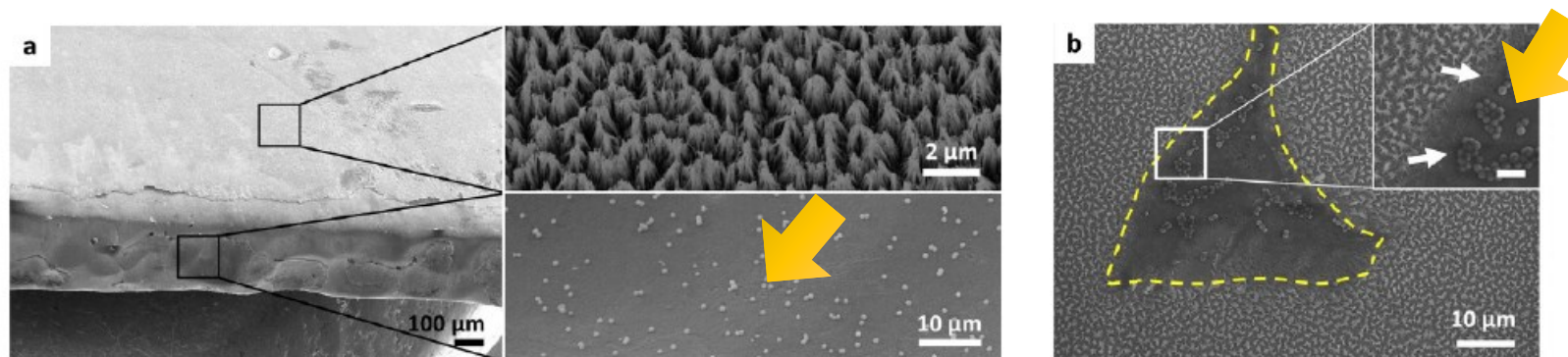
Rational for mature biofilm formation, even on previous so-called anti-biofilm surfaces

- **Conditioning film** (early macromolecule deposits) → screening of surface



Mathieu et al., 2014; Maul et al., 2015; Francius et al., 2017 Water Research

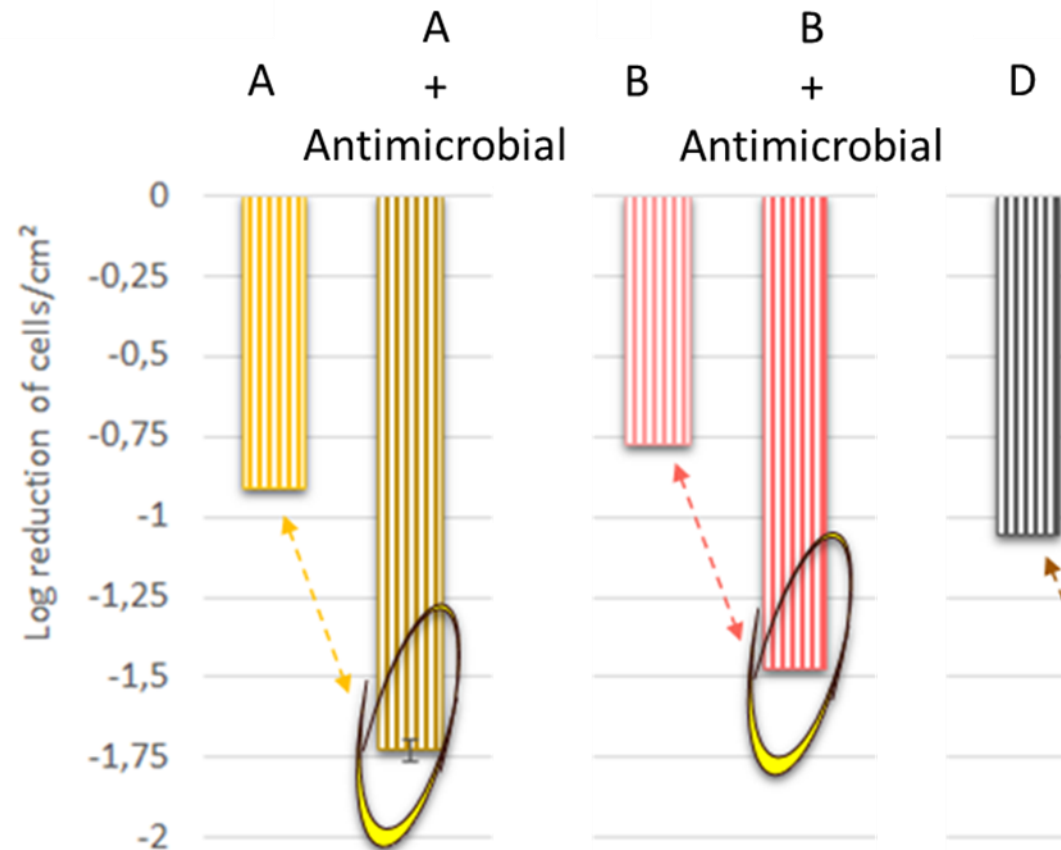
- **Surface defects** → colonization areas



Hinzal et al., Advance in Materials & interfaces, 2017

Surface modification, an efficient strategy on surface nettoyability

Cleaning conditions: chlorination (50 mg/L Cl₂ for 30 min)
+ hydrodynamic (increase from 1 to 5 Pa for 60 min)



Conclusions

- Surfaces antibiofilm, une réalité... dans la nature
- Matériaux antibiofilm, des avancées considérables mais limites/contraintes
 - Durée de vie limitée
 - Masquage des fonctions par écrantage / Perte d'activité
 - Réservoir limité, épuisement du principe actif
 - Vieillessement du matériau fonctionnalisé (érosion mécanique; dégradation par oxydation, ...)
- Dans le contexte eaux naturelles «Matériau zéro adhésion» : un rêve Accessible !
 - Cela nécessiterait
 - des surfaces avec des propriétés physico-chimiques extrême (motifs μm et nm , rugosité, composition...)
 - besoin d' une meilleure connaissance des « objets » véhiculés par l'eau : colloïdes mous, de taille de 0,1 à 5 μm et de propriétés de surface très différentes.
- Lutte anti-colonisation → approche multi-stratégies
 - matériau plutôt très **Hydrophobe**, bonne capacité auto-nettoyante, **texturation** (motifs)...
 - **couplé** à des pratiques de nettoyage/désinfection régulières



Stratégies Anti-Biofilms

Merci pour votre attention

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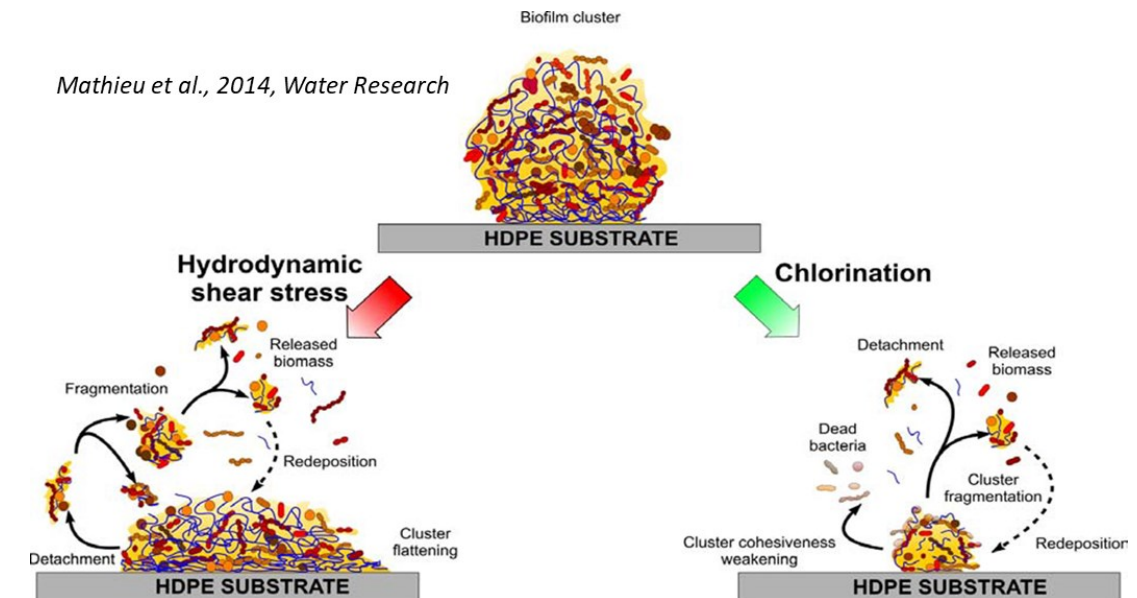


AMP

- Les peptides antimicrobiens sont des peptides de petite taille allant d'une dizaine à quelques dizaines d'acides aminés
- premiers peptides antimicrobiens (bactériocines) aient été découverts chez les bactéries vers le milieu des années 20
- premier isolement à partir d'une espèce pluricellulaire fut celui de la cécropine à partir du papillon *Hyalophora cecropia* en 1981
- sont généralement riches en acides aminés basiques (lysines et arginines) et en acides aminés hydrophobes (leucines, isoleucines, phénylalanines et tryptophanes).
- nombreux peptides ont été identifiés chez les plantes, les espèces animales invertébrées et vertébrées, dont l'être humain
- près de 2700 peptides antimicrobiens d'origine naturelle dont les séquences peptidiques sont établies (au moins partiellement) ont été recensés sur la base de données « Antimicrobial Peptide Database » (APD),
- plus de la moitié de ces peptides (56 %) est retrouvée chez les vertébrés

Chemical & physical treatments against biofilms have limited/partial effects

<u>Hydrodynamic flushing</u>	<50% removal 200µm clusters escape Contact points in biofilm x2	Abe et al. Water Research 2012 Mathieu et al. Water Research 2014
<u>Chlorination</u>	70 % removal Contacts points in biofilm -30% Release of OM from the walls Bacterial resilience (gamma proteobacteria) Defence against oxidative stress Rapid repopulation of the surfaces	Mathieu et al. Water Research 2014 Mathieu et al. Biofouling 2016
<u>New material easy to clean</u>	Nanopatterned fakir-like carpet	FUI 14 - Sirena+ Paper in preparation
<u>Heterogeneous Fenton-like</u>	Highly efficient even at pH 6 (iron from corrosion) Unapplicable = red waters	Gosselin et al. Water Research 2013
<u>Low magnetic electric fields (EMF)</u>	No effect on young drinking water biofilms	Gosselin et al. Biofouling 2018
<u>Low-frequency ultrasounds</u>	Promising technology	Mathieu et al. Ultrasonics Sonochemistry 1st revision 2019



Bacterial colonization and material properties - Major conclusions of the statistics (MLR, PCA)

- Q1

Are the characteristics of the materials at T24h dependent on those at T0?

→ More the naked material is hydrophobic, more it strongly traps hydrophobic polymers from drinking water.

- Q2

Is the colonization at T24h dependent on the physicochemical material characteristics?

→ No relation between the bacterial colonization (T24h) and the initial physicochemical characteristics of the material (T0)

→ No relation between the number of adhered bacteria (T24h) and the physicochemical characteristics of the material after T24h immersion in drinking water

→ More the surface is covered with macromolecules, more there is a chance that bacteria adhered in 24 h.

- Q3

Is the cleanability * of the materials dependent on their physicochemical characteristics?

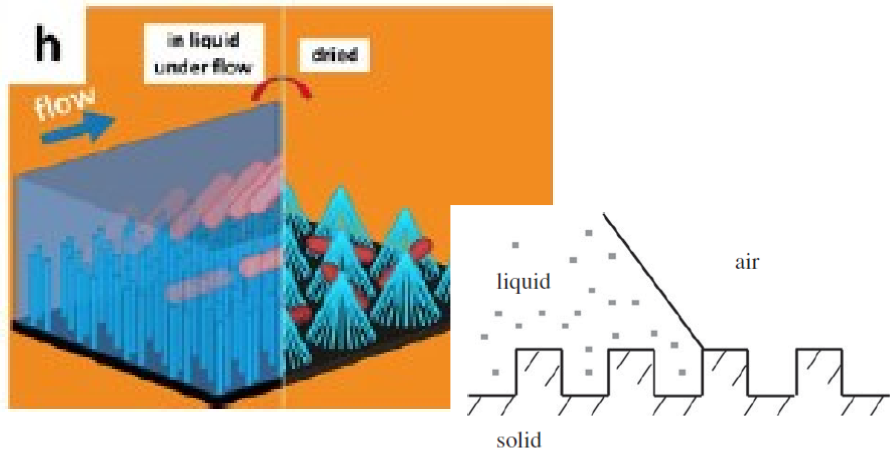
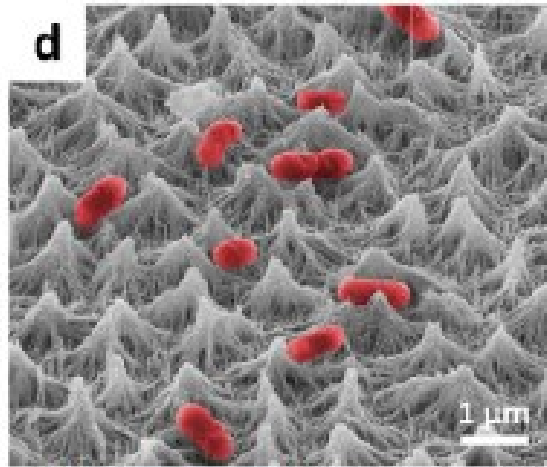
→ No relation between the initial physicochemical characteristics of the material (T0) and its cleanability

→ More the interaction force between the polymers and the surface is strong, less is the cleanability of the material

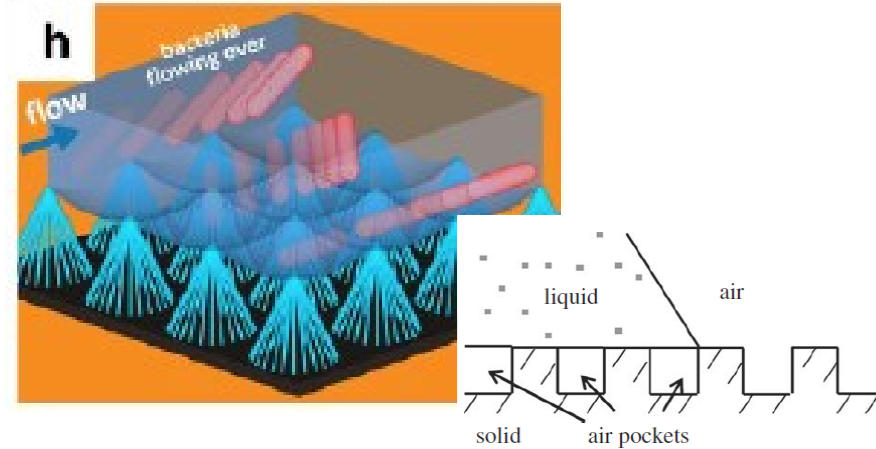
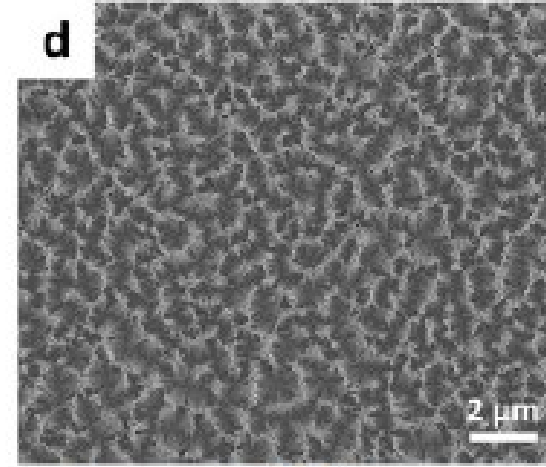
* Biofilm of 5 weeks -
Shear stress + 50 mg/L Cl₂ (1h)

Surface texturation, a key parameter

Hydrophilic nanopillard surface
+ *E. coli*



Hydrophobic nanopillard surface
+ *E. coli*



Des mécanismes de tolérance connus ...

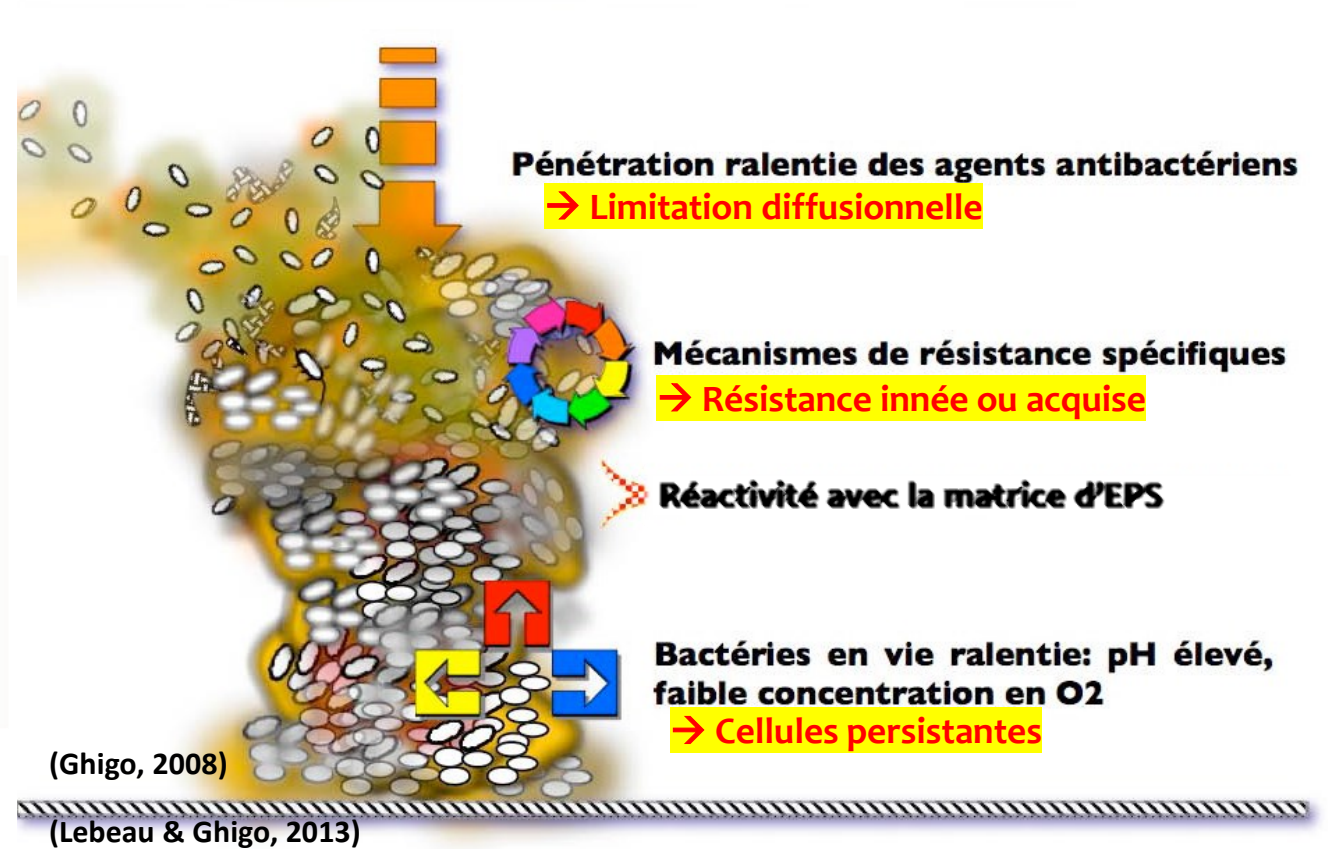
Zone réactionnelle

Piégeage / accumulation

Microorganismes (parfois pathogènes) sous abris !

Biocorrosion / fouling

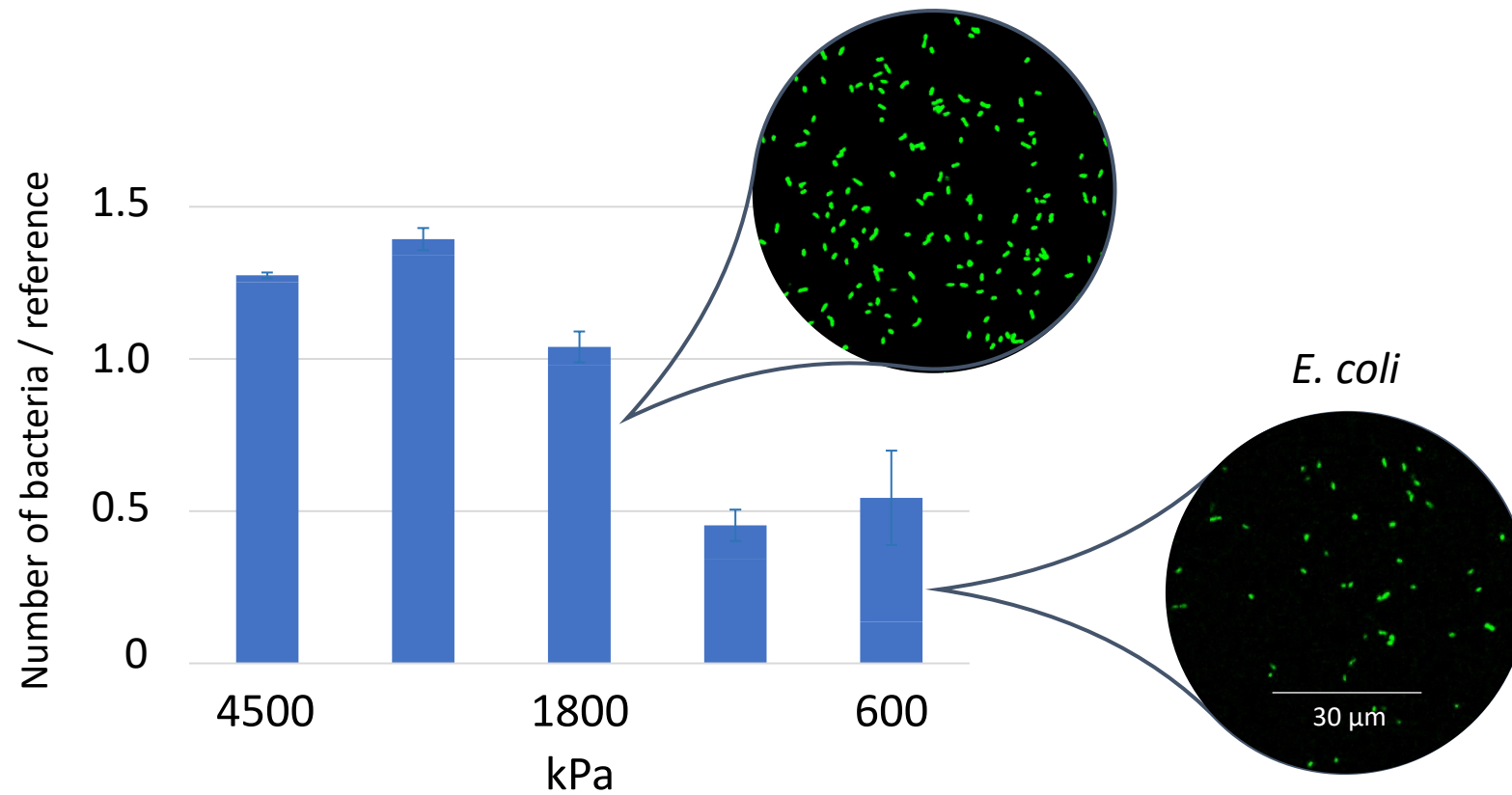
À l'origine de pathologies / maladies nosocomiales



→ Impacts technologiques, économiques et sanitaires

Softness / Stiffness

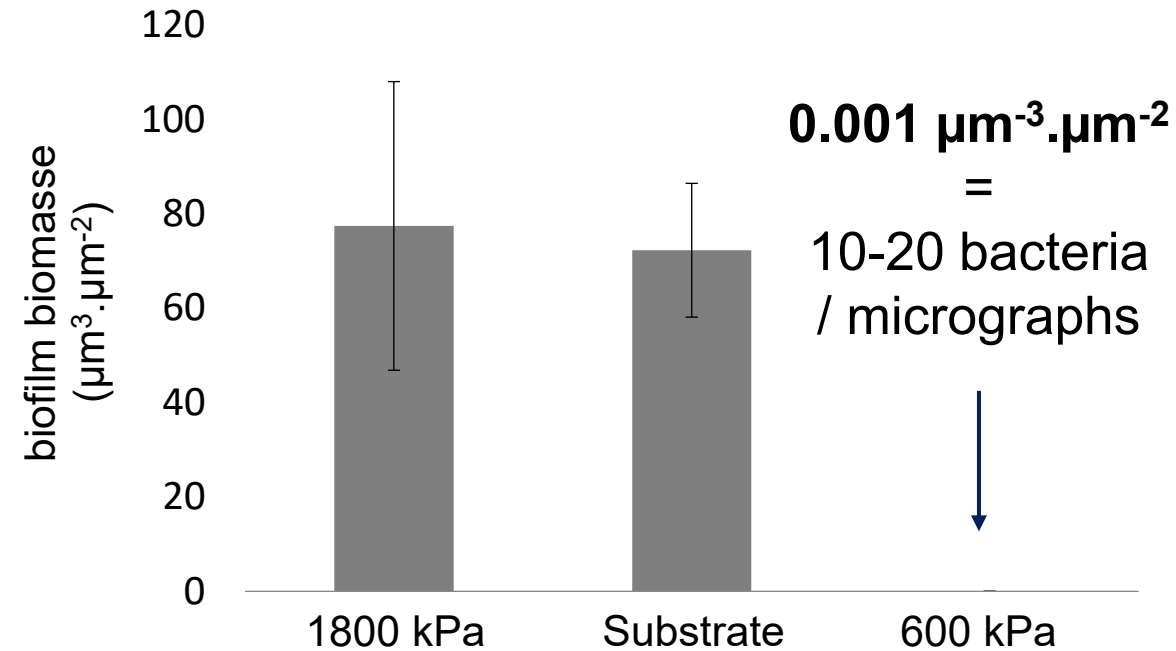
➔ Des mécanismes d'adhésion « mécano-sélectifs »



Veuillet et al., in preparation
Veuillet et al., Plasma Proc Polym, 2017
Veuillet et al., ECM journal, 2016

Softness / Stiffness

A biofilm-preventive approach (20h)



⇒ **Significant and high efficiency against biofilm formation!**