Biofilm : quelle évidence et quelles conséquences pour le traitement ?

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1re JEDI – 14 nov. 2019

Centre Hospitalier de Tourcoing

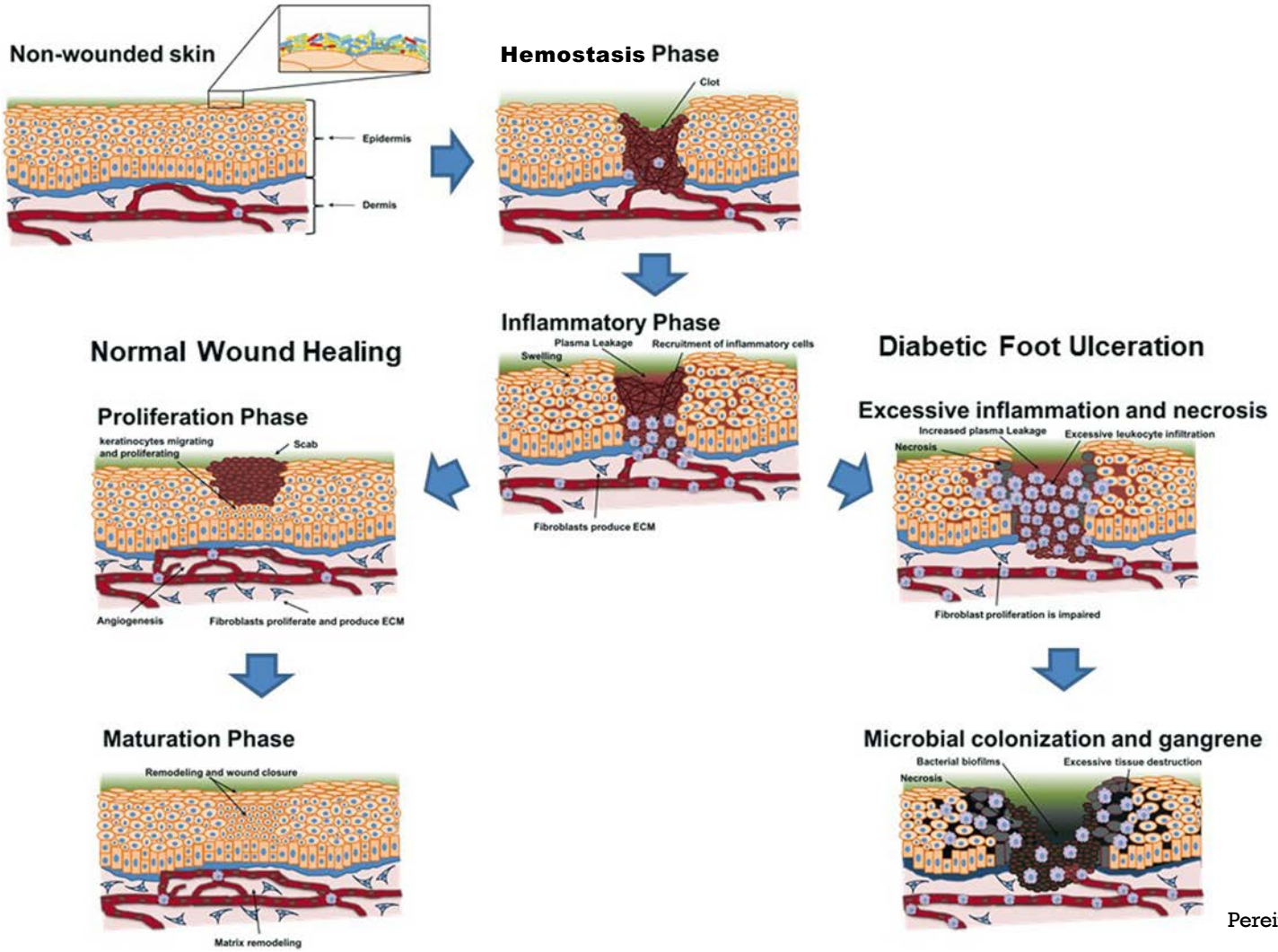
Université de Lille

CIIL
CENTER FOR INFECTION & IMMUNITY OF LILLE

Conflits d'intérêt

Aucun concernant cette présentation

Infection du pied diabétique : histoire naturelle



Phase de la guérison	Non diabétique	Diabétique
Réponse inflammatoire	✓	✗
Prolifération	✓	✗
Maturation	✓	✗

✓ normale

✗ déficiente ou anormale

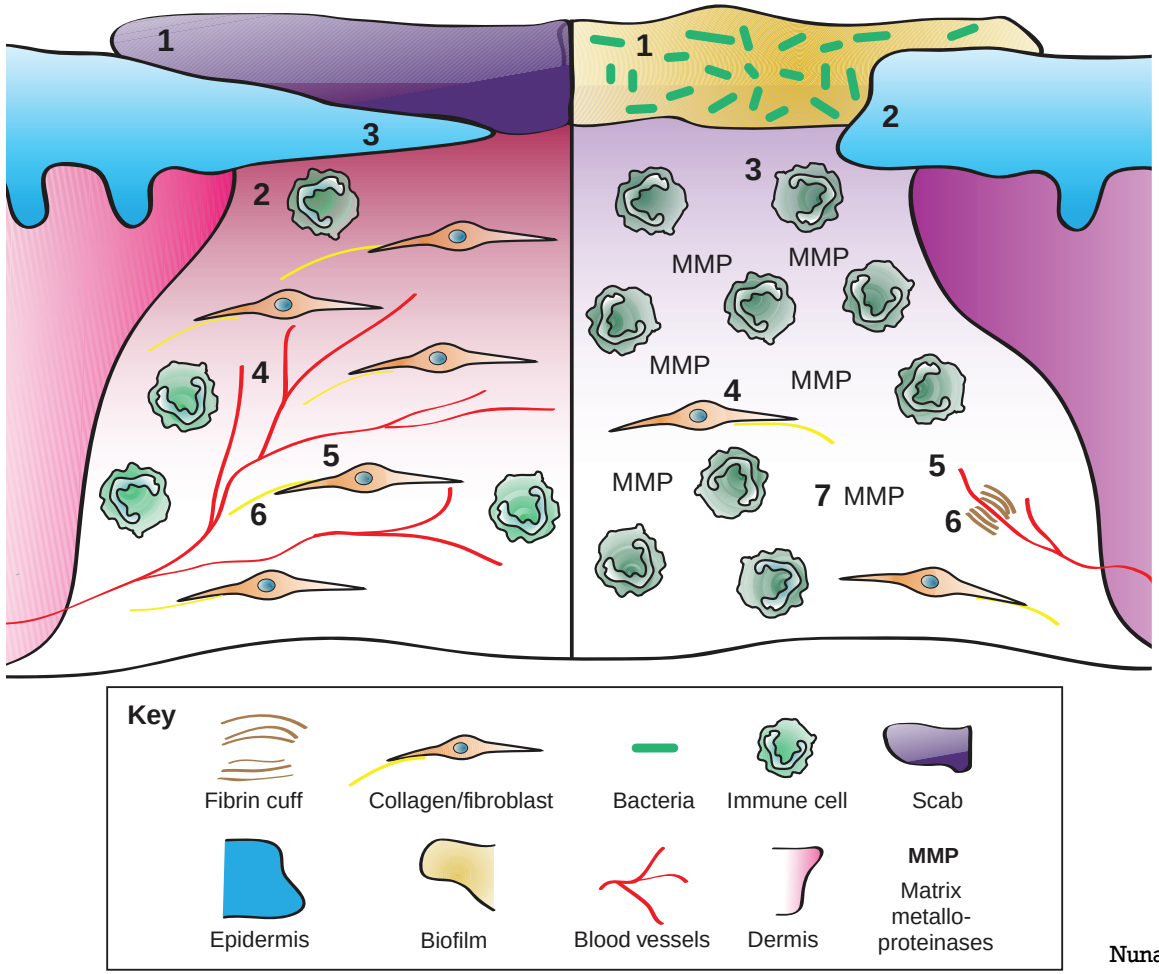
Pereira, *Front Microbiol* 2017

… à l'échelle cellulaire

Plaie en voie de guérison / *Plaie chronique infectée*

Inital phase:
1. Scab formation
2. Immune cell infiltration

Healing phase:
3. Re-epithelialisation
4. Angiogenesis
5. Fibroblast migration
6. Collagen deposition

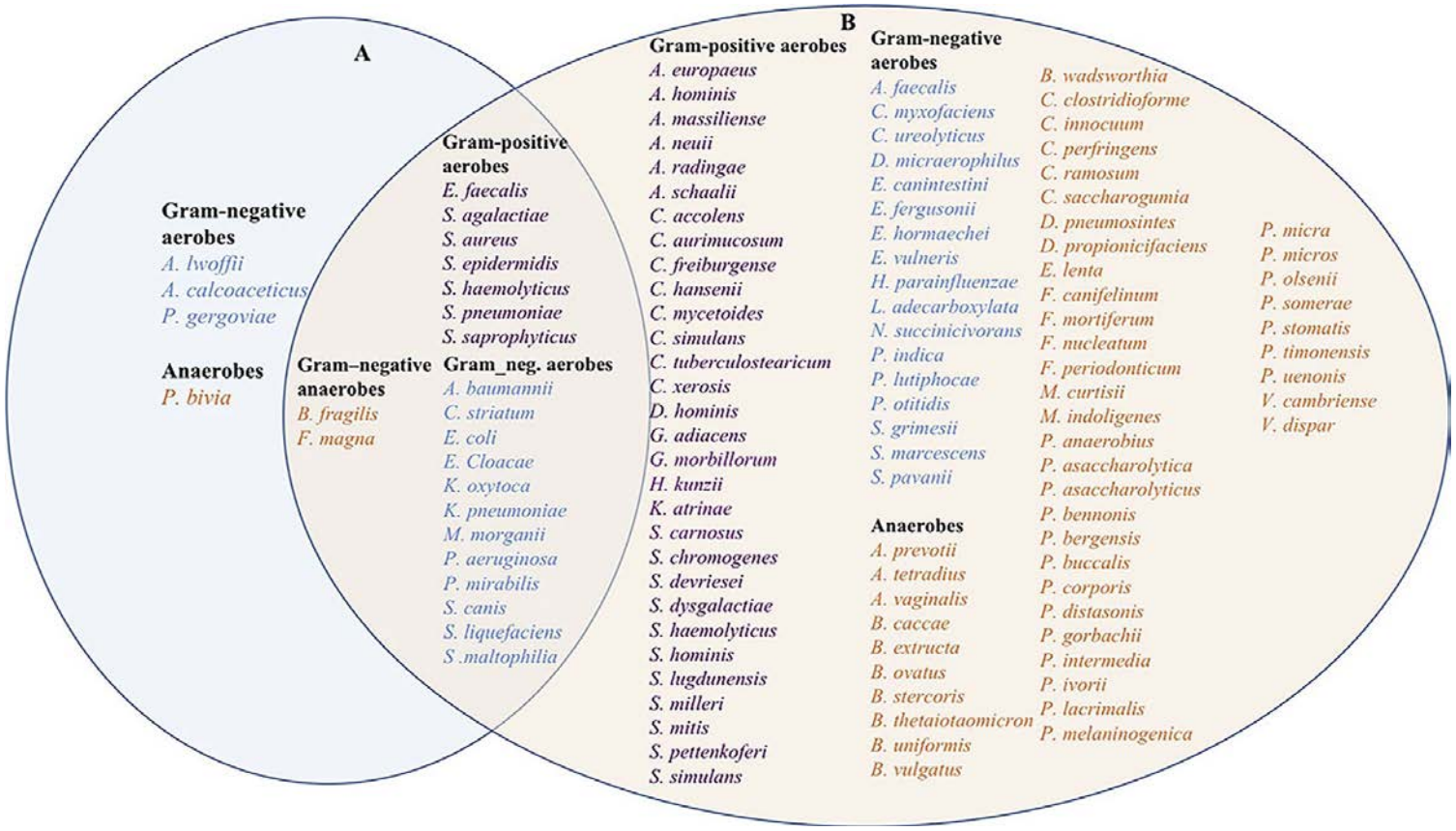


Chronic wound abnormalities:
1. Infection/biofilm
2. Hyperproliferative epidermis/ stalled re-epithelialisation
3. Persistent inflammation
4. Fibroblast senescence
5. Impaired angiogenesis
6. Fibrin cuffs (barrier to oxygen)
7. Elevated MMPs

Nunan, *Dis Model Mech* 2014

Aspects microbiologiques des IPD

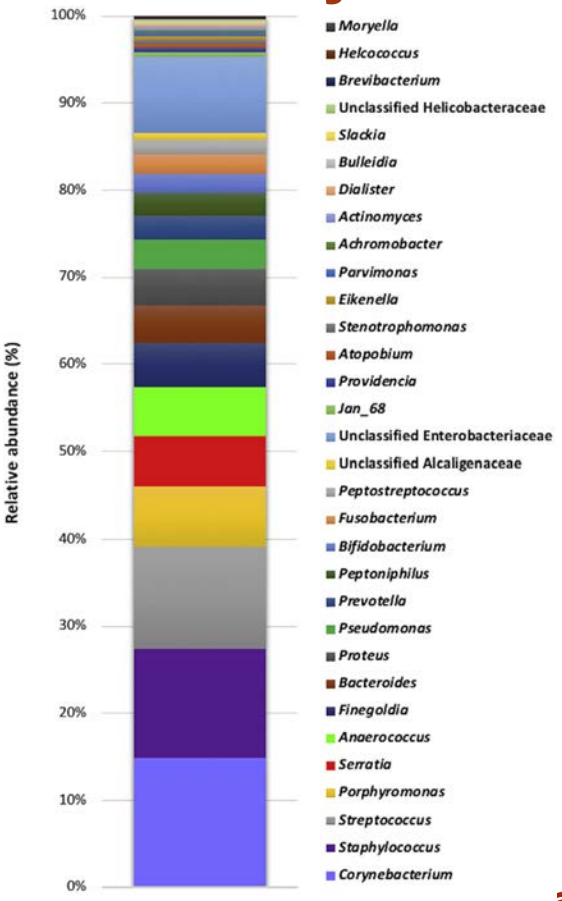
IPD



Jneid, *Hum Mic J* 2017
Johani, *Clin Microbiol Infect* 2019

Approche culturale et moléculaire (16S rRNA)

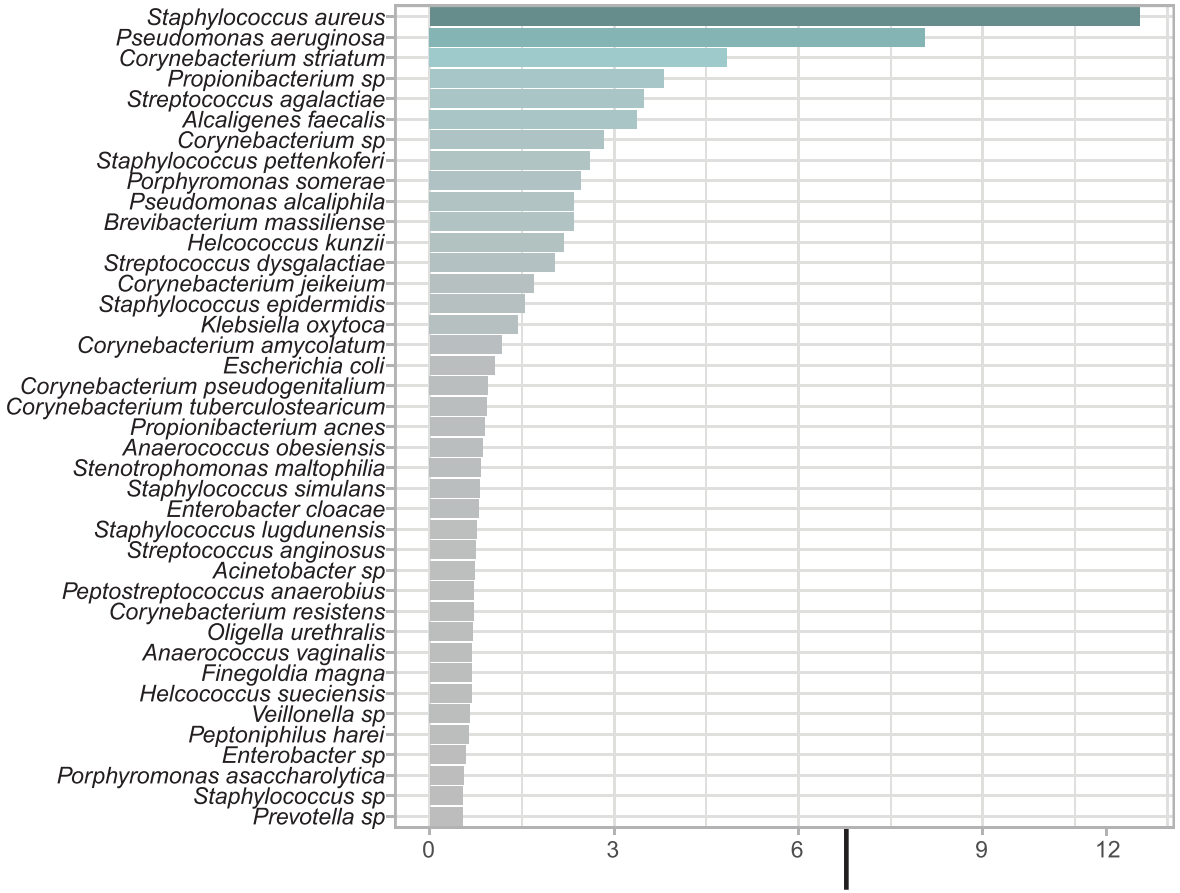
Ostéomyélites



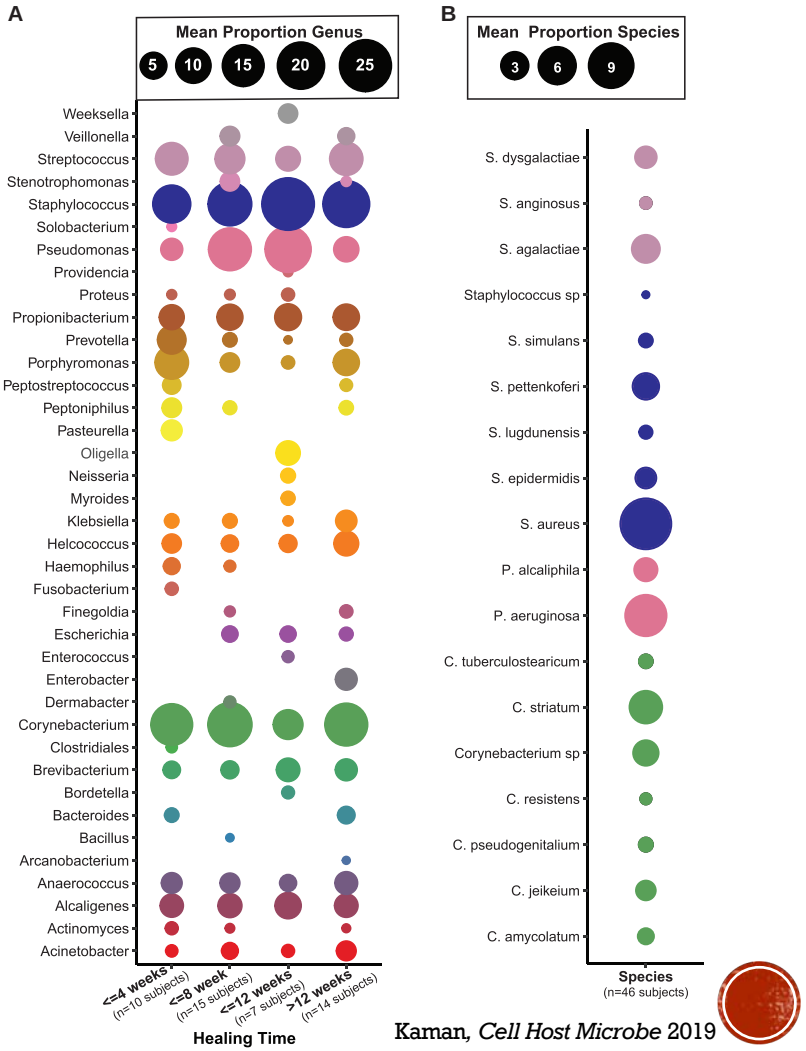
Approche moléculaire (16S rRNA)

Aspects microbiologiques des IPD

Ulcères du PD



Approche moléculaire
(Shotgun Metagenomic Sequencing)



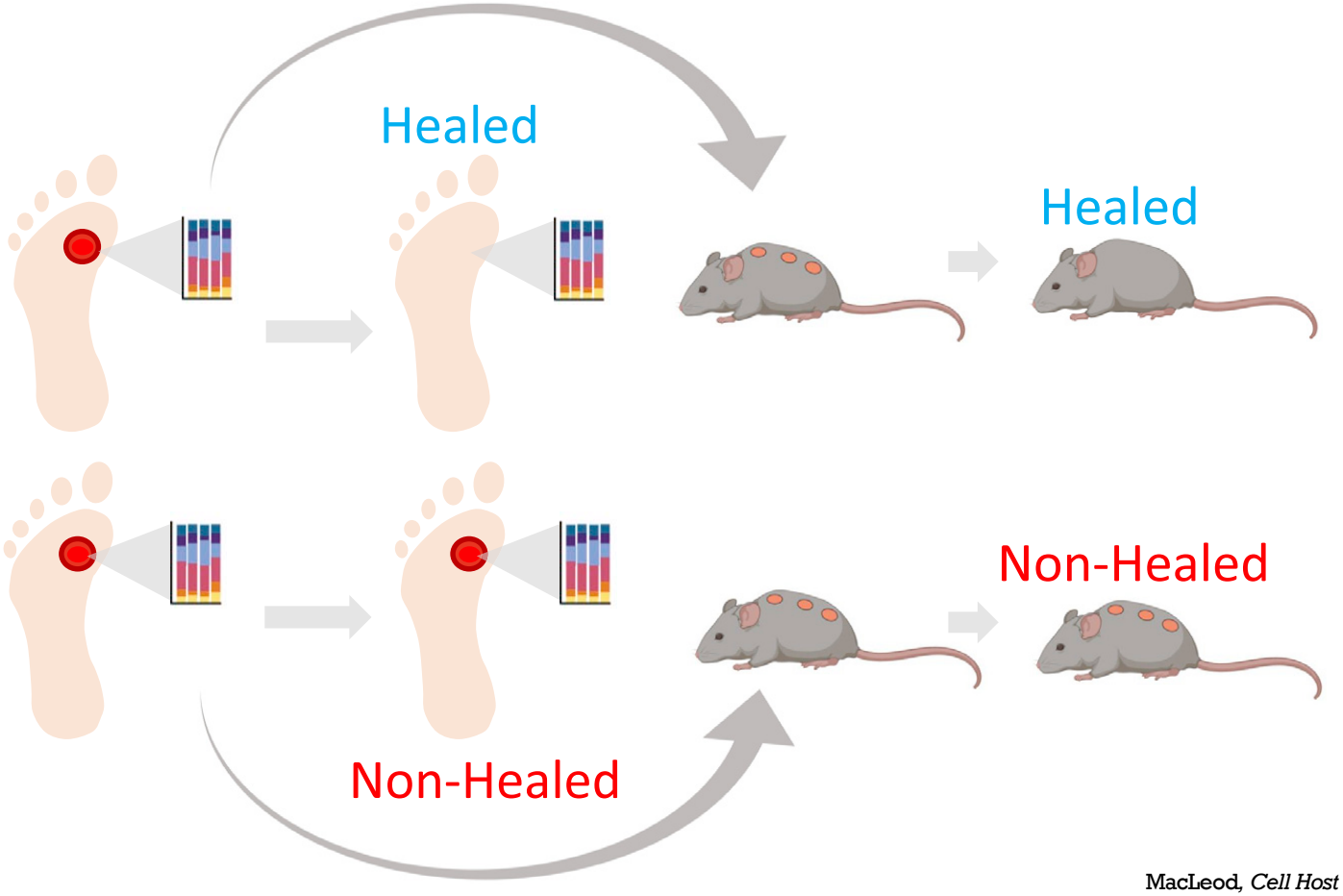
Kaman, *Cell Host Microbe* 2019

Commensal / Pathogène : où est la frontière ?

Bacteria	Positive Effects	Negative Effects
Staphylococcus epidermidis	Stimulates keratinocyte production of host AMPs (hBD3, RNase7) [22,74,75,88] Induces CD8+ T and IL-17A+ T cells [79] Enhances innate barrier immunity and limits pathogen invasion in absence of inflammation [6,74,80,89]	Occasionally pathogenic Implicated in production of biofilms [79,90–93]
Staphylococcus aureus	At a local level, super antigen production results in less skin inflammation and purulence due to decreased prod…actic facto… Amp…oduction of A…	Usually pathogenic Imp…nd heal… proc…[81]
Group A streptococcus (GAS)	Stim…ial diffe… Acti…cyte chem…nds [95]	Usu… Exp… recr… Proc…on thro… Con…i.e., impetigo, erysipelas, cellulitis [6]
Pseudomonas aeruginosa	Accelerates epithelialization and neovascularization in acute wounds Suppresses staphylococcal pathogens in polymicrobial wounds [84]	Usually pathogenic Implicated in production of biofilms and delayed wound healing in chronic wounds [90–93]
Corynebacterium jeikeium	Manganese acquisition and production of superoxide dismutase result in host epidermal protection from free radical oxygen species (ROS) [5]	Occasionally pathogenic Common cause of nosocomial skin infections [95]
Propionibacteria	Production of bacteriocins protect sebaceous ducts from other pathogenic inhabitants [77] induces expression of TLR2 and TLR4 in keratinocytes71	Occasionally pathogenic Overabundance associated with development of Acne [5]

Johnson, *Int J Mol Sci* 2018

Conséquence directe de la dysbiose sur la guérison



MacLeod, *Cell Host Microbe* 2019

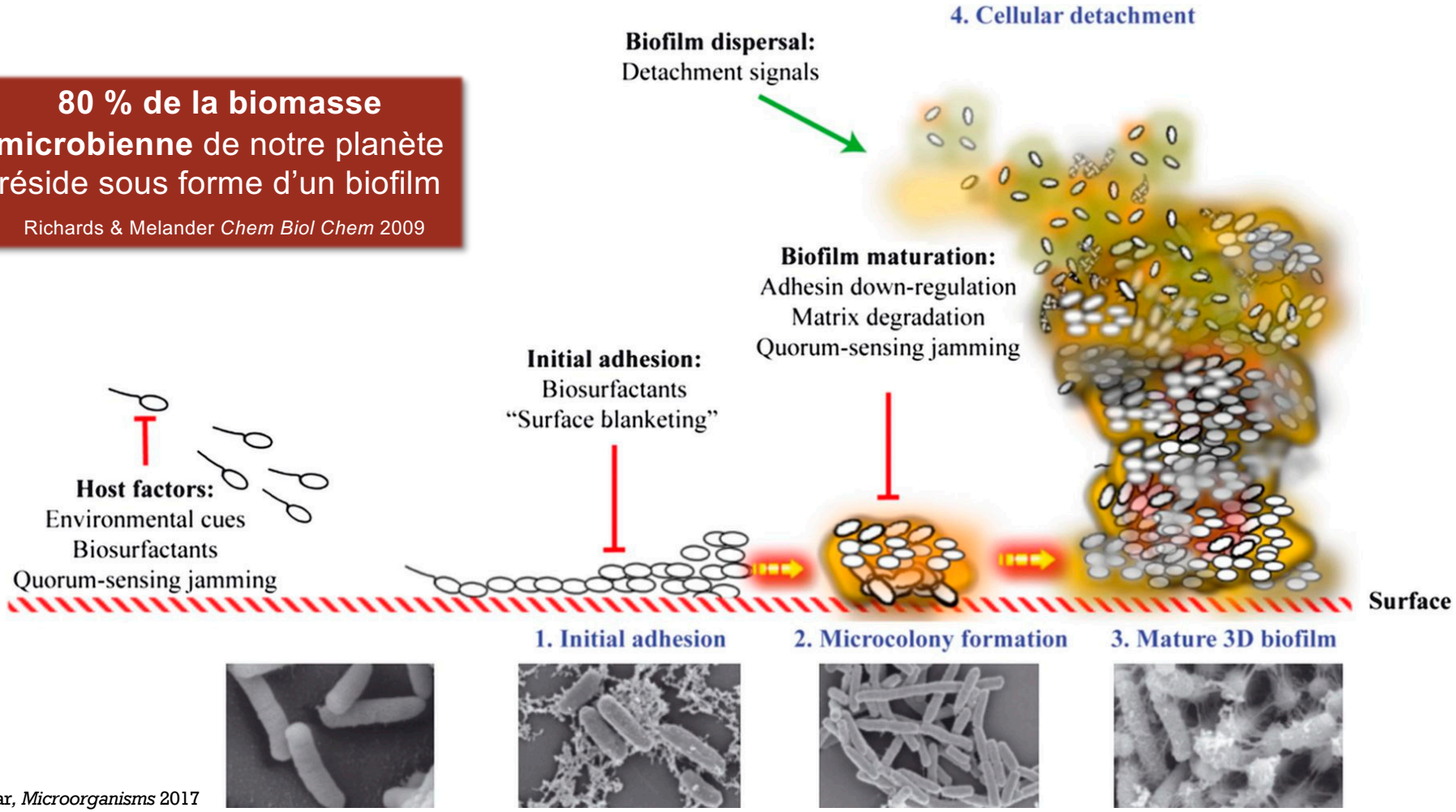
Le biofilm : c'est pas du cinéma !

- « *Agrégats de microorganismes dans lesquels les cellules sont enrobées dans une* ***matrice*** *autoproduite de* ***substances polymèriques extracellulaires*** *(EPS), adhérentes les unes aux autres et/ou à une surface* » Vert, *Pure Appl Chem* 2012
- À l'intérieur du biofilm, les bactéries :
  - peuvent **résister à la réponse immunitaire de l'hôte**
  - sont **moins sensibles aux antibiotiques et aux désinfectants** que les bactéries planctoniques
- La capacité à former un biofilm reconnue comme une **caractéristique propre** à de nombreux microorganismes.
- La **présence** de biofilms **lors d'infections** demande de nouvelles méthodes
  - de prévention
  - de diagnostic
  - de traitement

Le biofilm : c'est pas du cinéma !

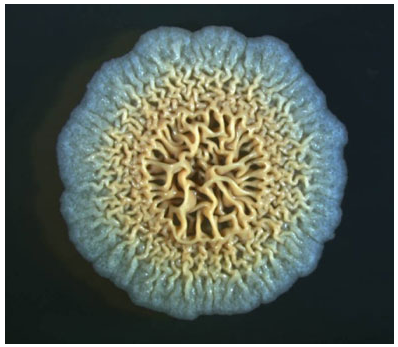
80 % de la biomasse microbienne de notre planète réside sous forme d'un biofilm

Richards & Melander *Chem Biol Chem* 2009

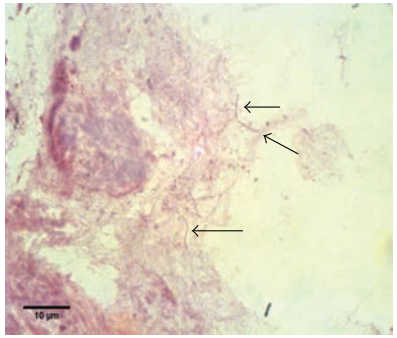


Omar, *Microorganisms* 2017

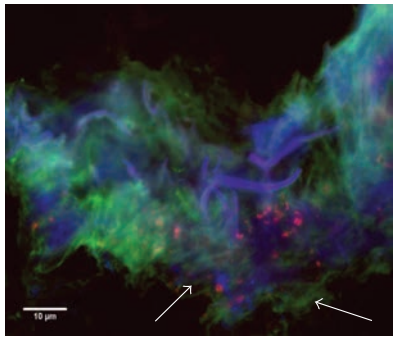
L'envers du décor …



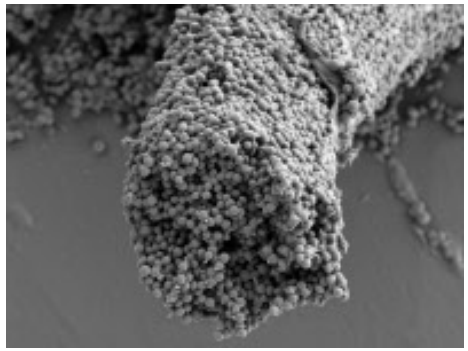
Aspect macroscopique



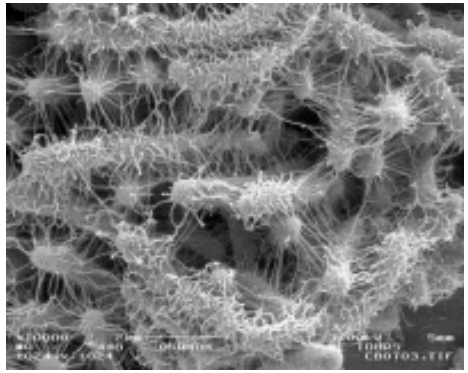
Coloration de Gram



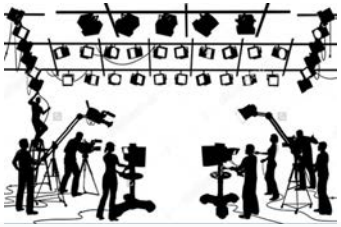
Microscopie confocale

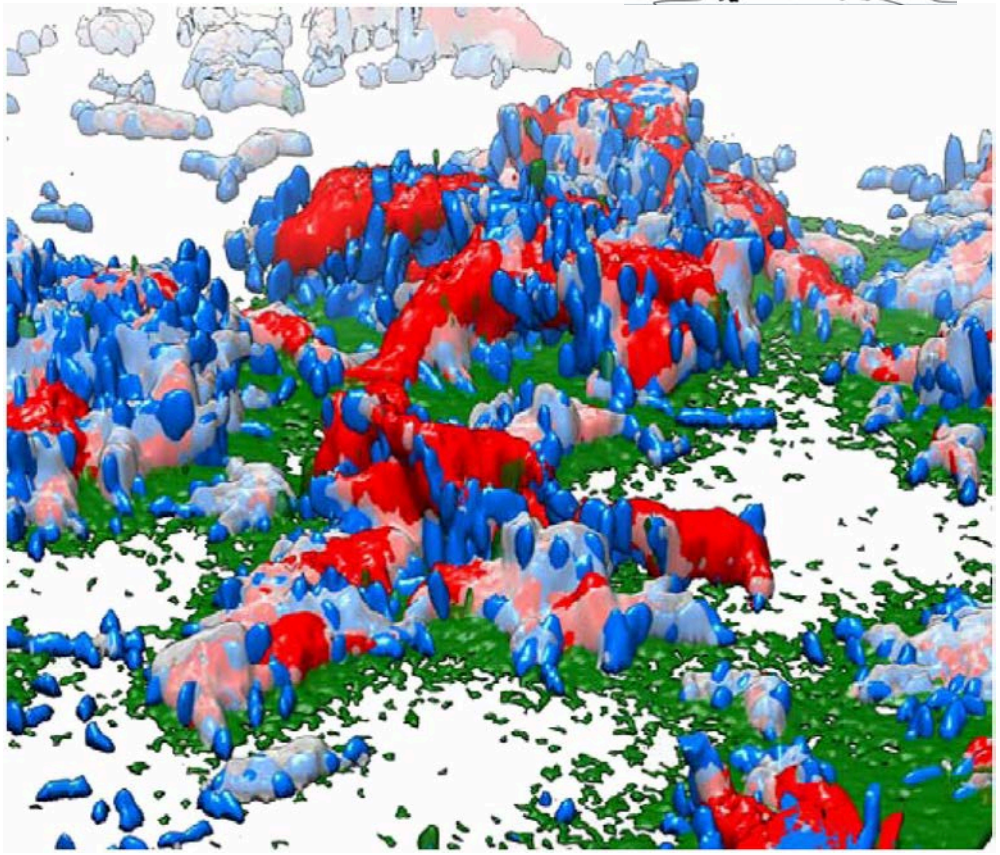


Staphylococcus aureus



Escherichia coli

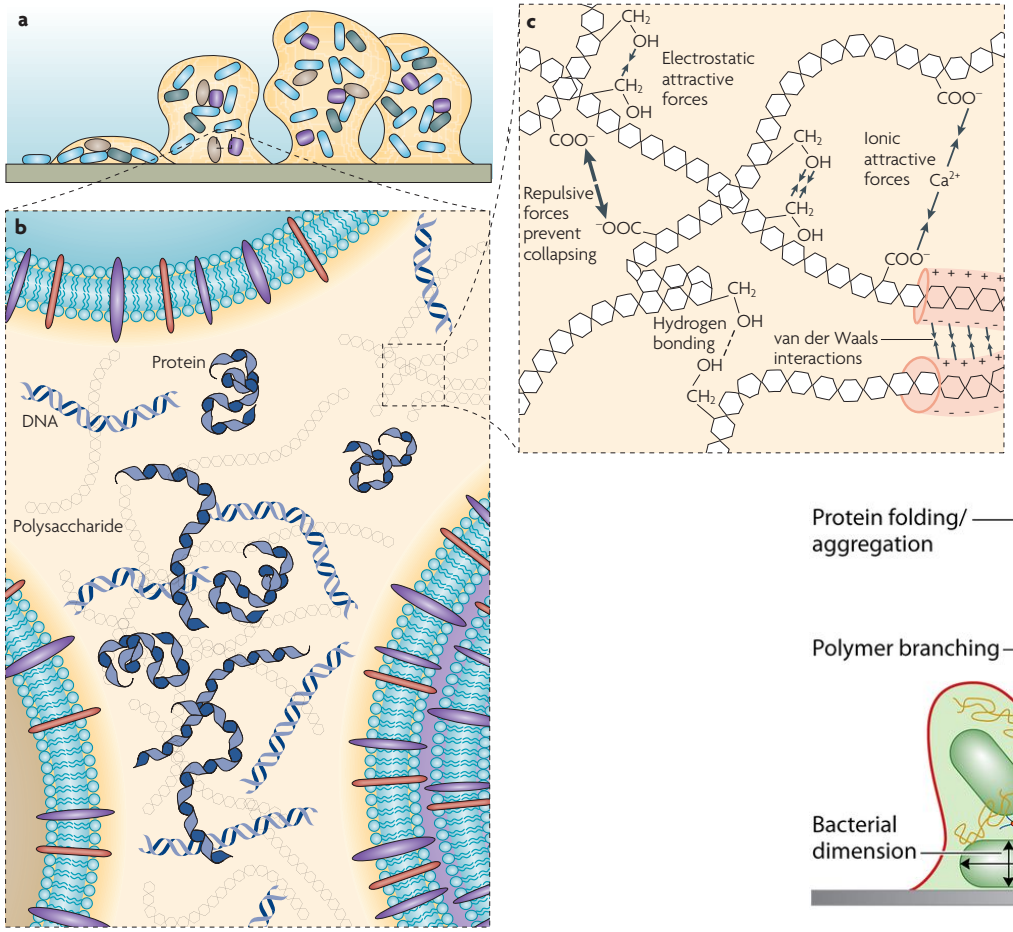




Vibrio cholerae

Berk, *Science* 2012
Schultz, *Proc Natl Acad Sci USA* 2012
Oates, *J Diabetes Res* 2014

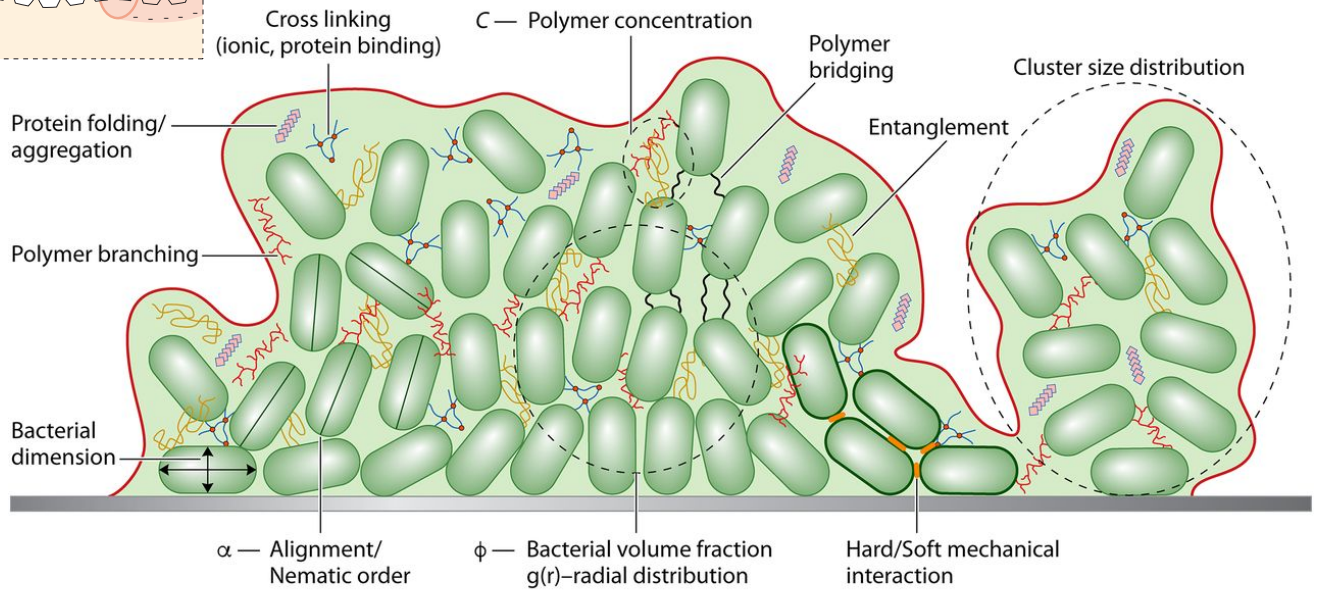
Composition du biofilm bactérien



Flemming & Wingender, *Nat Rev Microbiol* 2010

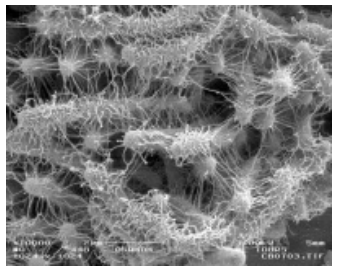
EPS de la matrice :
- exopolysaccharides
- ADN extracellulaire
- protéines

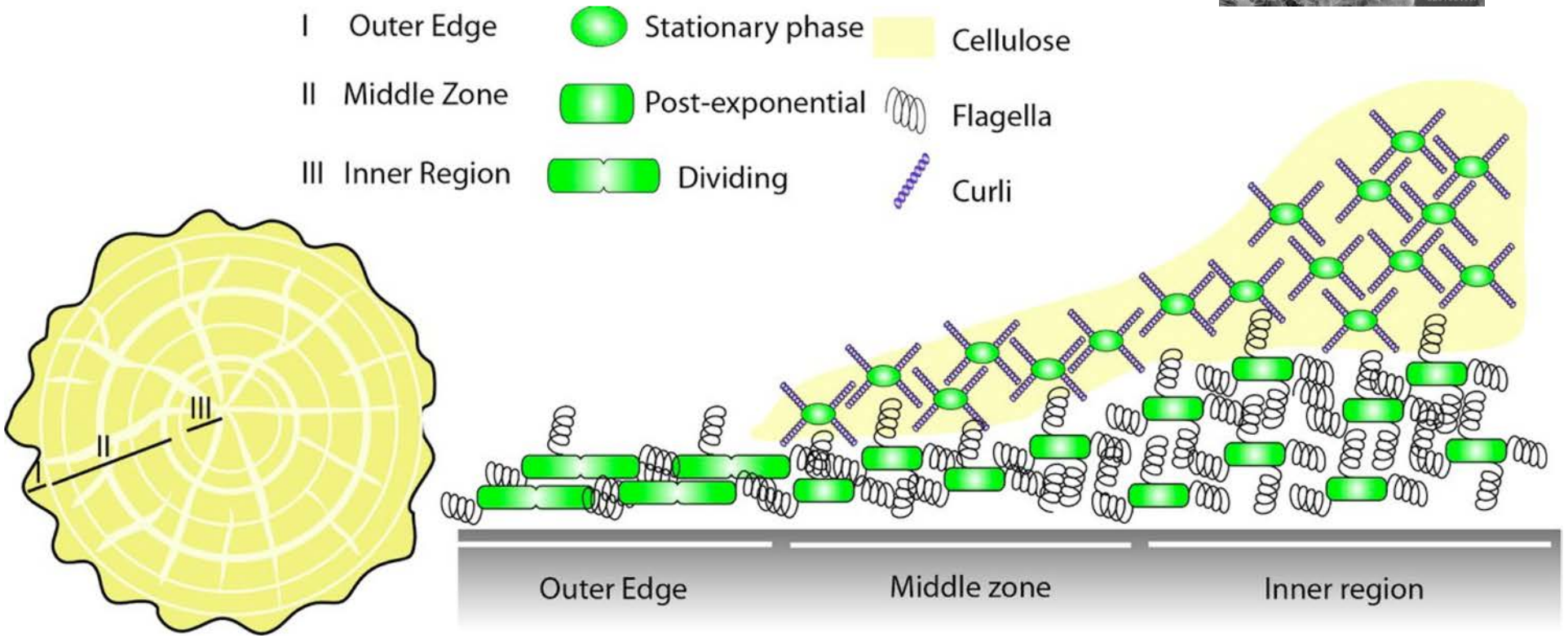
☞ **viscoélasticité**



Charlton, *J Bacteriol* 2019

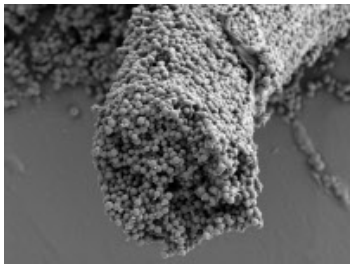
Structure du biofilm de *Escherichia coli*

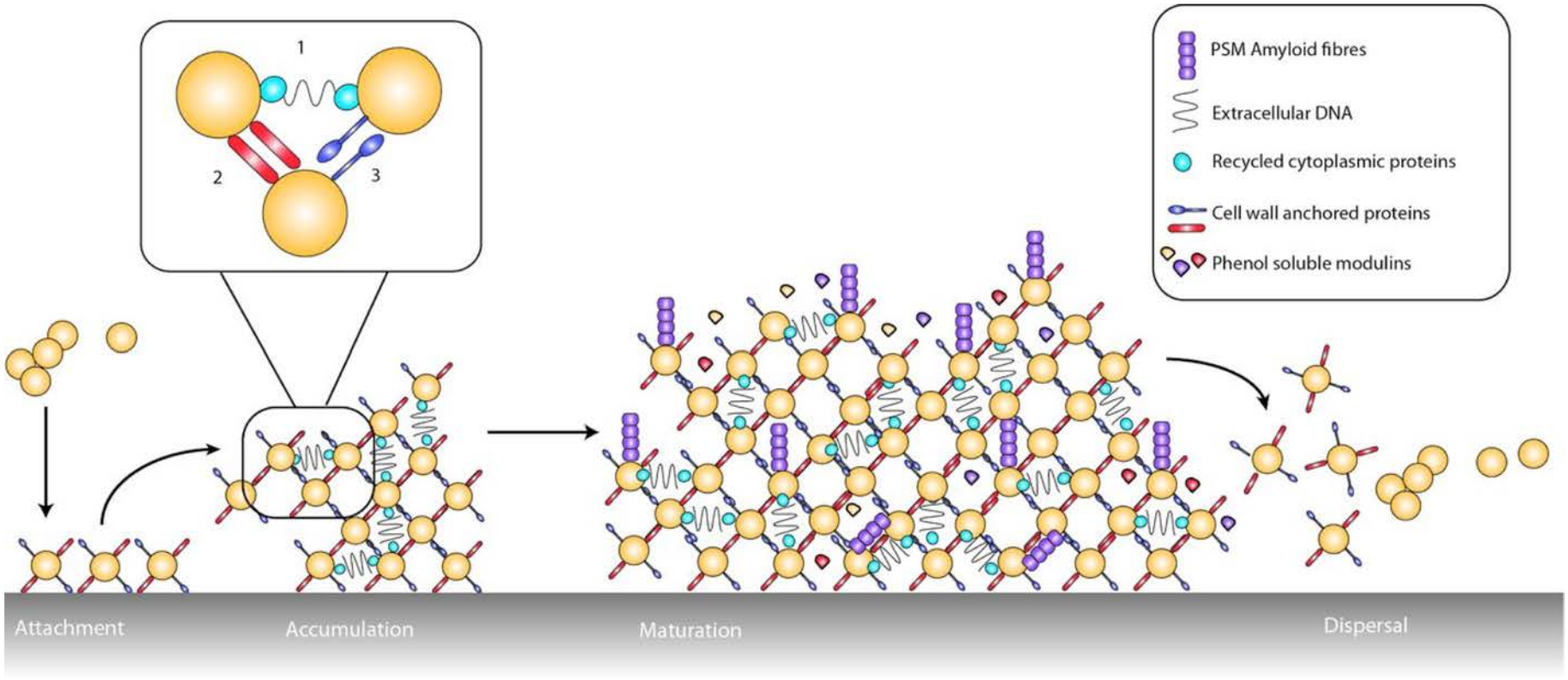




Hobley, *FEMS Microbiol Rev* 2015

Structure du biofilm de *Staphylococcus aureus*



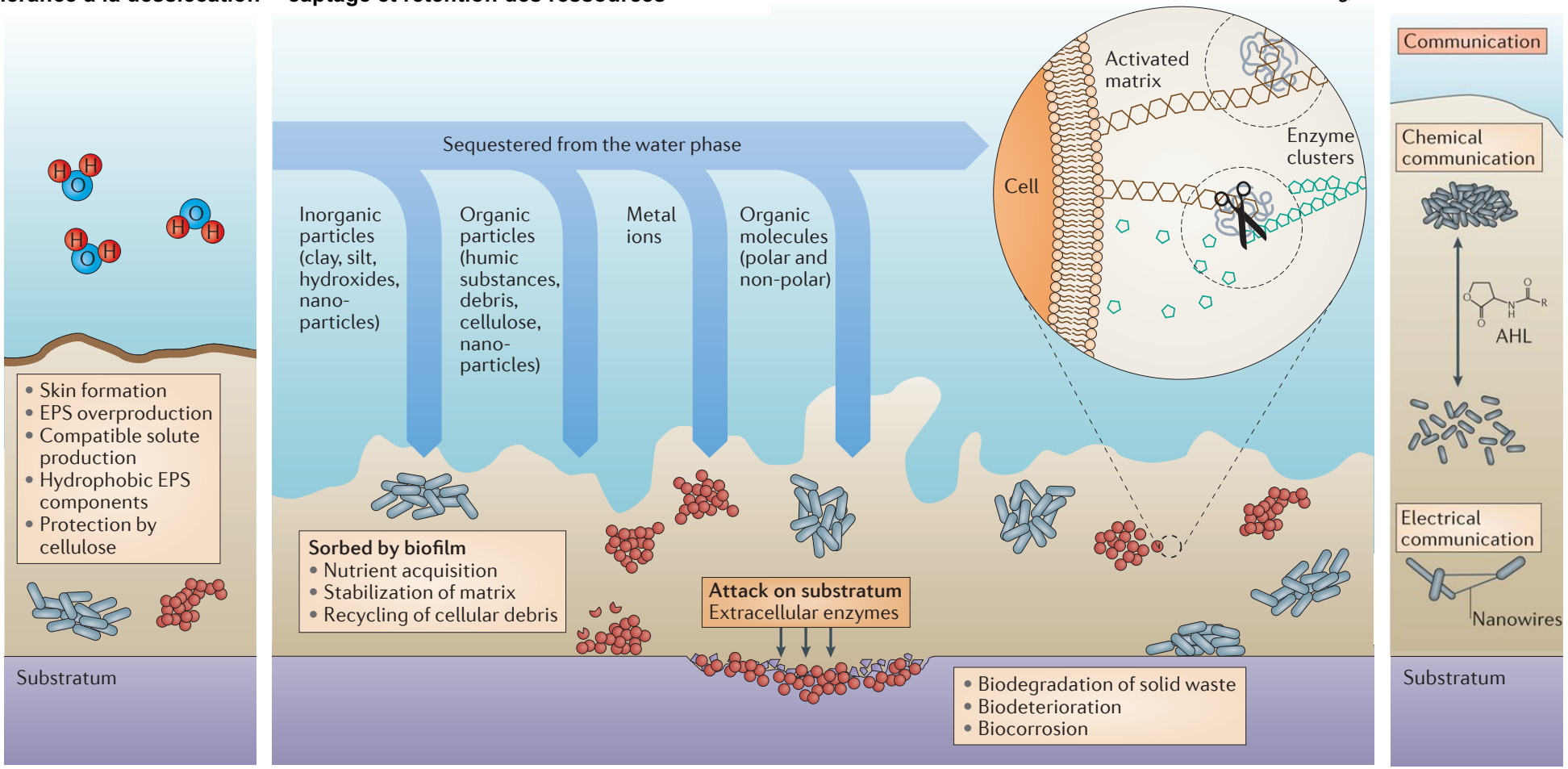


Hobley, *FEMS Microbiol Rev* 2015

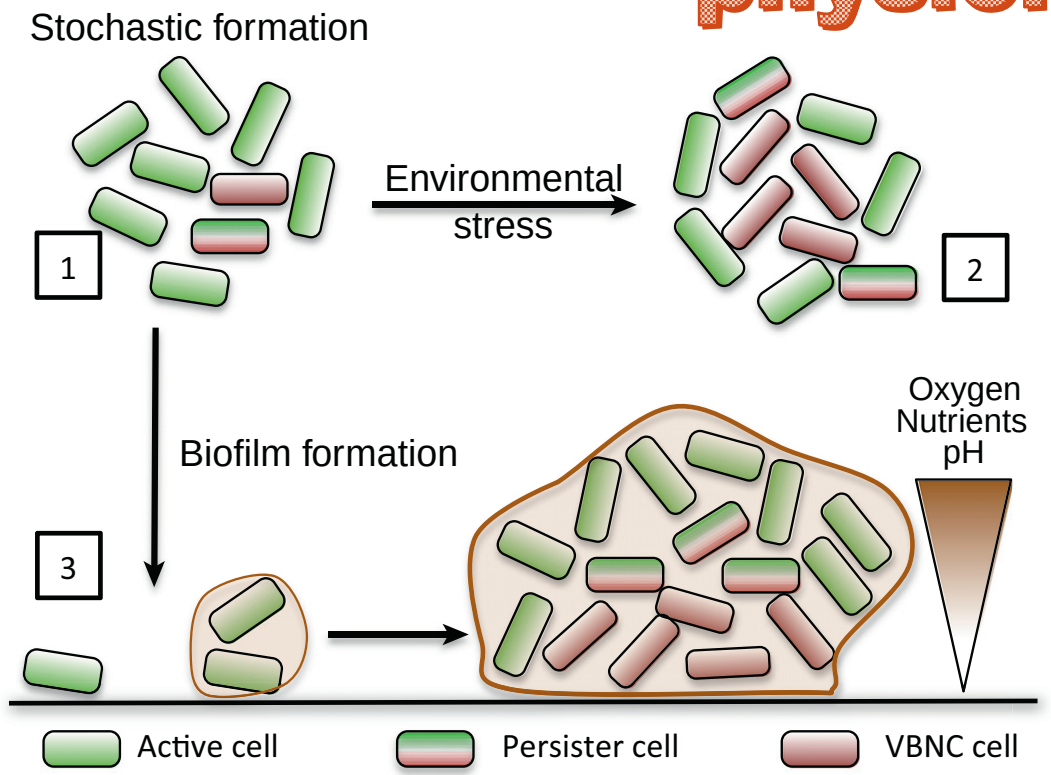
Propriétés physico-chimiques de la matrice

tolérance à la dessiccation **captage et rétention des ressources**

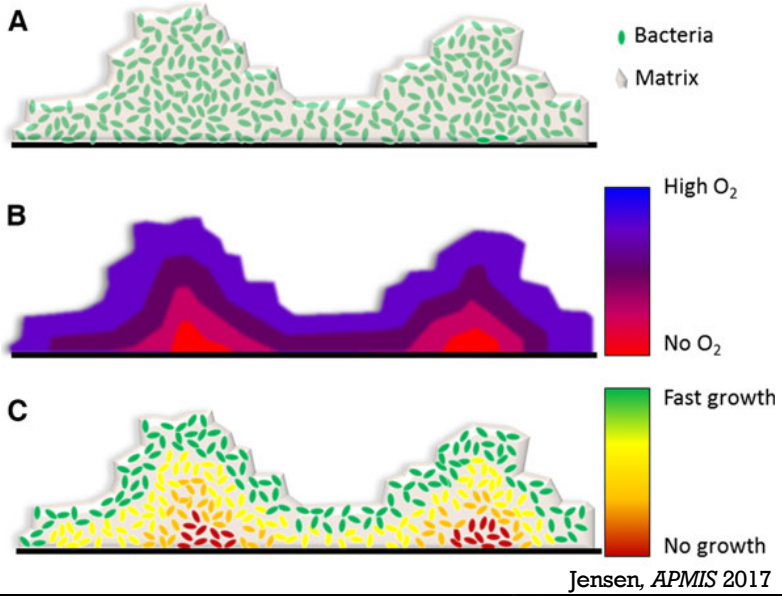
Flemming, *Nat Rev Microbiol* 2016



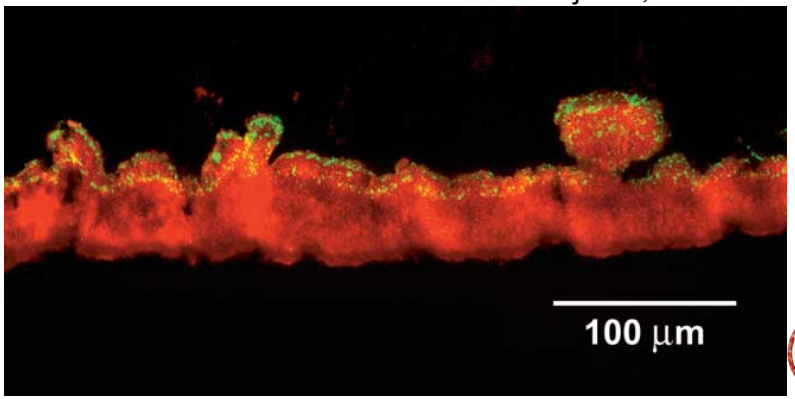
Différents niveaux d'activité physiologique



Ayrapetyan, *Trends Microbiol* 2014



Jensen, *APMIS* 2017

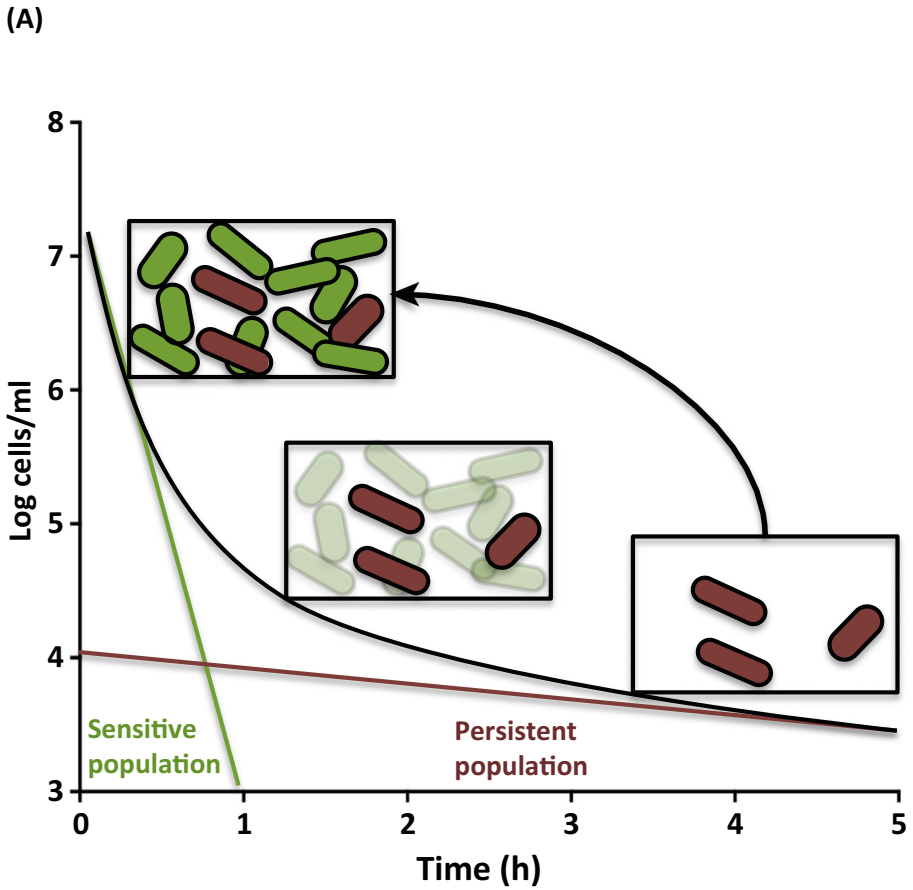


Fux, *Trends Microbiol* 2005

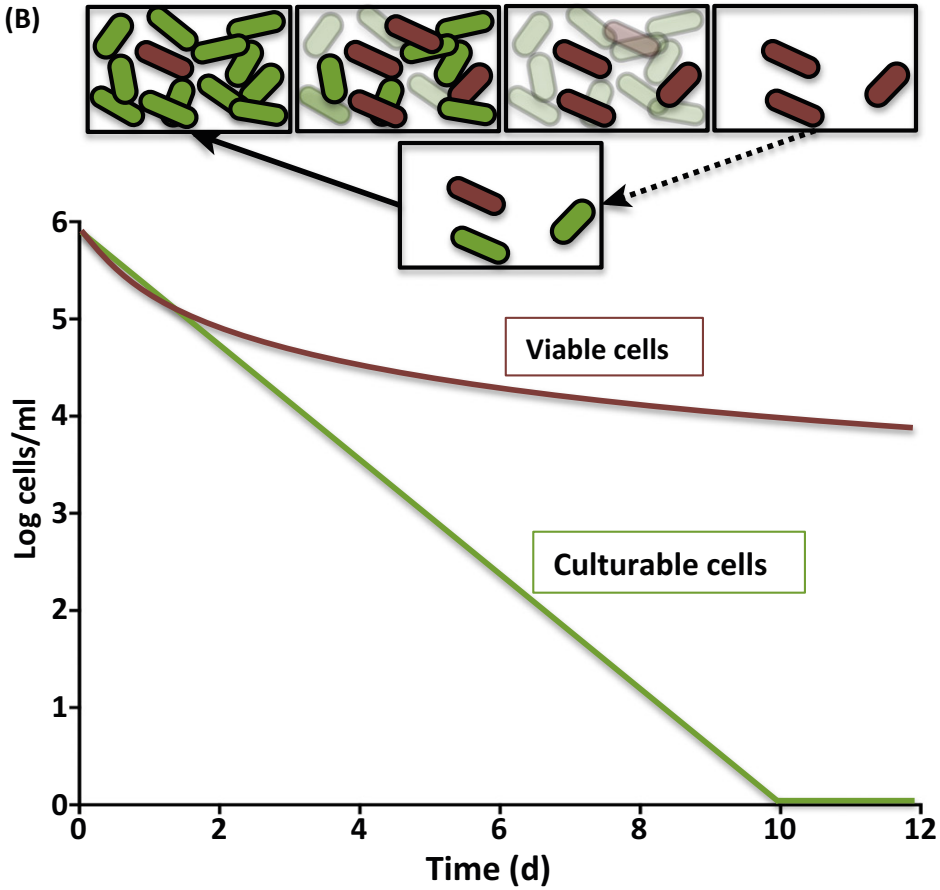
Persisters = cellules insensibles aux biocides donnant naissance à une population sensible après élimination de la substance toxique

VBNC = cellules qui ont perdu leur capacité à se développer sur un milieu de culture mais qui restent viables.

Conditions de sélection des persisters et VBNC



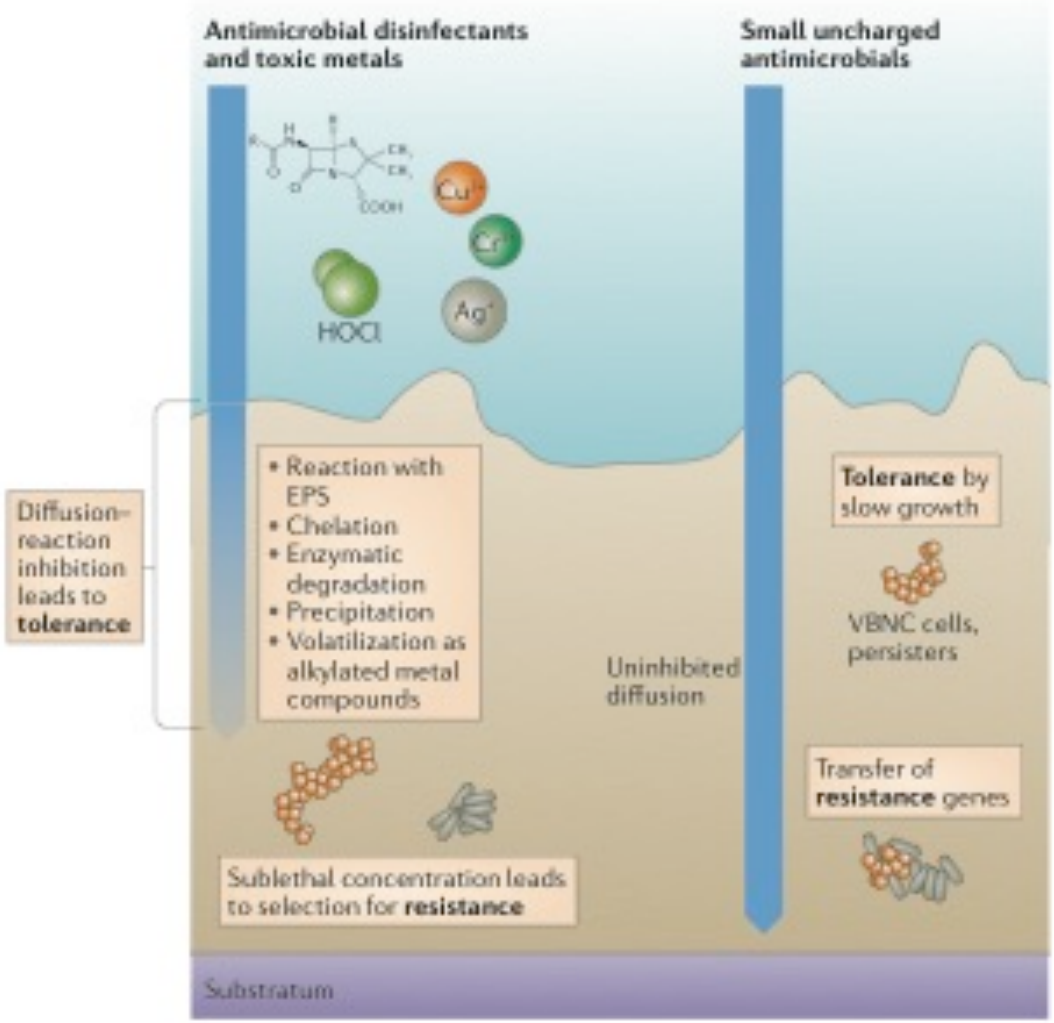
Persisters

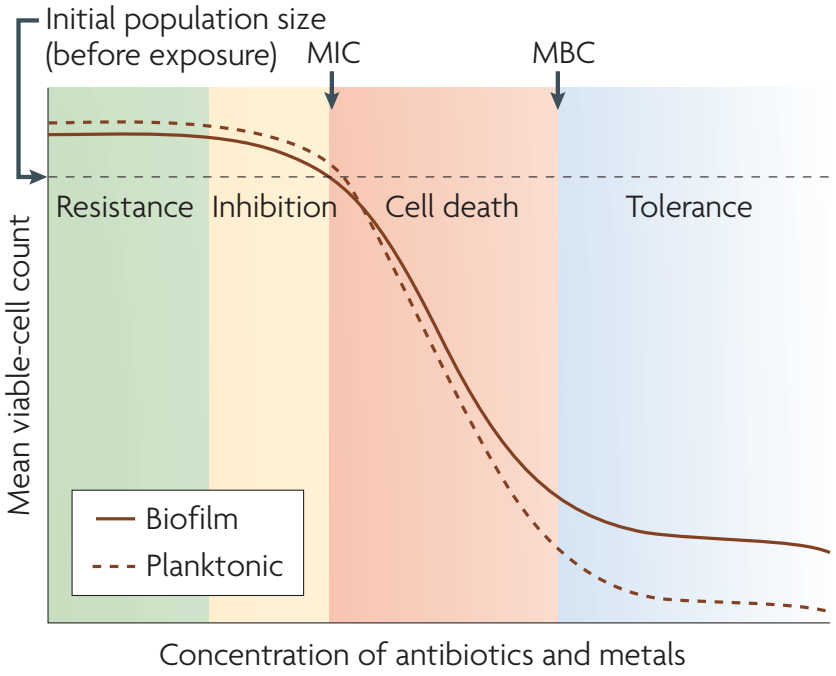


VBNC

Ayrapetyan, *Trends Microbiol* 2014

Conséquences sur l'activité des anti-infectieux

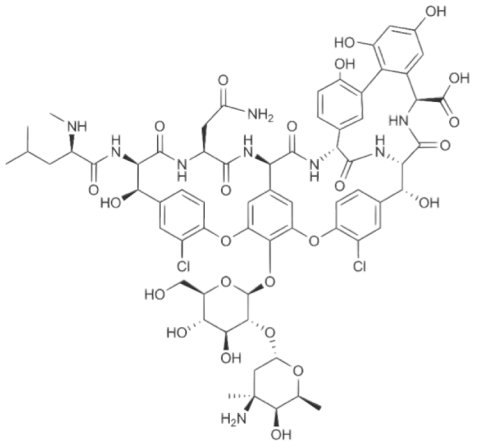




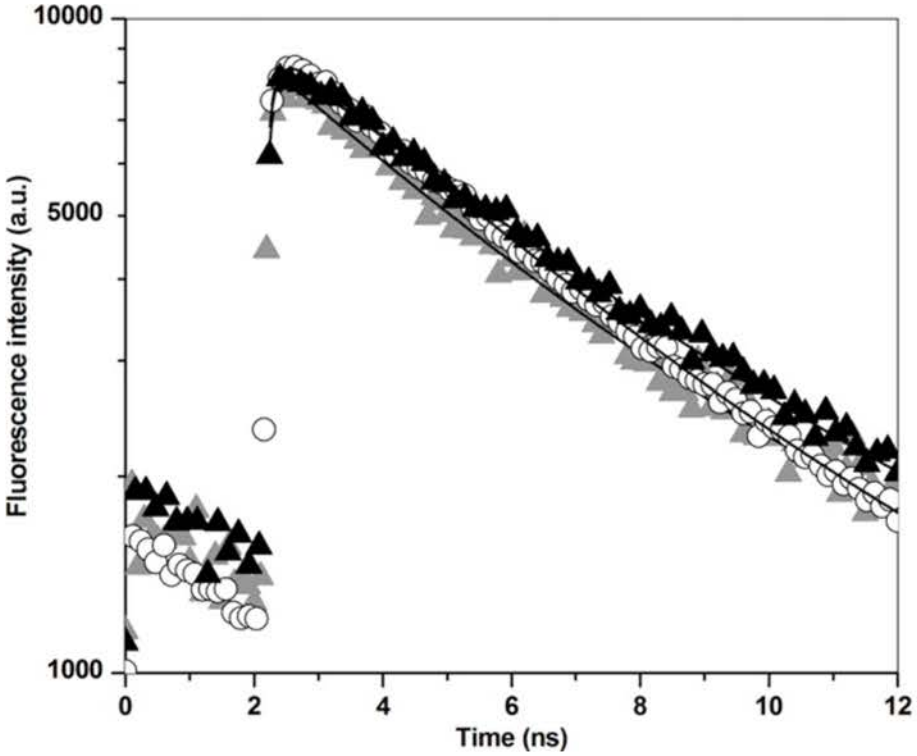
Notion de **tolérance**
phénotype **non héréditaire**

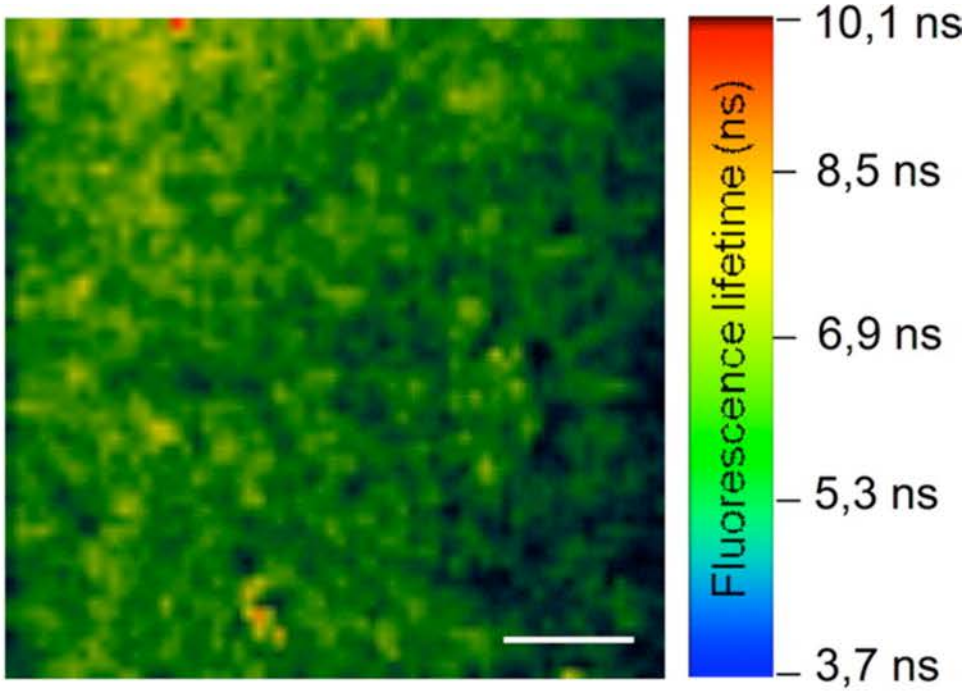
Flemming, *Nat Rev Microbiol* 2016
Harrison, *Nat Rev Microbiol* 2007

Comportement de la vancomycine dans un biofilm de *S. aureus*



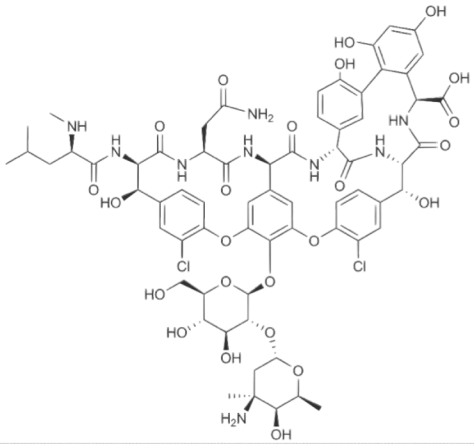
Diffusion non empêchée



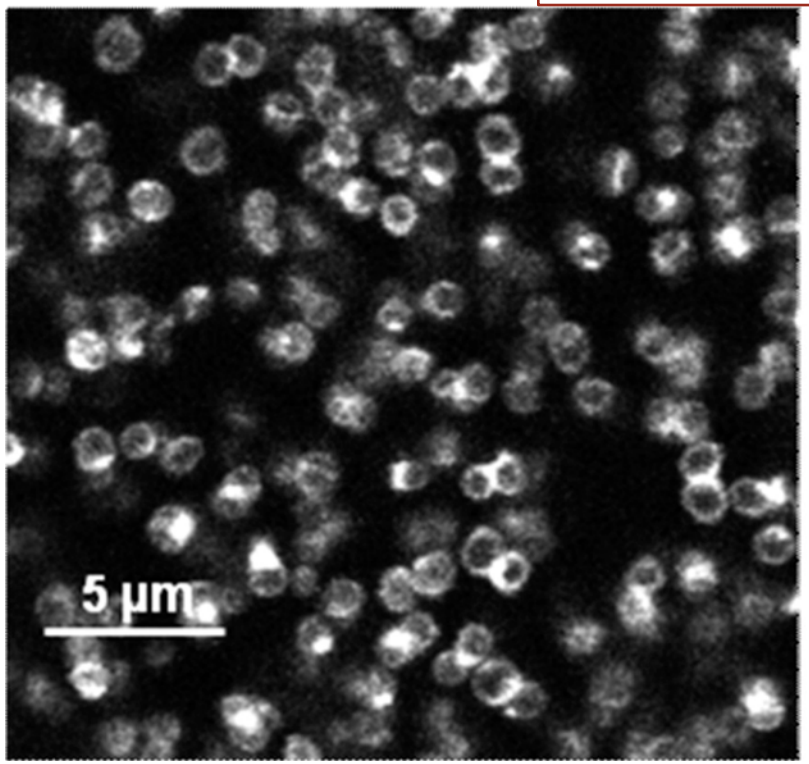


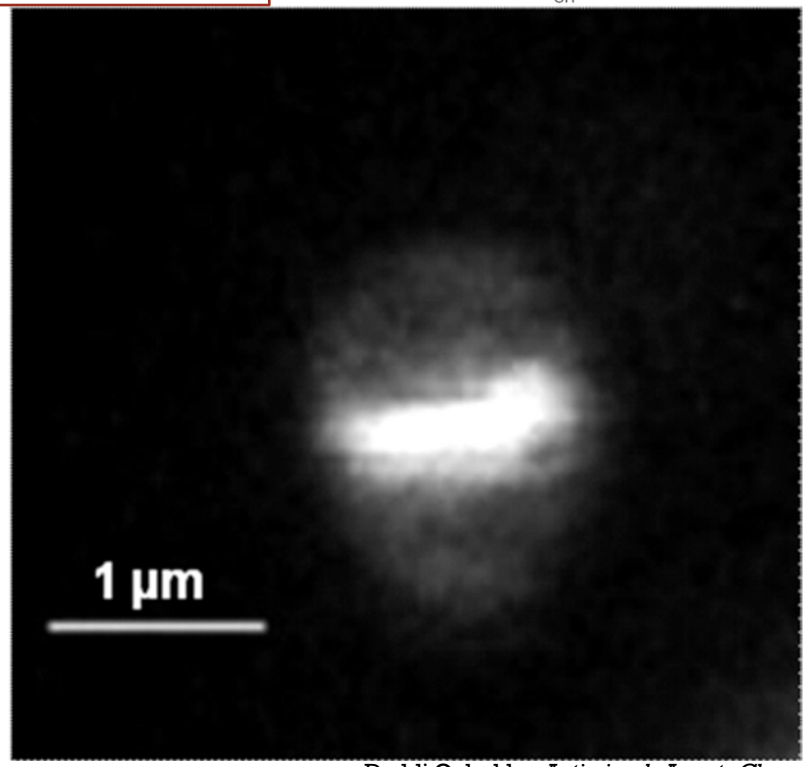
Daddi Oubekka, *Antimicrob Agents Chemother* 2012

Comportement de la vancomycine dans un biofilm de *S. aureus*



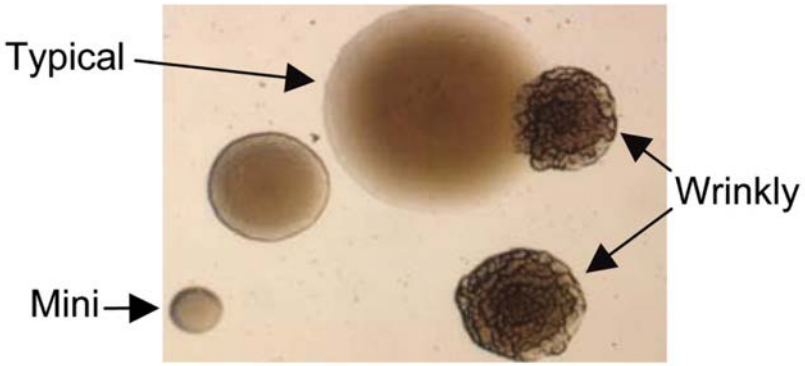
Atteinte de la cible

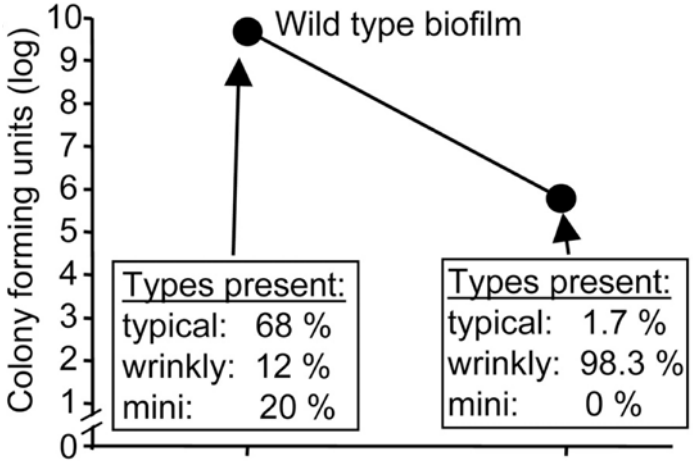


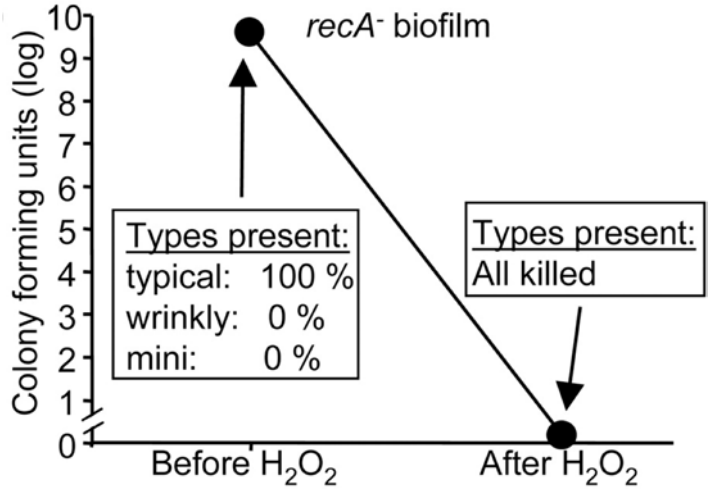


Daddi Oubekka, *Antimicrob Agents Chemother* 2012

Tolérance et hétérogénéité phénotypique



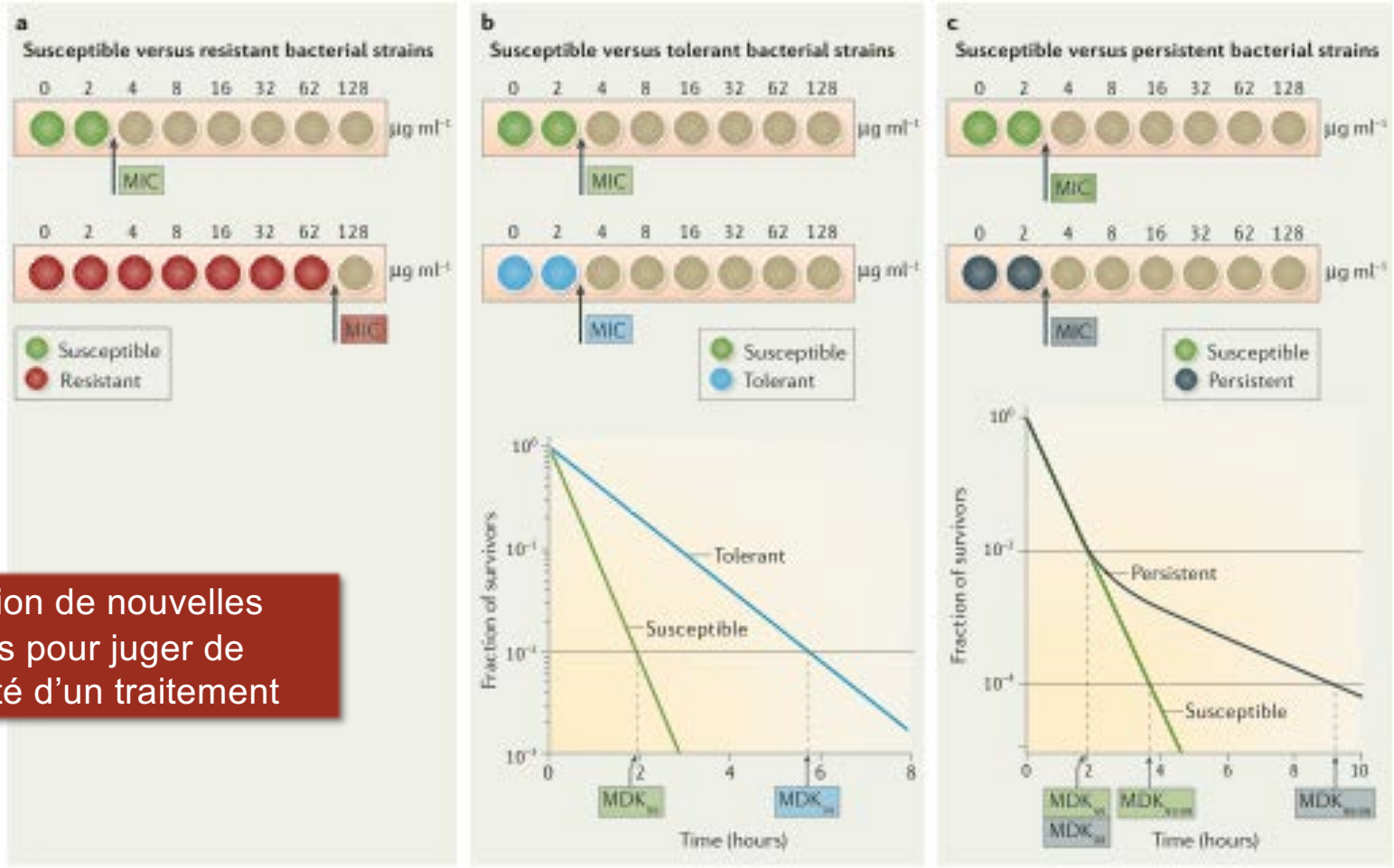




Biofilm = **continuum d'états phénotypiques** différents pour une même souche

Boles, *Proc Natl Acad Sci USA* 2004

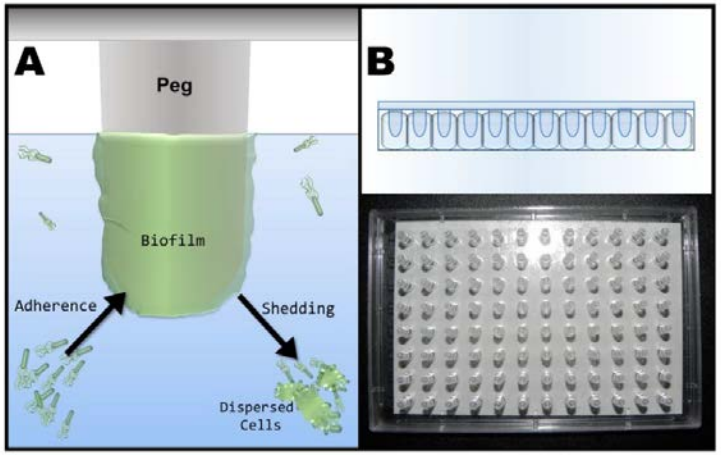
Résistance, tolérance, persistance : la CMI est-elle toujours adaptée ?



Apparition de nouvelles notions pour juger de l'efficacité d'un traitement

Brauner, *Nat Rev Microbiol* 2016

Intérêt de l'utilisation de nouveaux paramètres pharmacodynamiques



Dispositif de Calgary ou MBEC assay®

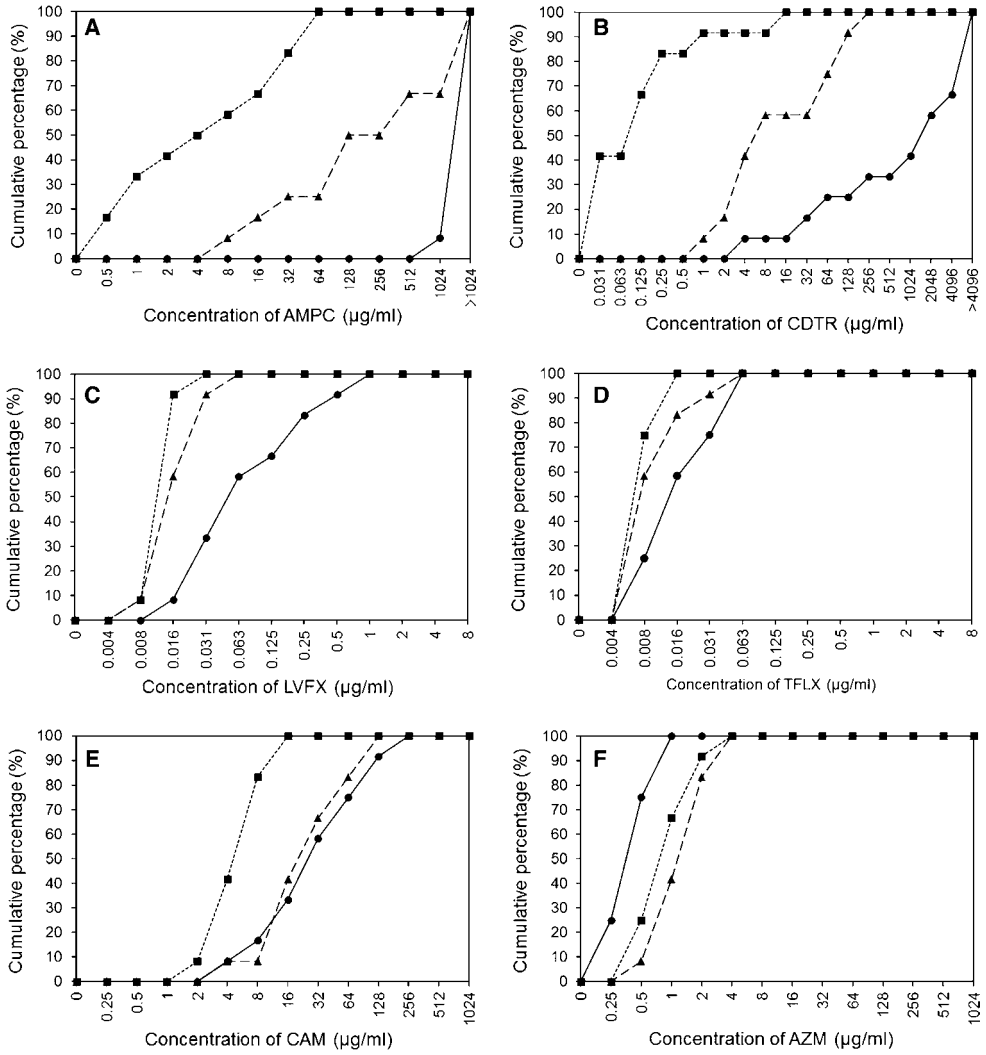
Biofilms de *P. aeruginosa*

Antibiotic	MIC (mg/L)	MBIC (mg/L)	MBC (mg/L)	MBEC (mg/L)	BBC (mg/L)	BPC (mg/L)	Biofilm model
AZT	4[a]	>128[a]	8	>1024[b]	–	–	Calgary device
CAZ	2[a]/1[c]/2[d]	128[a]/128[d]	2[c]/4[d]	>1024[b]	16[c]/1024[d]	16[d]	Calgary device
MER	≤1[a]/0.5[c]	4[a]	1[c]	–	8[c]	–	Calgary device
IMP	2[d]/1[e]	64[d]/32[e]	4[d]/4[e]	1024[e]/>1024[b]	256[d]	32[d]	Calgary device
CIP	0.5[a]/0.125[c]/1[d]	1[a]/1[d]	0.25[c]/1[d]	4[b]	2[c]/64[d]	1[d]	Calgary device
TOB	2[a]/2[d]	4[a]/8[d]	2[d]	2[b]	64[d]	4[d]	Calgary device
COL	2[d]/2[e]	16[d]/16[e]	1[d]/8[e]	128[e]	64[d]	2[d]	Calgary device
AZM	128[f]/128[d]	2[a]/16[d]	>128[d]	–	512[d]	8[d]	Calgary device
CXA-101	0.5[c]	–	0.5[c]	–	0.5[c]	–	Calgary device

AZM, azithromycin; AZT, aztreonam; BBC, biofilm bactericidal concentration; BPC, biofilm-prevention concentration; CAZ, ceftazidime; CIP, ciprofloxacin; COL, colistin; IMP, imipenem; MBC, minimal bactericidal concentration; MBEC, minimal biofilm-eradication concentration; MBIC, minimal biofilm inhibitory concentration; MER, meropenem; TOB, tobramycin.

Macià, *Clin Microbiol Infect* 2014

Étude comparative MIC, MBC et MBEC



Souches de ***Haemophilus influenzae***
isolées de paracentèses
chez des enfants avec **OMA** DTT
(n=12)

FQ et macrolides > ß-lactamines

Takei, *J Infect Chemother* 2013

MERCI !