SAPIENZA
UNIVERSITÀ DI ROMA

Dipartimento di
BIOLOGIA E BIOTECNOLOGIE
CHARLES DARWIN

Biofilm and quorum sensing

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24 CFU/cm^2 on a toilet seat and flush

[Cooper et al, 2016]

Supermarket Carts Carry More Than Groceries

Germs like *E. coli* , *Yersinia enterocolitica, P. aeruginosa* and other bacterial spp have been found on shopping carts (150 CFU/cm^2)

(6 times more germs than found on a standard toilet seat)

[Gebra and Maxwell, 2012; Irshaid et al, 2014]

Smartphones: Think of All the Places They've Been

The smartphone was found to reach 254.9 CFU/cm^2
(10 times more germs than a standard toilet seat)

200 HCWs were screened. 94.5 % of the phones were contaminated by bacteria, many of which were resistant to multiple antibiotics.
S. aureus were 52% and 37.7% were MRSA.

Profession	N (Mean ± SD)
Assistant doctor	79 (19.0 ± 35.8)
Healthcare personnel*	68 (18.4 ± 41.3)
Senior doctor	15 (12.8 ± 15.1)
Nurse	38 (10.7 ± 28.7)

[Ulger et al, 2009]

Toothbrush Terror! Can Your Toothbrush Make You Sick?

10^3 CFU/cm^2 **Coliform were more than 5%**

(41 times more germs than found on a standard toilet seat)

[Donofrio et al., 2012]

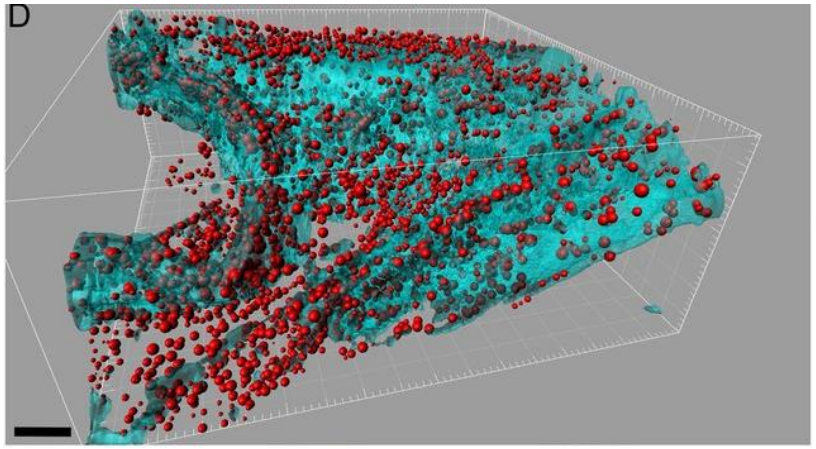
Your Office, Along With Other Communal Surfaces, Are Likely Teeming With Germs

The average desktop harbours 20,961 germs per square inch and that's in addition to 3,295 on the keyboard and 1,676 on a mouse and a staggering 25,127 on the phone.

The typical office desk harbours more than 10 million bacteria (4000 times more germs than found on a standard toilet seat)

[Mendell et al, 2002]

Are We Aware of Microbial Hotspots in Our Household?



(approximately 42 000 times more germs than found on a standard toilet seat)

10^{7} CFU/cm^{2} **Coliform were more than 20%**

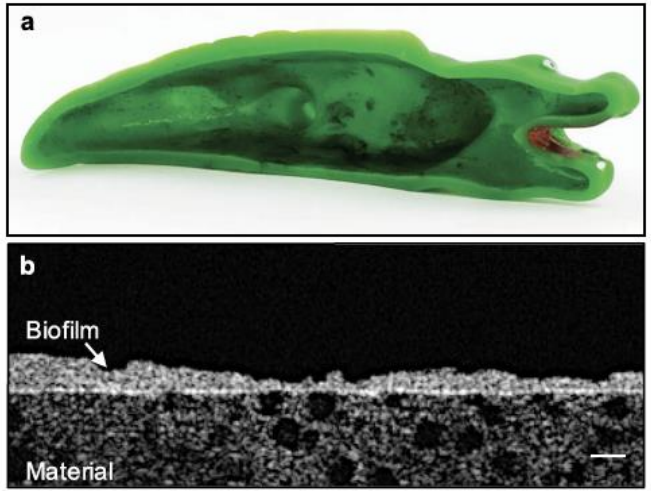
[Donofrio et al., 2012]

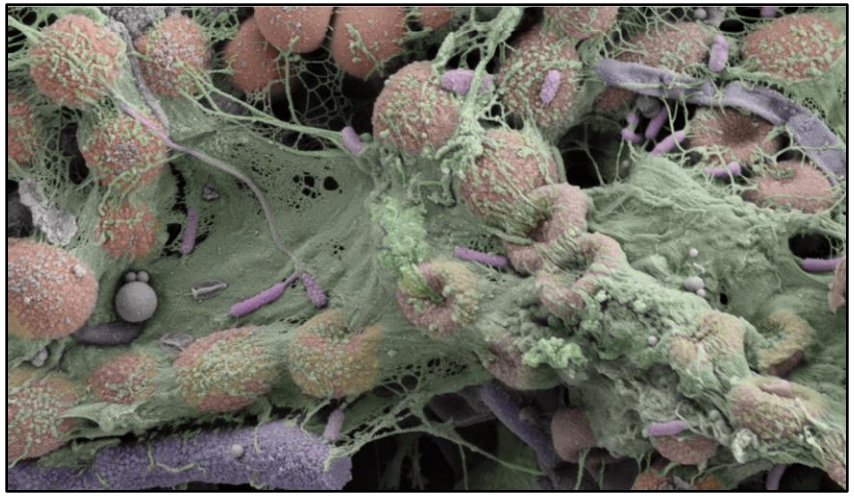
Ugly ducklings — the dark side of plastic materials

The average total bacterial number was 9.5 x 10^6 CFU/cm2.
(400 000 times more germs than found on a standard toilet seat)

Bacterial community compositions showed the presence of many rare taxa in real bath toys.

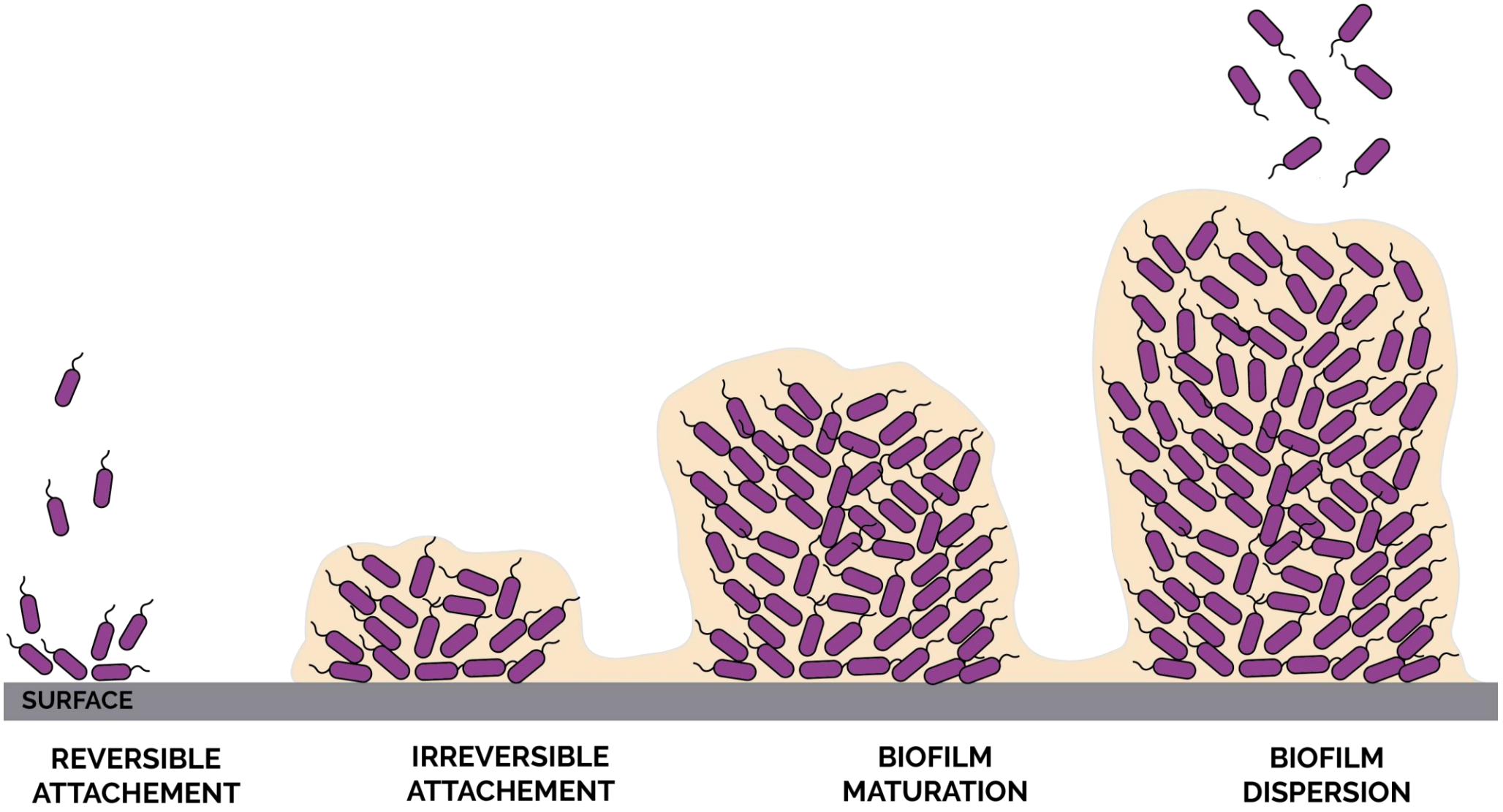
Fungi were identified in 58% of all real bath toys.



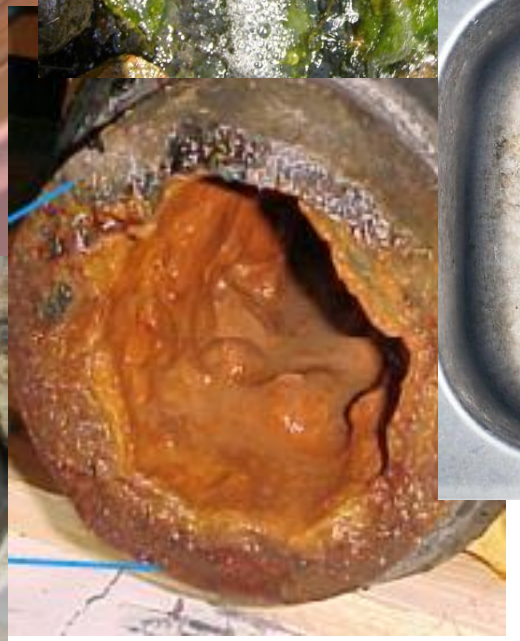


[Neu et al, 2018]

The Biofilm Lifestyle



Biofilms grow virtually everywhere

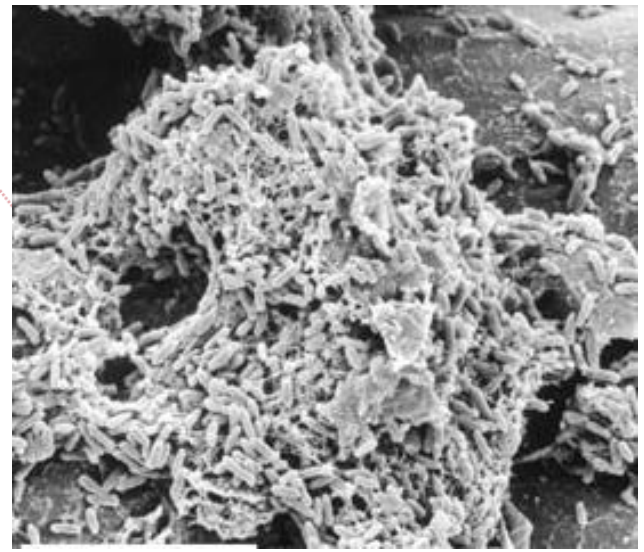


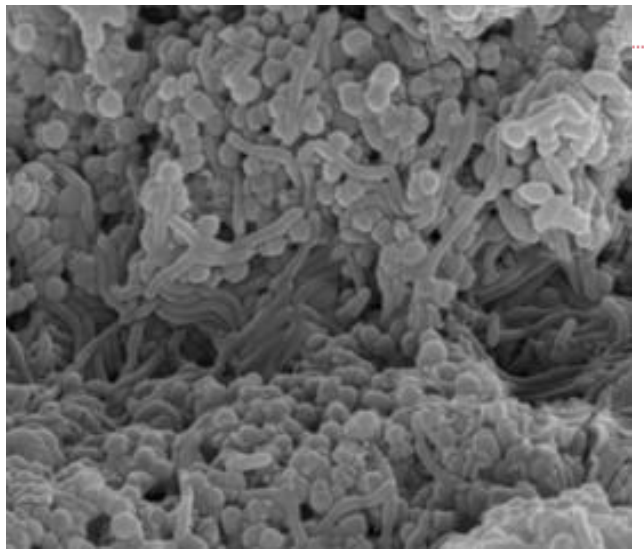
Biofilm is the dominant mode of growth of the microbiota

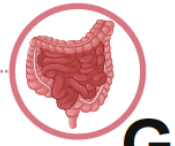
Biofilm is the dominant mode of growth of the microbiota

Respiratory

Actinobacteria
Firmicutes
Proteobacteria
Bacteroidetes





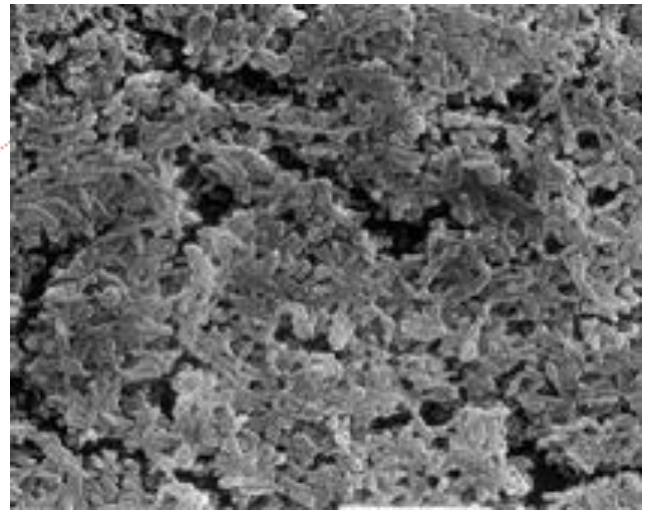


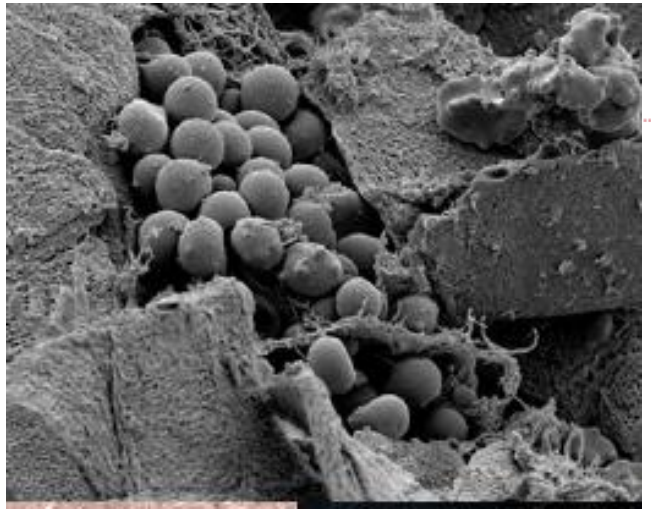
Gut

Actinobacteria
Bacteroidetes
Firmicutes
Lactobacillae
Streptococci
Enterobacteria

Vagina

Lactobacilli

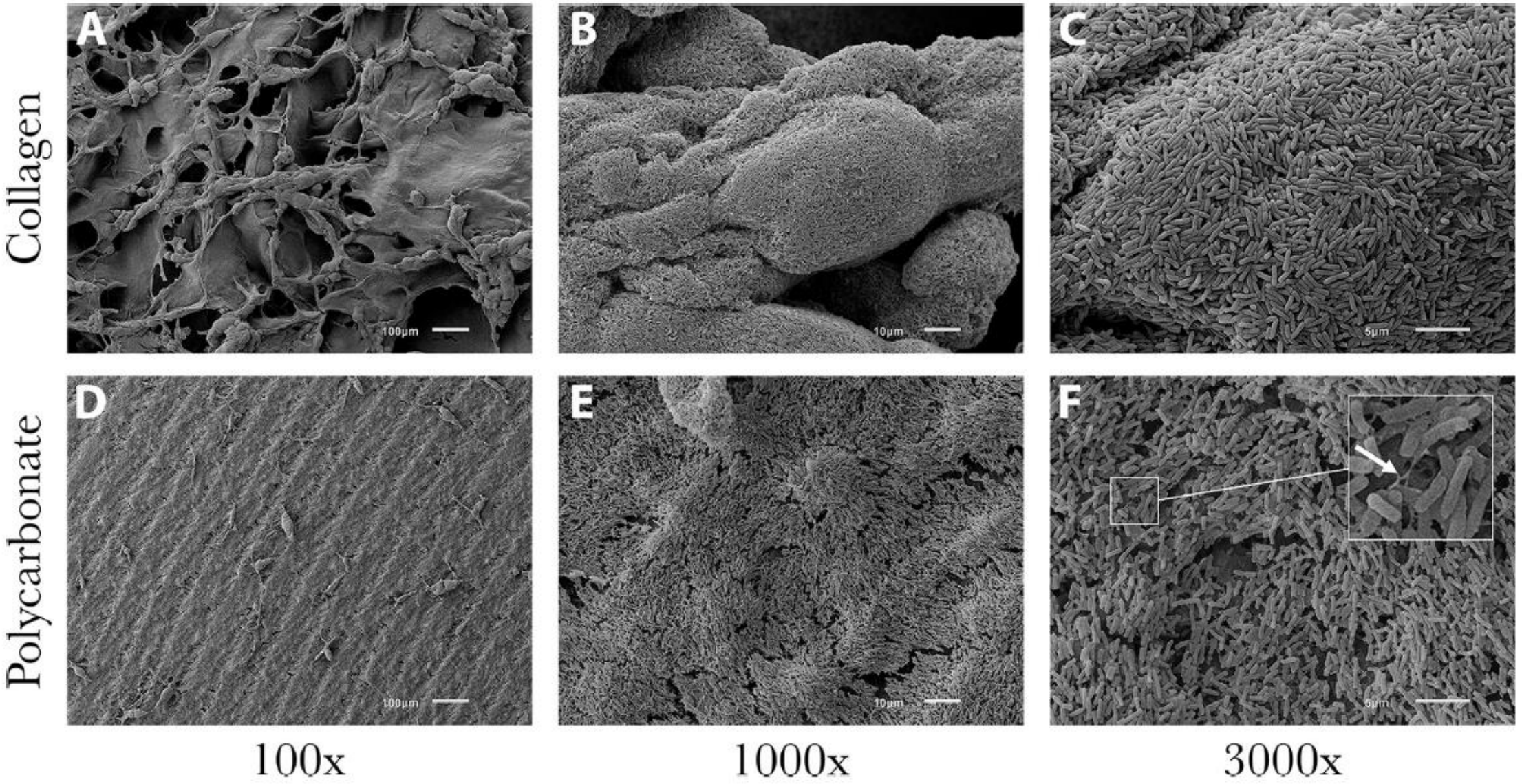




Skin

Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Proteobacteria

Biofilm growth on different substrates



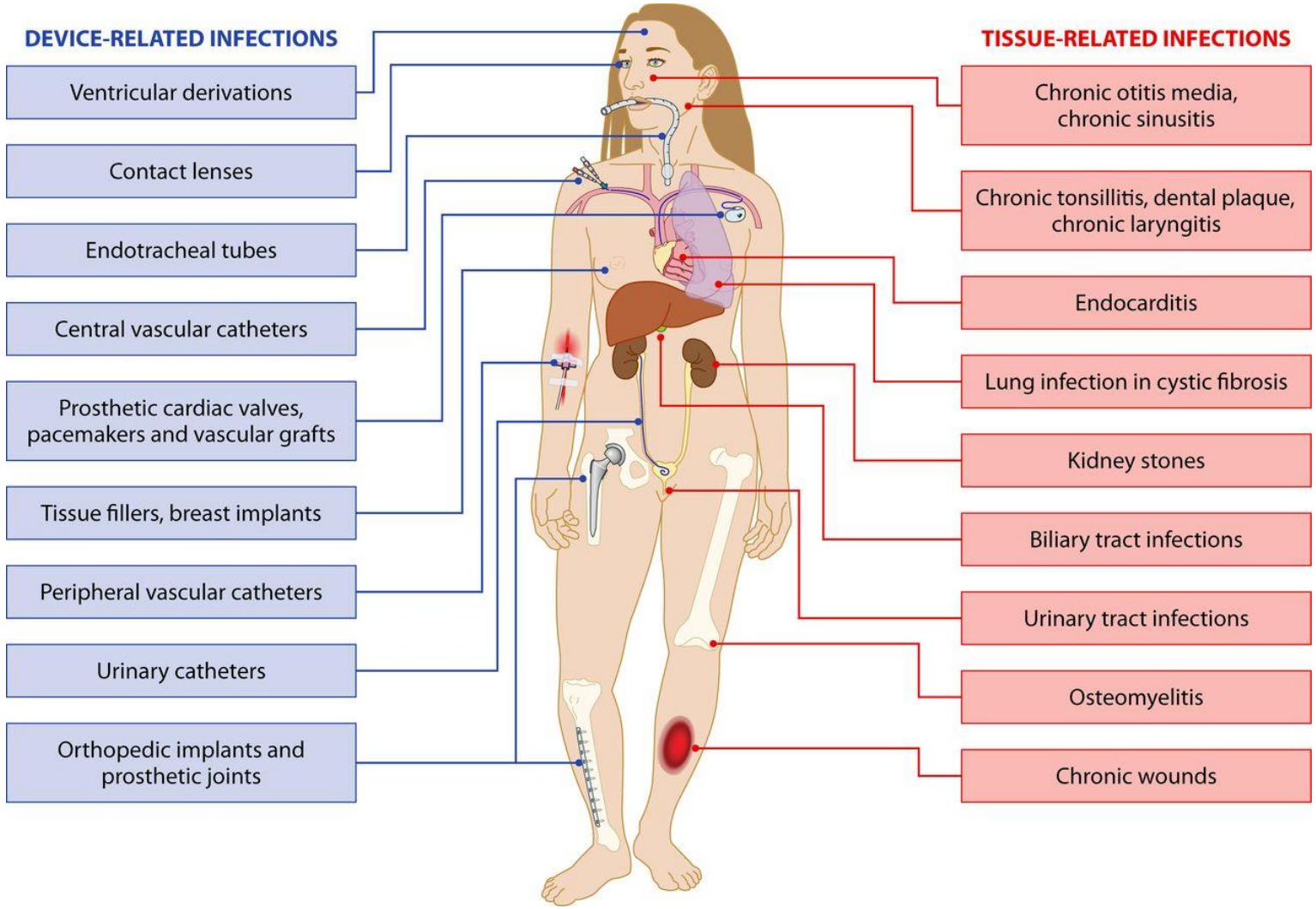
Biofilm-related infections represents more than 80% of all human infections

Biofilms pose a serious problem for public health because of the increased resistance to antibiotics

Microbial cells within biofilms have shown 10–1000 times more antibiotics resistance than the planktonic cells

An additional resistance mechanism that escapes conventional clinical analysis

Biofilm-related Infections



Economic significance of biofilms

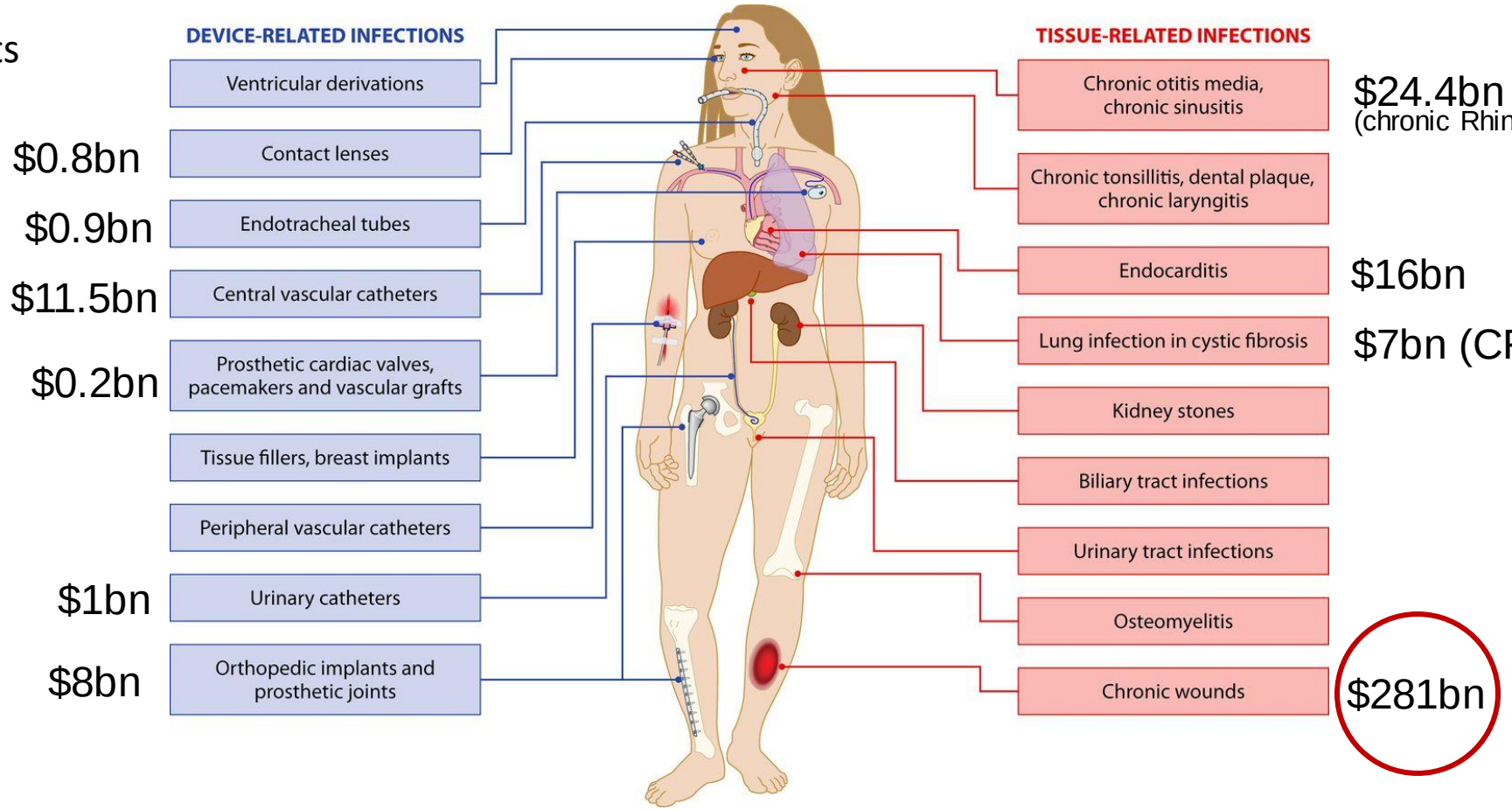
In 2019, pre-COVID-19 pandemic, it was estimated that biofilms have an economic significance of $5000bn a year.

$41.5bn
Cleaning products

$91bn
Personal care

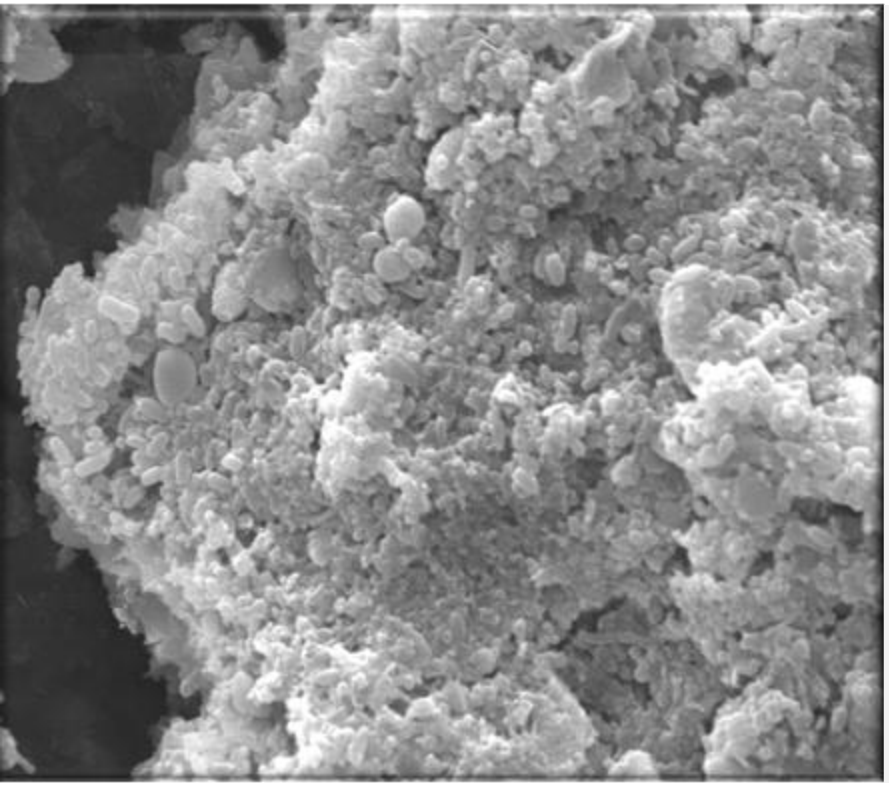
$212bn
Homecare

$47bn
Oral care



What is a biofilm?

Biofilm is an association of microorganisms in which microbial cells adhere to each other on a surface within a self-produced matrix



The biofilm matrix of is composed by the extracellular polymeric substance (EPS)

85% Matrix (extracellular polysaccharides, proteins, lipids and DNA)**15%** Bacterial cells

Polysaccharidic components: PIA/PNAG, colanic acid, alginate, glucose and mannose, cellulose and b-1,6-GlcNac polymer

Discovery

1676 - Van Leeuwenhoek was the first to observe microorganisms on tooth surfaces by making use of his simple microscopes and thus was the one who made the discovery of microbial biofilms.

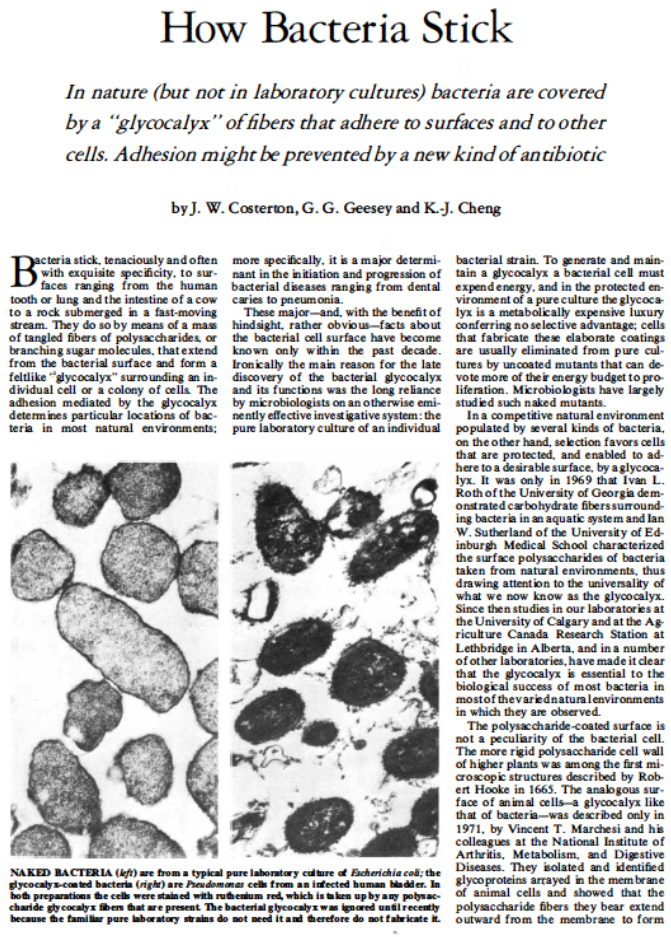
In 1868 German biologist **Ernst Haeckel** hypothesized that life originated from **primordial slime** at the bottom of the ocean

1935 - **Claude E. Zobell**: the first to description of biofilm in marine bacteria.
He observed bacterial cells in intimate contact with the solid surface. It is believed that after coming into contact with a solid surface, physiologically active sessile bacteria secrete a cementing substance.

Detailed assessment of biofilms had to await the development of the electron microscope, which permitted high-resolution photo-microscopy at magnifications that were much higher than that of light microscope.

1978 - **Bill Costerton:** was a pioneer in biofilm research and his work significantly advanced the understanding of these complex microbial communities. He defined the term **biofilm**

Revealing a world of biofilms — the pioneering research of Bill Costerton



How Bacteria Stick

In nature (but not in laboratory cultures) bacteria are covered by a "glycocalyx" of fibers that adhere to surfaces and to other cells. Adhesion might be prevented by a new kind of antibiotic

by J. W. Costerton, G. G. Geesey and K.-J. Cheng

Bacteria stick, tenaciously and often with exquisite specificity, to surfaces ranging from the human tooth or lung and the intestine of a cow to a rock submerged in a fast-moving stream. They do so by means of a mass of tangled fibers of polysaccharides, or branching sugar molecules, that extend from the bacterial surface and form a feltlike "glycocalyx" surrounding an individual cell or a colony of cells. The adhesion mediated by the glycocalyx determines particular locations of bacteria in most natural environments; more specifically, it is a major determinant in the initiation and progression of bacterial diseases ranging from dental caries to pneumonia.

These major—and, with the benefit of hindsight, rather obvious—facts about the bacterial cell surface have become known only within the past decade. Ironically the main reason for the late discovery of the bacterial glycocalyx and its functions was the long reliance by microbiologists on an otherwise eminently effective investigative system: the pure laboratory culture of an individual bacterial strain. To generate and maintain a glycocalyx a bacterial cell must expend energy, and in the protected environment of a pure culture the glycocalyx is a metabolically expensive luxury conferring no selective advantage; cells that fabricate these elaborate coatings are usually eliminated from pure cultures by uncoated mutants that can devote more of their energy budget to proliferation. Microbiologists have largely studied such naked mutants.

In a competitive natural environment populated by several kinds of bacteria, on the other hand, selection favors cells that are protected, and enabled to adhere to a desirable surface, by a glycocalyx. It was only in 1969 that Ivan L. Roth of the University of Georgia demonstrated carbohydrate fibers surrounding bacteria in an aquatic system and Ian W. Sutherland of the University of Edinburgh Medical School characterized the surface polysaccharides of bacteria taken from natural environments, thus drawing attention to the universality of what we now know as the glycocalyx. Since then studies in our laboratories at the University of Calgary and at the Agriculture Canada Research Station at Lethbridge in Alberta, and in a number of other laboratories, have made it clear that the glycocalyx is essential to the biological success of most bacteria in most of the varied natural environments in which they are observed.

The polysaccharide-coated surface is not a peculiarity of the bacterial cell. The more rigid polysaccharide cell wall of higher plants was among the first microscopic structures described by Robert Hooke in 1665. The analogous surface of animal cells—a glycocalyx like that of bacteria—was described only in 1971, by Vincent T. Marchesi and his colleagues at the National Institute of Arthritis, Metabolism, and Digestive Diseases. They isolated and identified glycoproteins arrayed in the membrane of animal cells and showed that the polysaccharide fibers they bear extend outward from the membrane to form

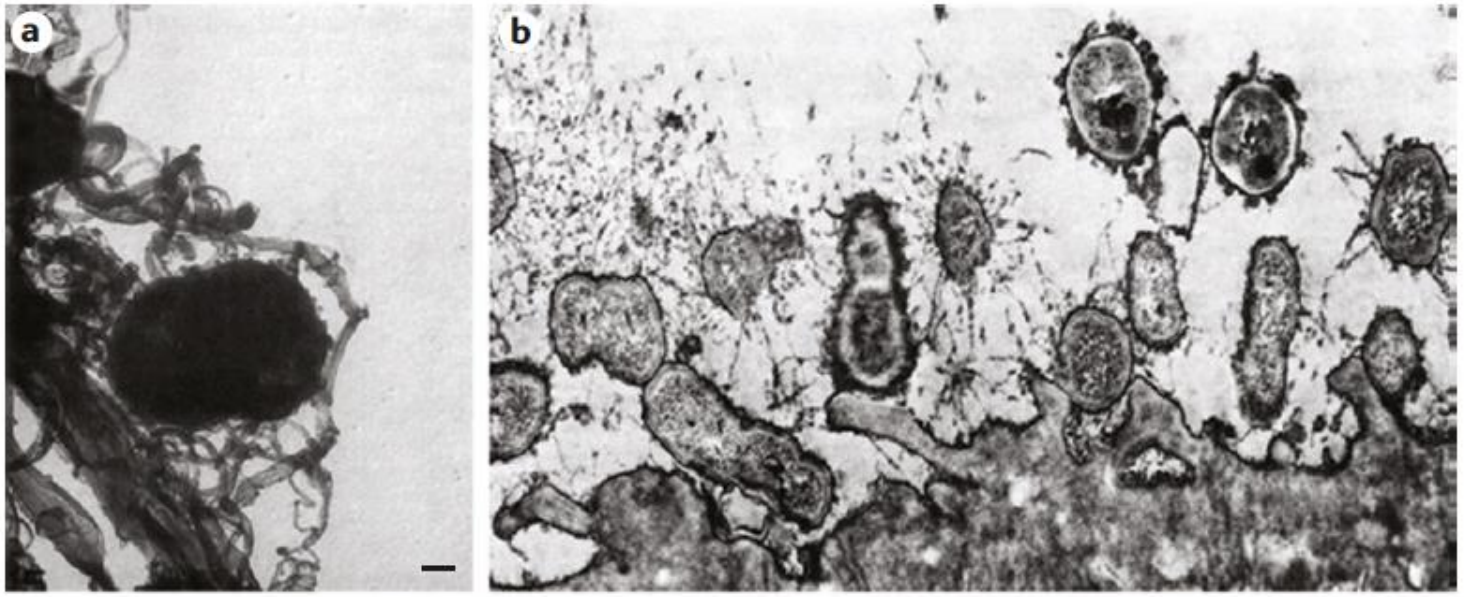
NAKED BACTERIA (*left*) are from a typical pure laboratory culture of *Escherichia coli*; the glycocalyx-coated bacteria (*right*) are *Pseudomonas* cells from an infected human bladder. In both preparations the cells were stained with ruthenium red, which is taken up by any polysaccharide glycocalyx fibers that are present. The bacterial glycocalyx was ignored until recently because the familiar pure laboratory strains do not need it and therefore do not fabricate it.

Costerton et al., How bacteria stick. *Sci. Am.* **238**, 86–95 (1978).

In 1970 he noted that bacteria were attached to the gut or to cellulose fibers via a complex matrix (the glycocalyx), and had little in common with the same species cultivated in the laboratory.

In the latter part of his career, Bill returned to imaging EPS ultrastructure with the discovery of nanowires, nanotubes, membrane vesicles and extracellular DNA networks

Revealing a world of biofilms — the pioneering research of Bill Costerton



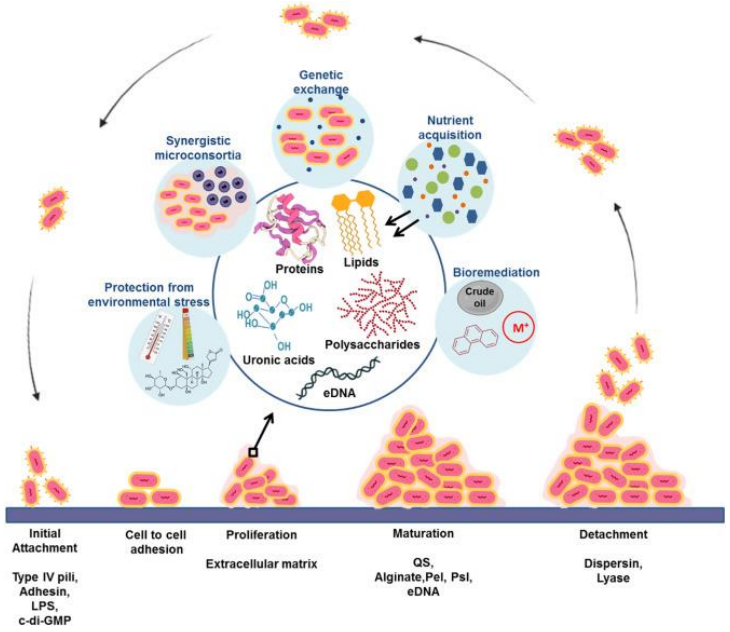
"Bacteria stick, tenaciously and often with exquisite specificity, to surfaces ranging from the human tooth or lung and the intestine of a cow to a rock submerged in a fast-moving stream."

Biofilm theory

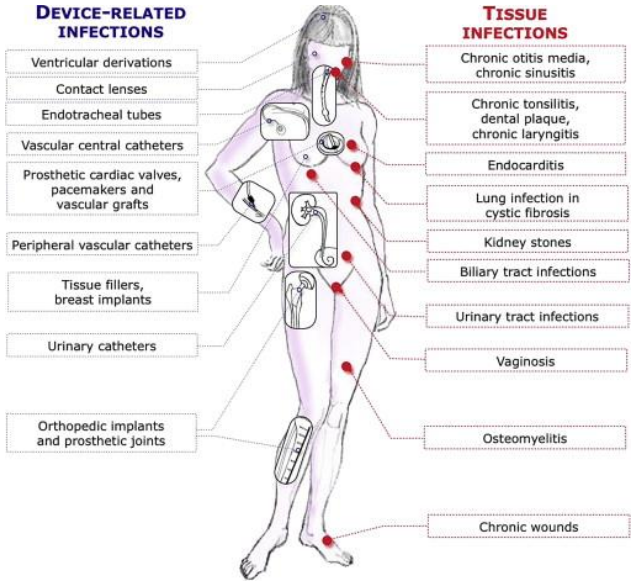
In 1978 Bill Costerton warned that chronic infections in patients with indwelling medical devices were caused by bacteria growing in well-developed glycocalyx-enclosed biofilms and that bacteria within biofilms resist antibiotic therapies and immune host defenses.

Costerton opened **two lines** of scientific endeavor:

The study of the **biochemistry and genetics of biofilm** formation and function



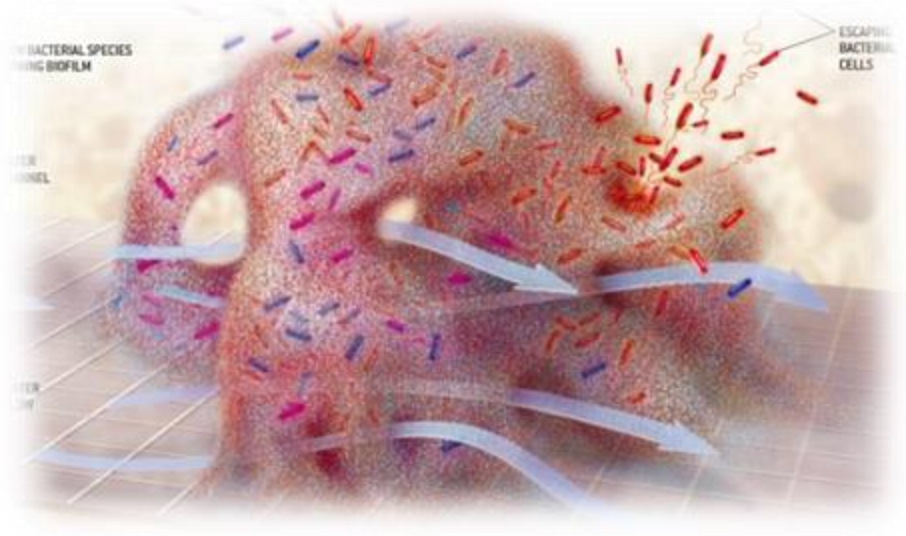
Medical diagnosis and treatment of biofilm-associated infections.



Biofilms: The social life of microorganisms

Planktonic bacteria

Bacterial biofilm



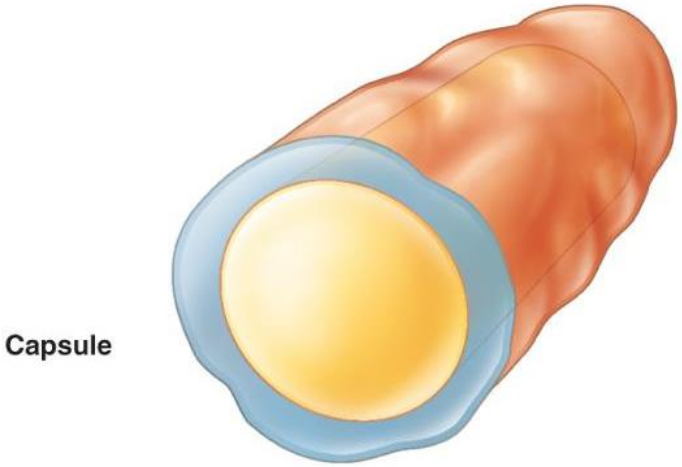
The expression of approximately 40% of the bacterial genome might be affected by biofilm formation

Gene expression changes with time

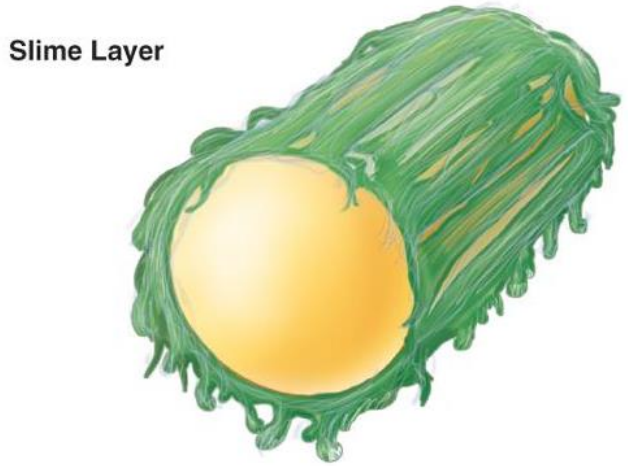
The glycocalyx: Capsule and Slime

Definition and Composition:

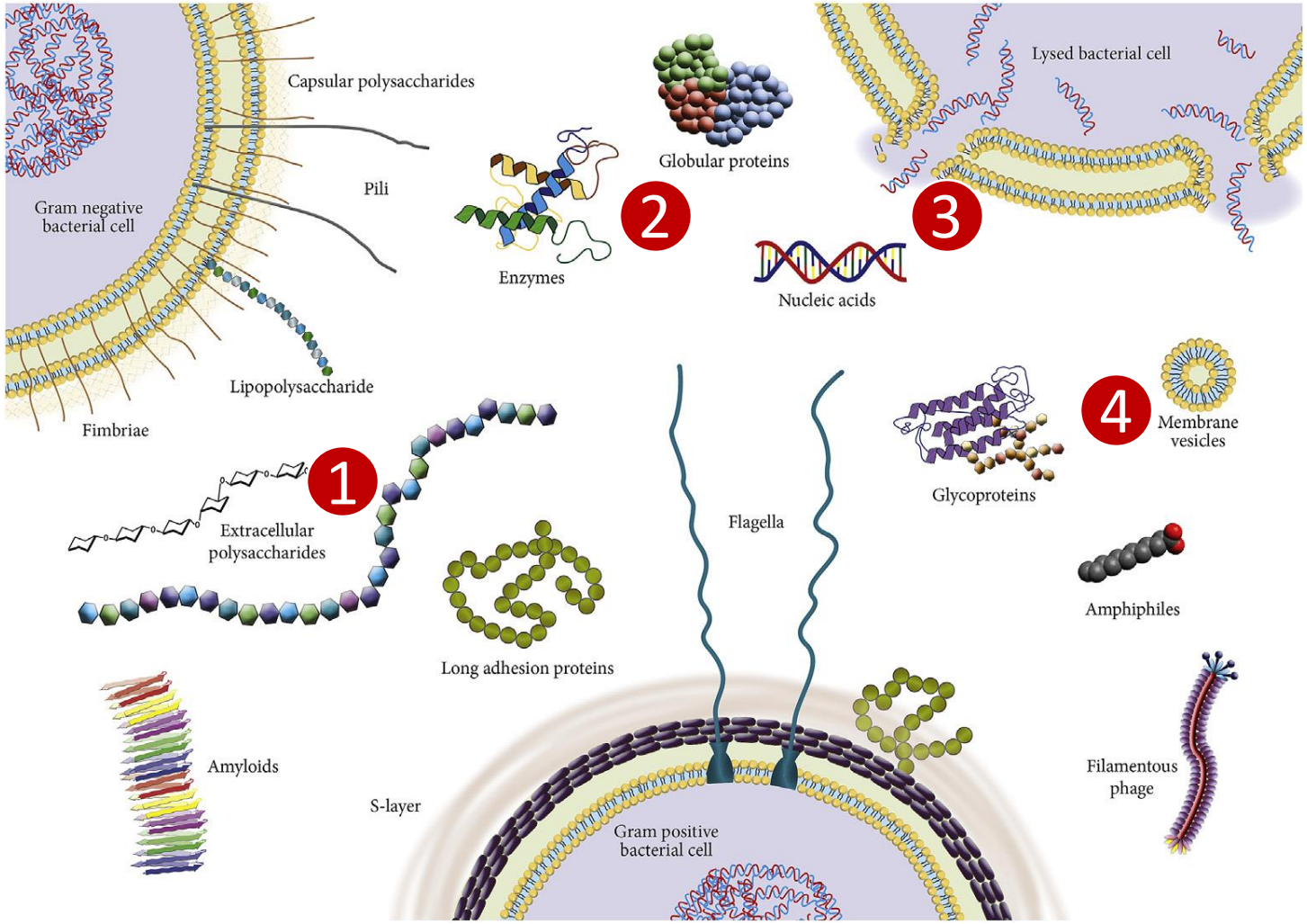
Capsule: a well-defined, gel-like layer surrounding the bacterial cell. It's primarily composed of polysaccharides.
The composition and structure of the capsule can vary among different bacterial species.



Slime: a more diffuse, unorganized layer that is not as tightly associated with the bacterial cell as a capsule. It consists of extracellular polymers, including polysaccharides, proteins, nucleic acids, and lipids.
It's often part of what's called biofilm matrix



Biofilm Matrix in Structured Microbial Communities



The biofilm matrix of is composed by the extracellular polymeric substance (EPS)

1 extracellular polysaccharides,
2 proteins (enzymes)
3 extracellular DNA (exDNA)
4 lipids

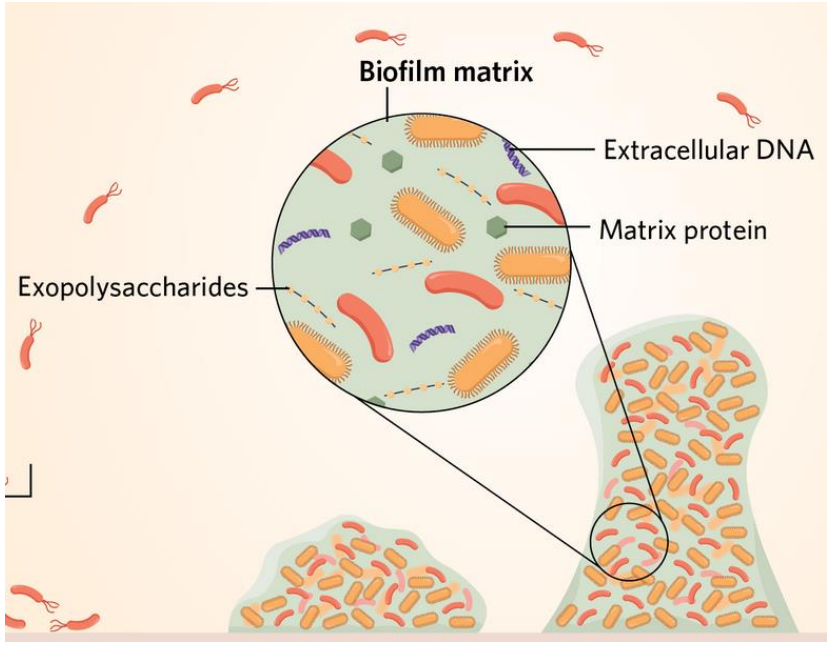
Many biofilm matrix polysaccharidic components have been identified: PIA/PNAG, colanic acid, alginate, glucose and mannose, and cellulose

Biofilm Matrix structure

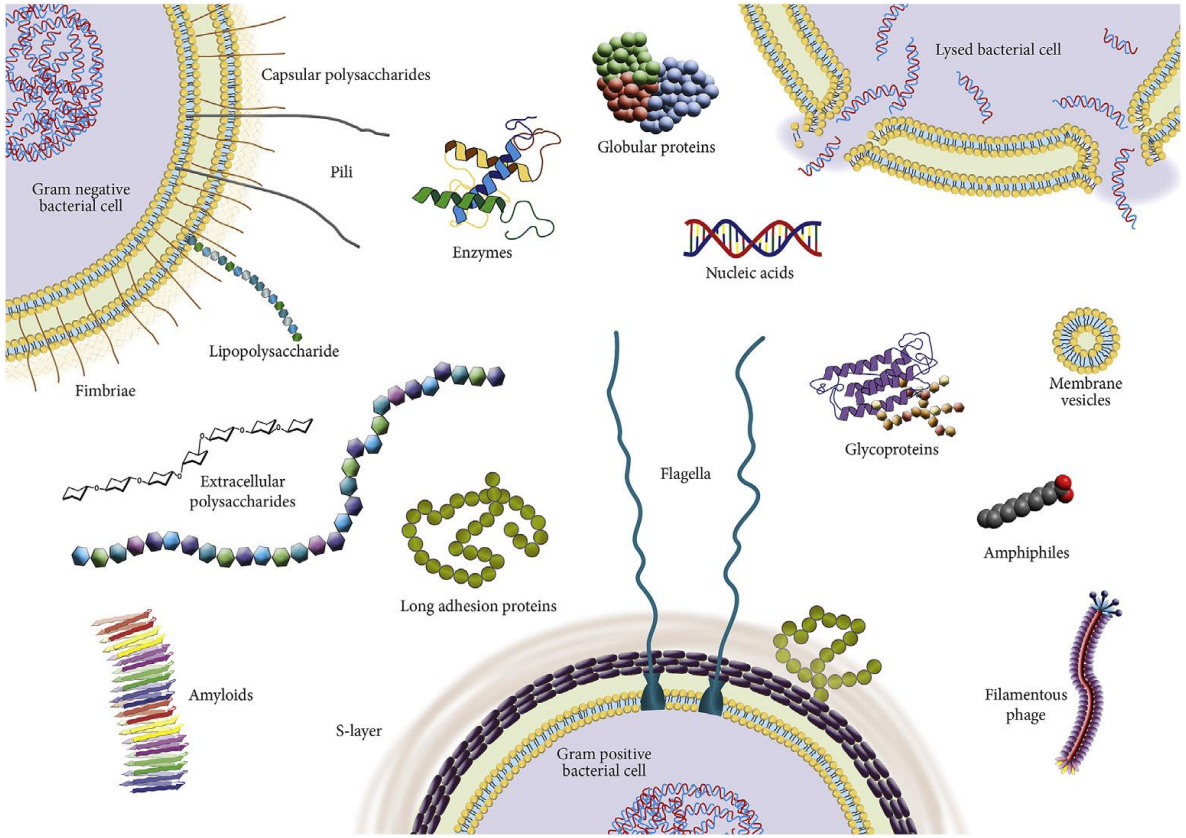
1. **Extracellular Polymeric Substances (EPS)**: This is the most abundant component, typically making up 50-90% of the biofilm's organic matter. EPS are primarily composed of:
   - **Polysaccharides**: These can vary widely but generally constitute a significant portion of the EPS.
   - **Proteins and Peptides**: Including enzymes and structural proteins, they can account for a substantial part of the EPS.
   - **Nucleic Acids**: Mainly eDNA, which can be important for the structural integrity of the biofilm and horizontal gene transfer.
   - **Lipids**: These are usually a minor component but can be significant in some biofilms.
   - **Water**: is 97% which is retained within the EPS matrix. This high water content is crucial for nutrient transport and waste removal within the biofilm.

2. **Microbial Cells**: usually constitute a relatively small fraction of the biofilm's total biomass, often around 10-25%.

3. **Inorganic Compounds**: minerals and other trace elements. The concentration depends on the environment and the type of microorganisms present.



Extracellular polymeric substances of biofilms: Suffering from an identity crisis



EPS compounds originate from different community members and a specific organism can produce different polymers as a function of time or condition.

EPS produced by a given microbial population can persist long after the population producing it has disappeared

Different components contribute to the function and organization of the biofilm matrix.

Many of the biopolymers produced by the cells are processed by extracellular enzymes embedded in the extracellular matrix.

It is currently not possible to track the production of specific EPS components over time or attribute them to the specific host organism in mixed species biofilm communities

Functions of microbial EPS

1. EPS can be produced by bacteria, cyanobacteria, microalgae, yeasts, fungi, and protists.

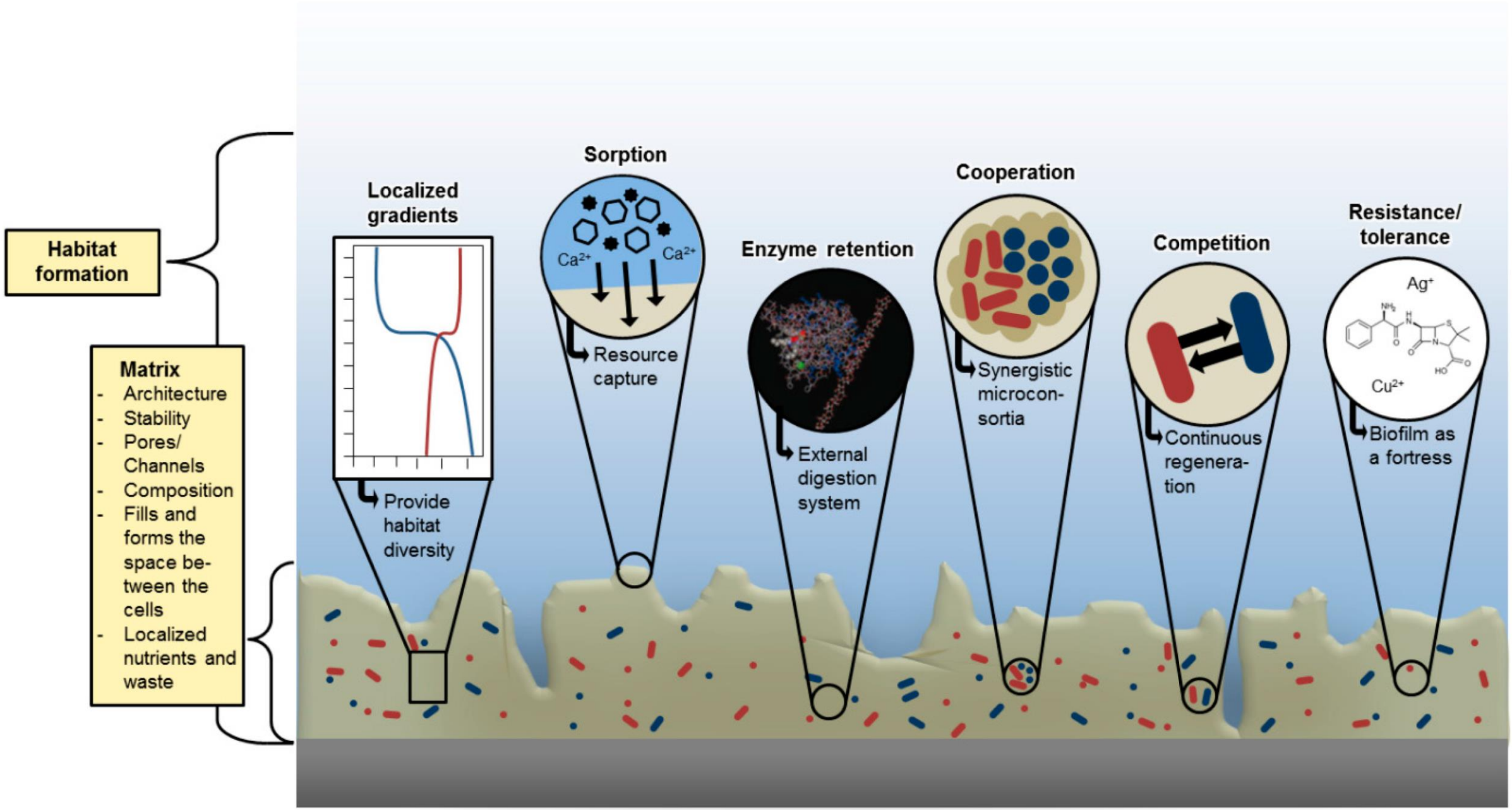
2. EPS are responsible for the **cohesion of microorganisms** and adhesion of biofilms to surfaces, influencing spatial organization, allowing interactions among microorganisms, and acting as adhesives between cells

3. EPS biosynthesis is an **energy-demanding process**. Therefore, their production requires selective advantages in the environment of the producing microorganism.

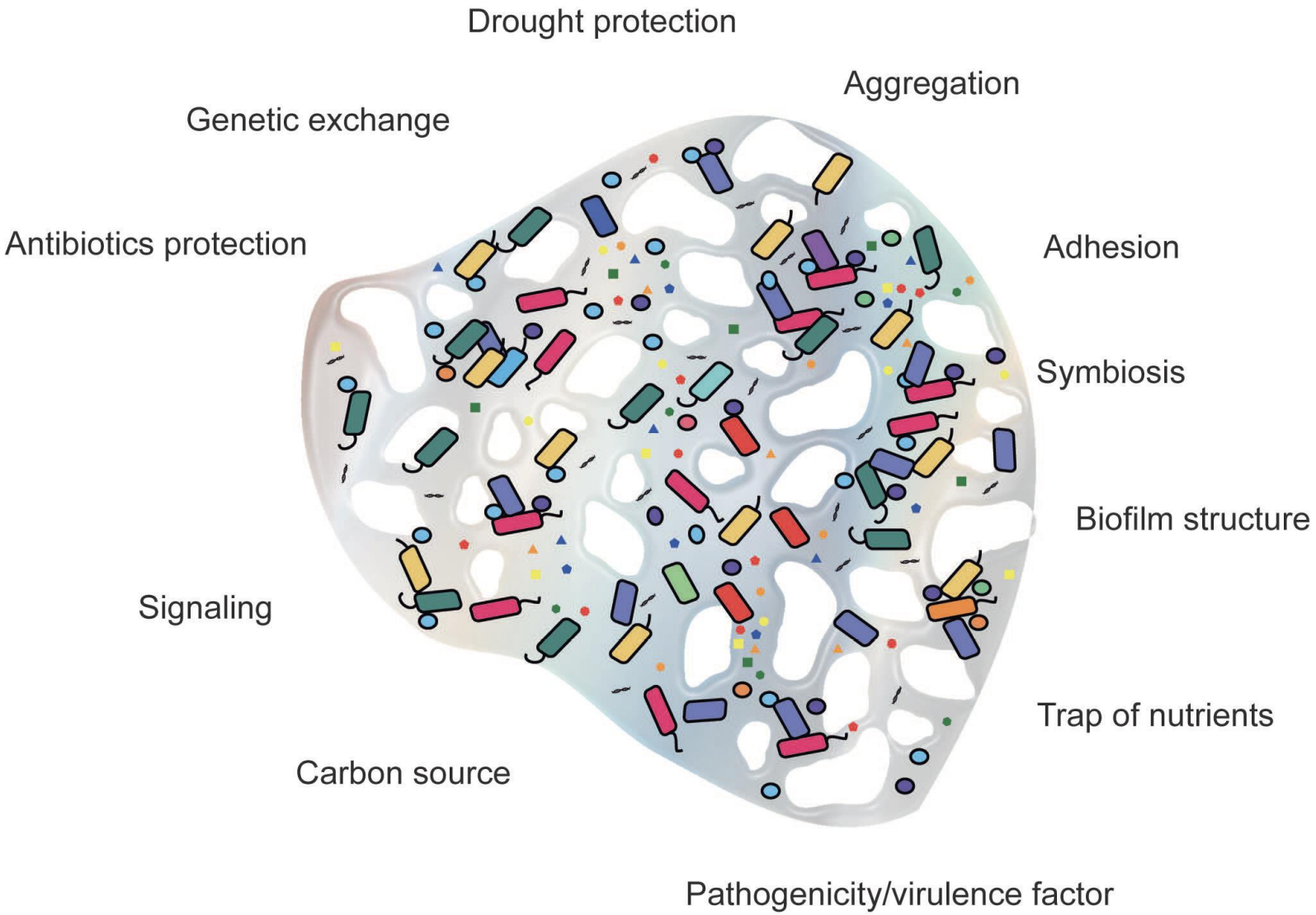
4. In natural environments, most microorganisms live in aggregates, such as flocs and biofilms, for which EPS are **structurally and functionally essential**.

5. Most of the functions attributed to EPS are related to **protection** of the producing microorganism. Drought, temperature, pH, and salinity can trigger the production of EPS as a response to environmental stresses.

Life in the EPS



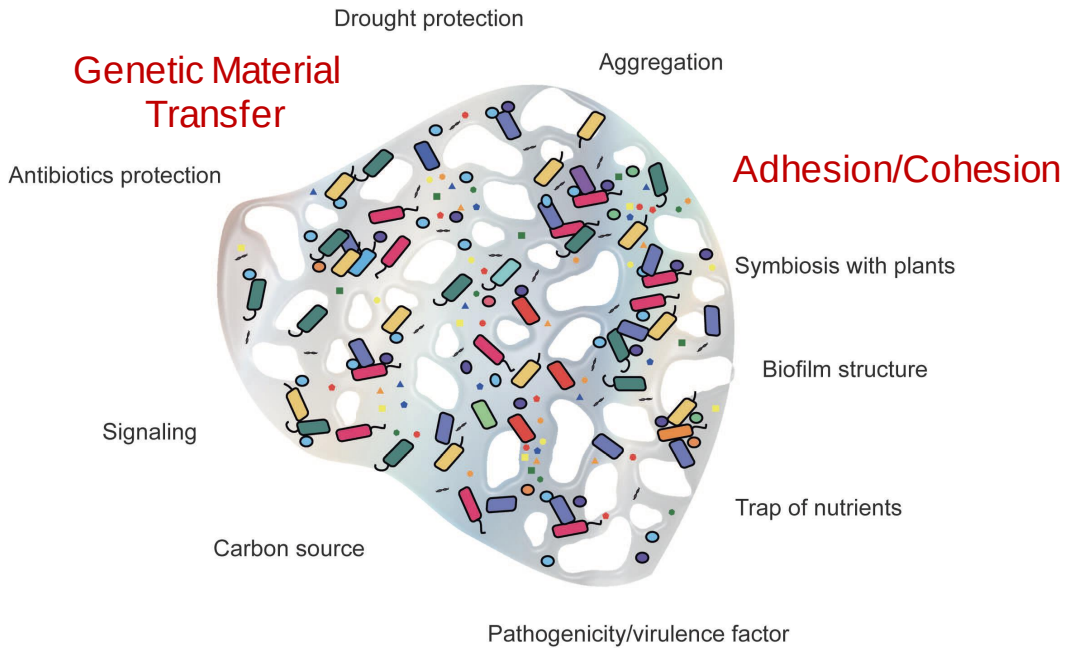
Functions of microbial EPS



https://www.frontiersin.org/articles/10.3389/fmicb.2018.01636/full#:~:text=Extracellular%20polymeric%20substances%20(EPS)%20are,triggered%20primarily%20by%20environmental%20signals.

EPS in interactions with other microorganisms and environment

Adhesion/Cohesion/Genetic Material Transfer



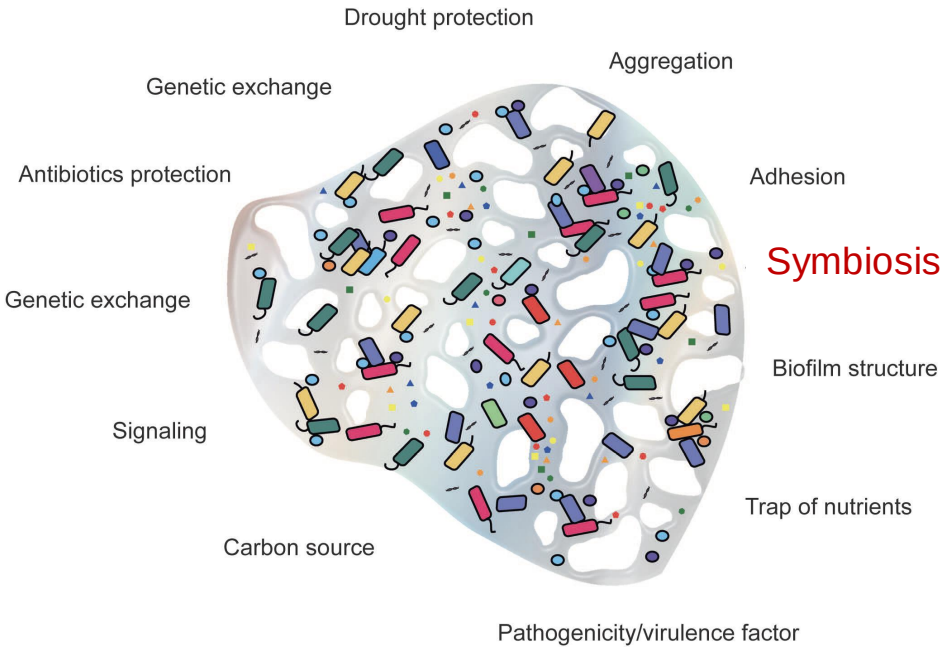
EPS are responsible for the cohesion of microorganisms and adhesion of biofilms to surfaces, influencing spatial organization, allowing interactions among microorganisms, and acting as adhesives between cells.

The polymers mechanically stabilize the microbial aggregates via several types of interactions between the macromolecules, including electrostatic interactions, and hydrogen bonds.

Together with different protein adhesins, EPS are involved in the initial steps of microbial adhesion to surfaces.

eDNA: adhesion, stability, protection, nutrition, horizontal gene transfer

EPS in Microbe–Host Interactions



Symbiosis

EPS in the establishment of symbiosis between nitrogen-fixing bacteria (*Rhizobium* genus) and plants.

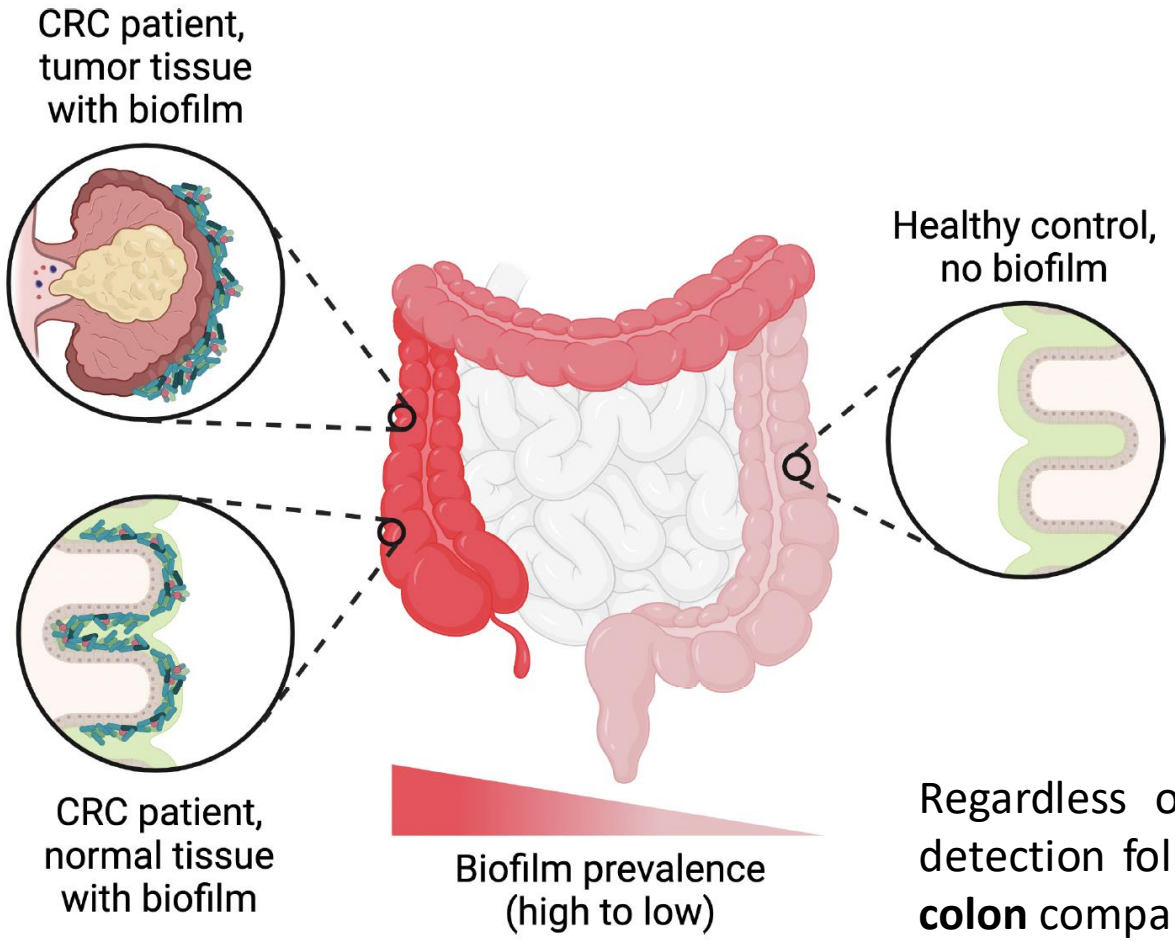
Rhizobium is a genus of bacteria associated with the formation of **root nodules on plants**. These bacteria live in symbiosis with legumes. They take in nitrogen from the atmosphere and pass it on to the plant, allowing it to grow in soil low in nitrogen

Rhizobial surface polysaccharides are fundamental for nodule formation by some legumes

Lotus japonicus produces a receptor that binds to and permits infection by only bacteria that produce EPS with a specific structure;

- mutants with truncated EPS are less successful in infection.
- The expression of this receptor demonstrates that the plant is capable of recognizing the structure of EPS produced by rhizobia.

Bacterial-derived exopolysaccharides on gastrointestinal microbiota, immunity and health

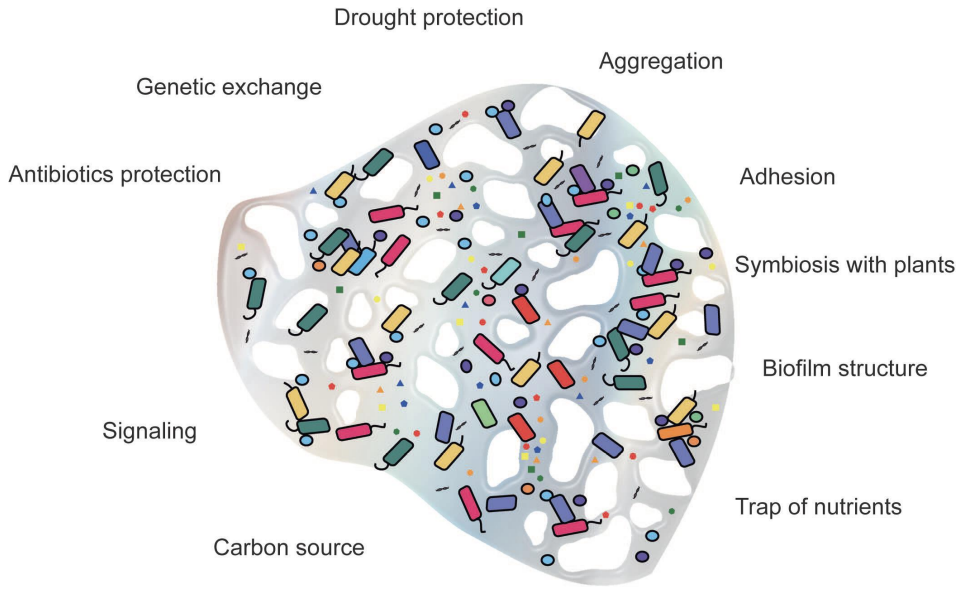


- EPS influences the epithelial barrier integrity
- EPS interacts with the host immune system
- EPS protect commensal/beneficial bacteria against immune responses.

Adherent biofilms on the colonic epithelium are found in a higher proportion of patients with colorectal cancer (CRC), inflammatory bowel disease, etc compared to healthy controls.

Regardless of the patient's underlying condition, the frequency of biofilm detection follows a decreasing trend with higher frequencies in the **ascending colon** compared to the **transverse** and **descending colon**

EPS as Pathogenicity/Virulence Factors



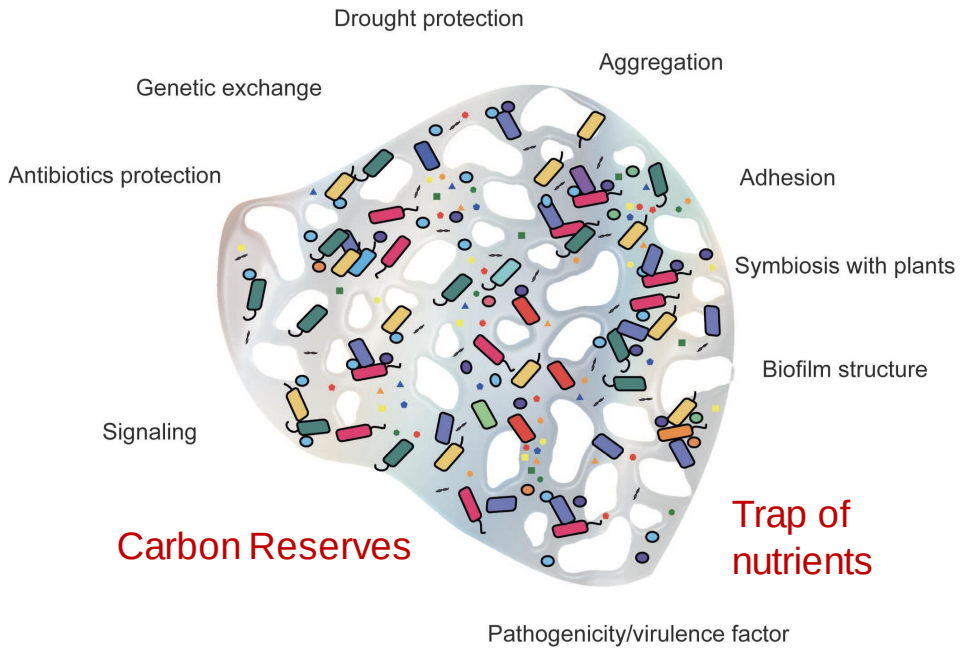
Pathogenicity/Virulence Factors

Alginate, the EPS produced by *P. aeruginosa*, protects the bacteria against the **inflammatory process** of the host (free radicals, antibodies, and phagocytosis)

Tolerance: the ability of bacteria to survive transient exposure to an antibiotic without undergoing genetic change. Tolerant bacteria are not killed by the antibiotic but are inhibited and can resume growth once the antibiotic is removed.

Persistance: bacteria within a biofilm exhibit a heightened ability to survive in the face of adverse conditions.
This persistence is a key factor in the chronic nature of many biofilm-associated infections

EPS and Nutrition
Carbon Reserves – Trap of nutrient

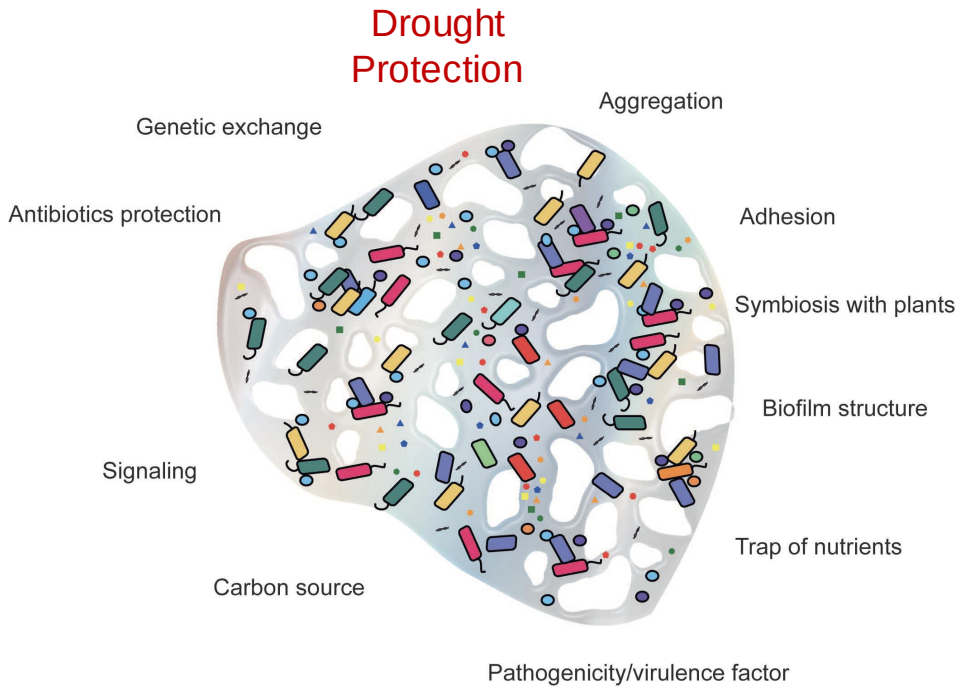


Extracellular polymeric substances produced by microorganisms might act as carbon reserves

EPS can accumulate other nutrients and molecules. The retention of extracellular enzymes in the EPS matrix promotes the formation of an extracellular digestion system that captures compounds from the water phase and permits their use as nutrient and energy sources.

In soils, microbial EPS can sorb, bind or entrap many soluble and insoluble metal species, as well as clay minerals, colloids, and oxides, which also have metal binding properties

Protection Against Abiotic and Biotic Stresses
Drought Protection/Salt Tolerance



A high **water-holding capacity** was observed for the EPS

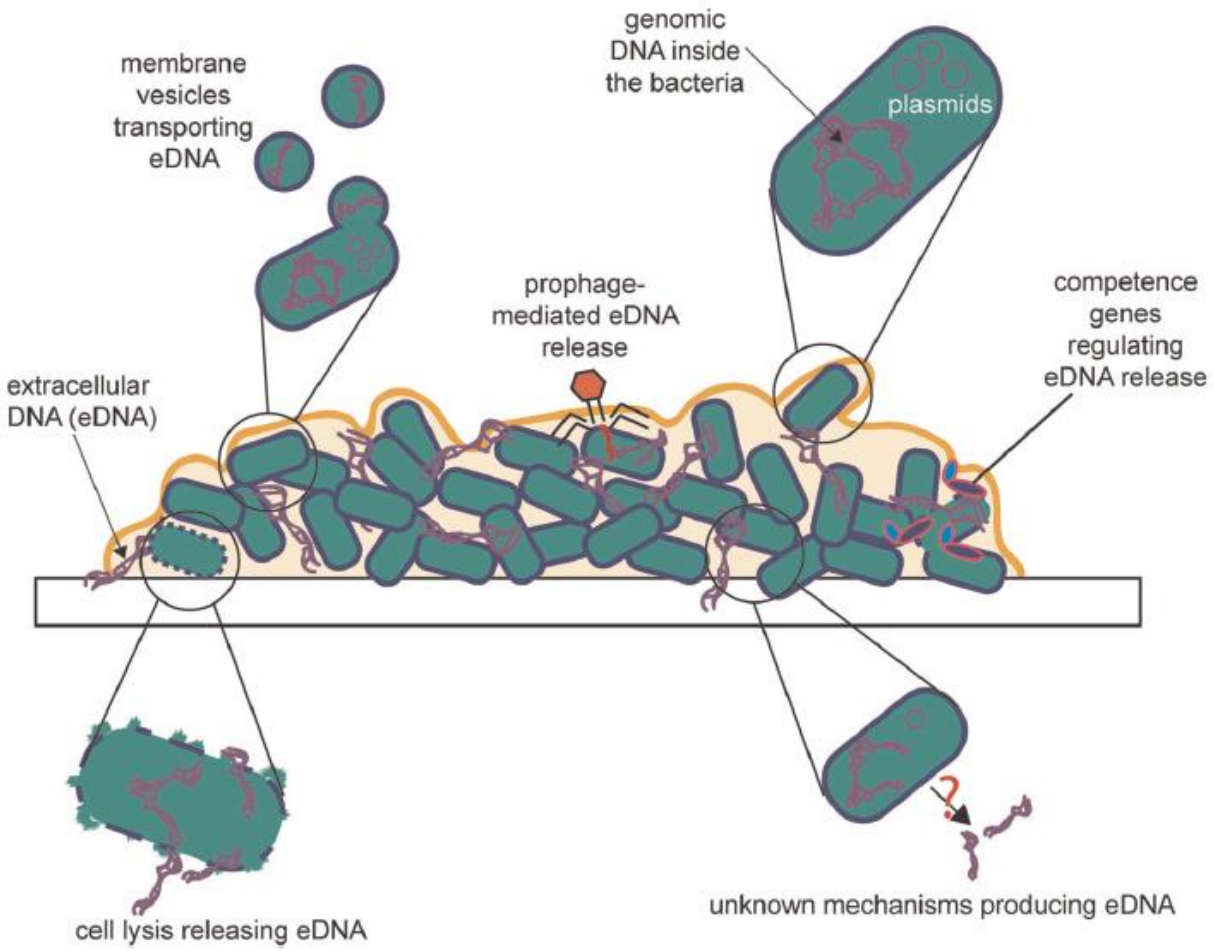
EPS exhibits significant structural modifications during **desiccation** and may be an important protection factor, trapping a reservoir of water and nutrients for bacterial survival.

Microbial polymers are involved in **tolerance to salt stress**, not only for the producer microorganisms but also for the associated plants. The production of polymer by NaCl-tolerant isolates can decrease *Na* uptake by plants by trapping and decreasing the amount of ions.

The production of EPS is an important factor in the protection against Low/High temperatures.

Protection against antibiotics.

eDNA production and release



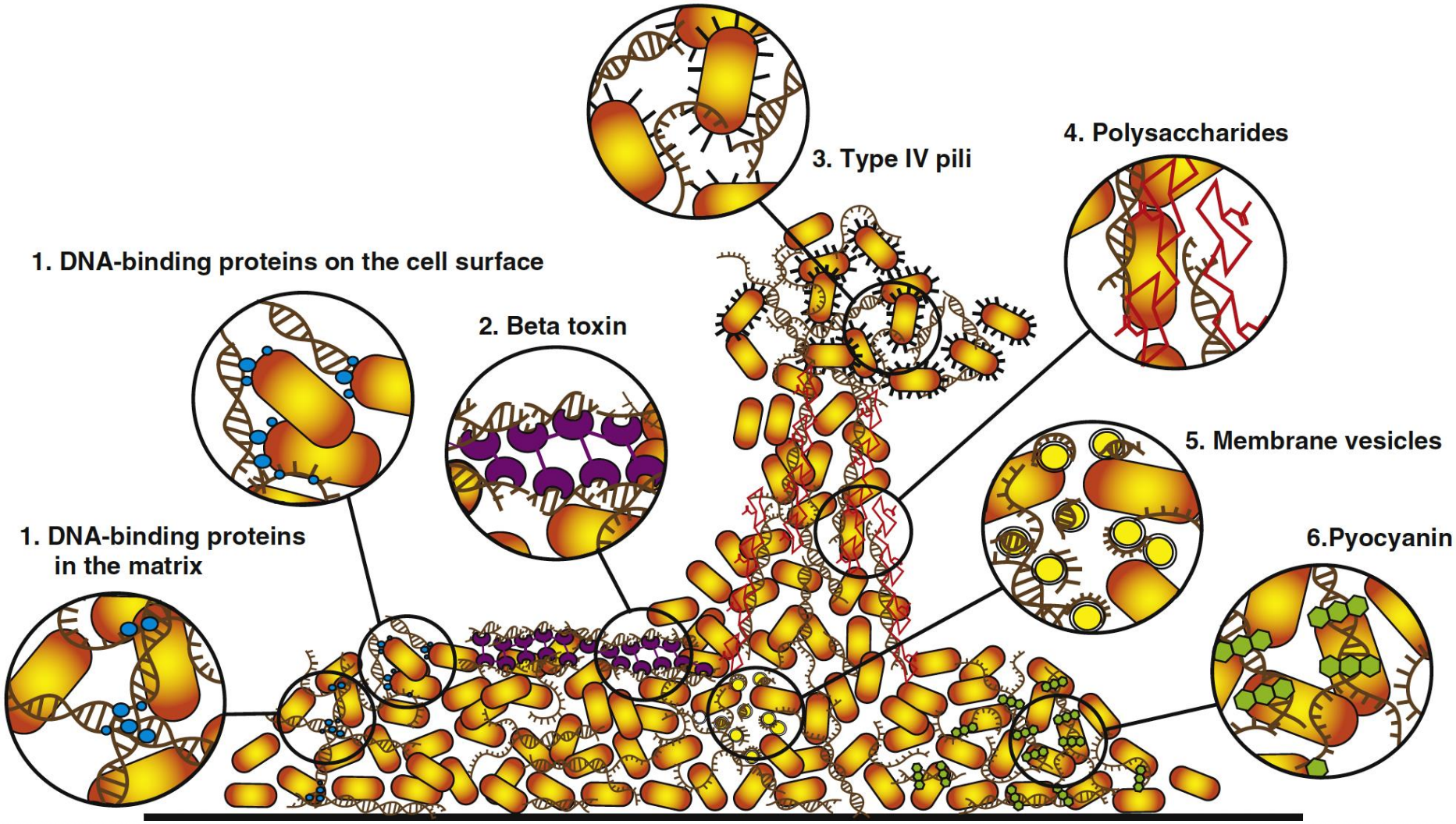
Lysis-related (Autolysis or cell death)

Membrane vesicles

Prophage-mediated eDNA release

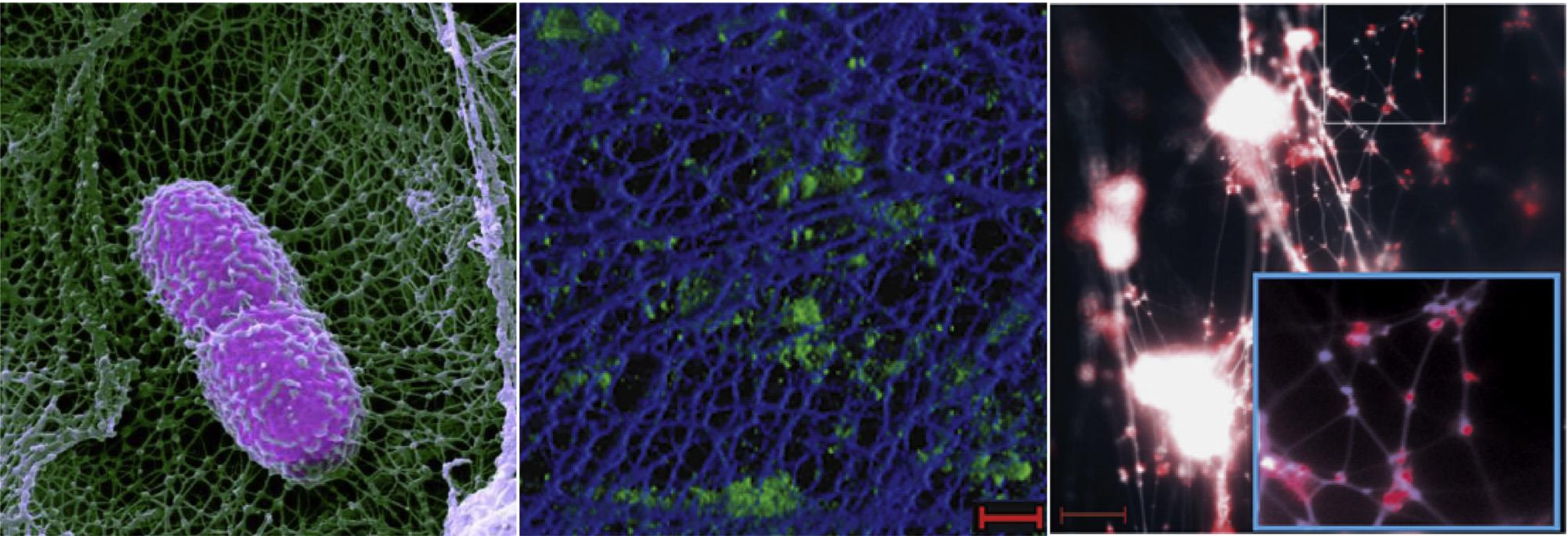
Unknown pathways

eDNA functions



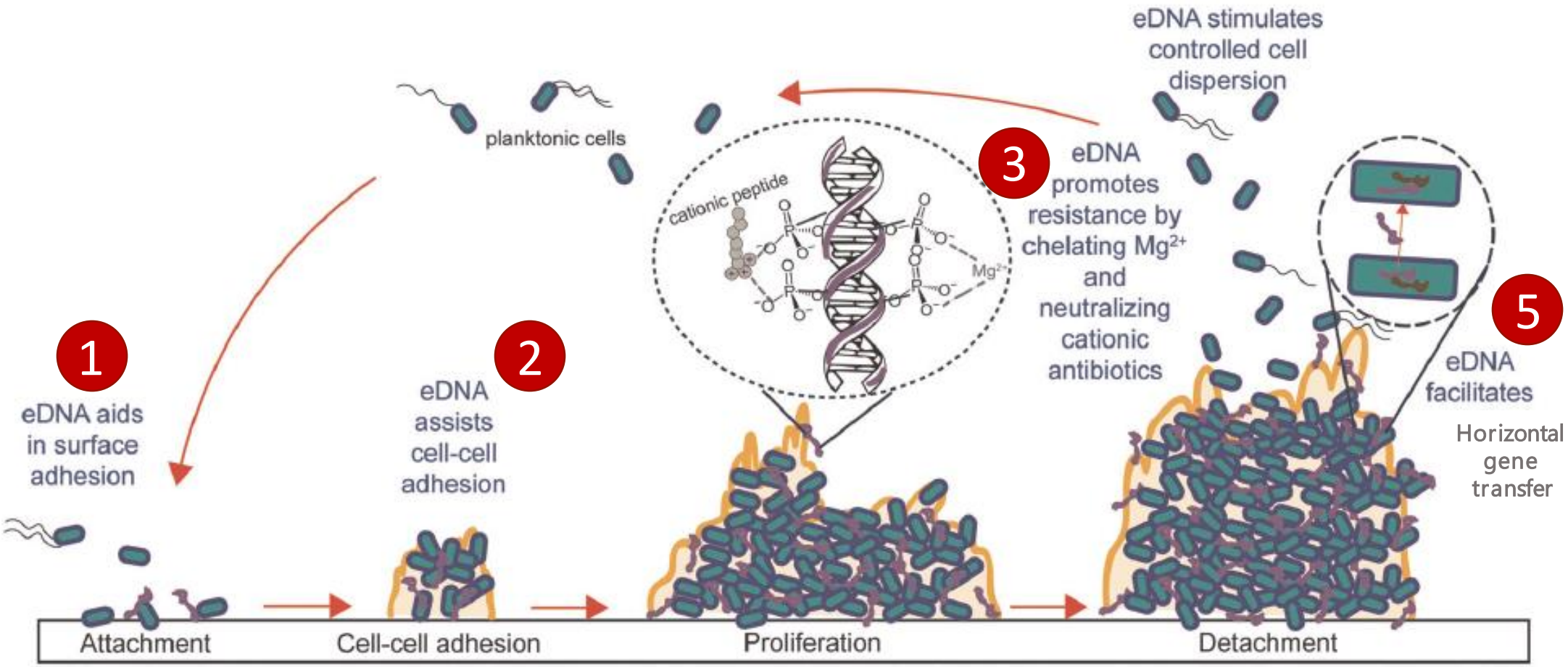
Interactions between eDNA and other matrix components. **(1)** DNA-binding proteins (Defensin or Integration Host Factor) may localize to the extracellular matrix where they form junctions of eDNA strands [36••,37,38•,71]. DNA-binding proteins may also be surface-associated (e.g. NhbA and IgAp [72••] and hypothetically also proteins from the DNA binding and uptake system used in competence [66]). **(2)** Beta toxin, a secreted virulence factor, crosslinks in the presence of eDNA and forms an insoluble nucleo-protein complex [40]. **(3)** Type IV pili bind to eDNA and guide motility [42]. **(4)** Polysaccharides co-localize with eDNA [15•,35••]. **(5)** DNA interacts with biofilm-derived membrane vesicles [74]. **(6)** Pyocyanin intercalates with eDNA to promote cell-to-cell interactions [73•].

eDNA functions in biofilm

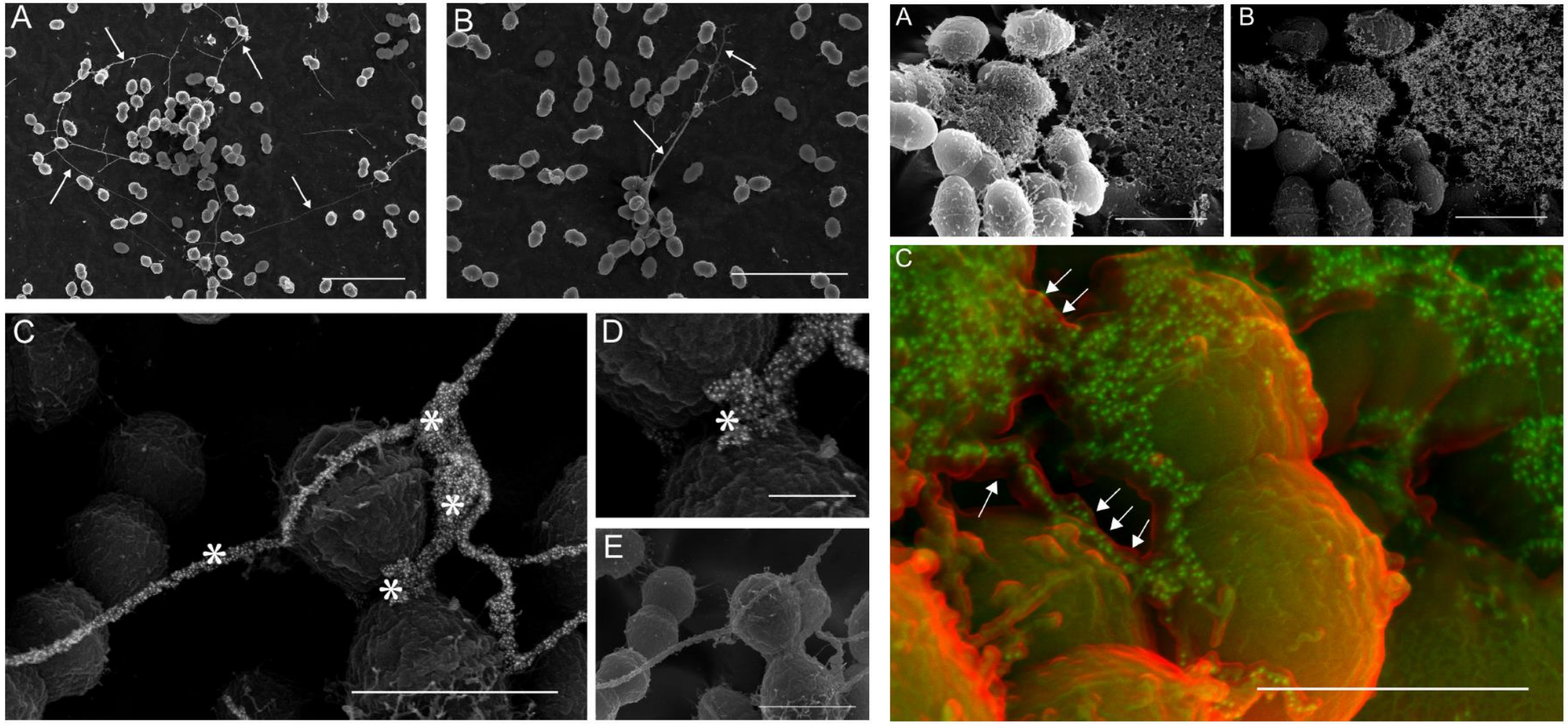


(a) *Klebsiella pneumoniae* caught in the DNA of a NET in a mouse lung
(b) eDNA filaments (blue) in a biofilm formed in vivo by *Haemophilus influenzae*
(c) Immunohistochemical labeling of the DNABII protein IHF (red) shows that IHF connects eDNA strands of *H. influenzae* biofilm formed in the middle ear of a rodent

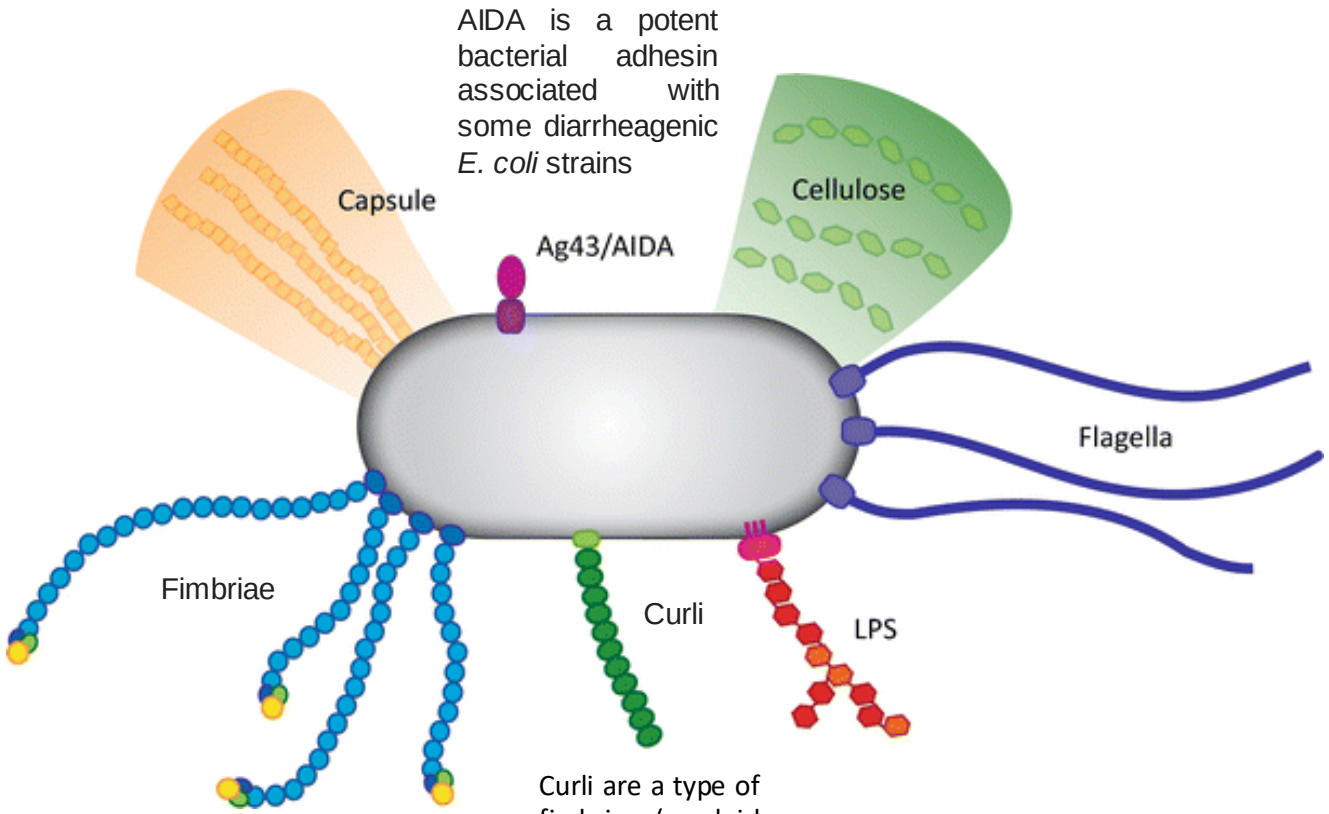
eDNA functions in biofilm life cycle



Enterococcus faecalis produces abundant eDNA in the absence of cell lysis during biofilm formation



Attachment Phase: Bacterial adhesins



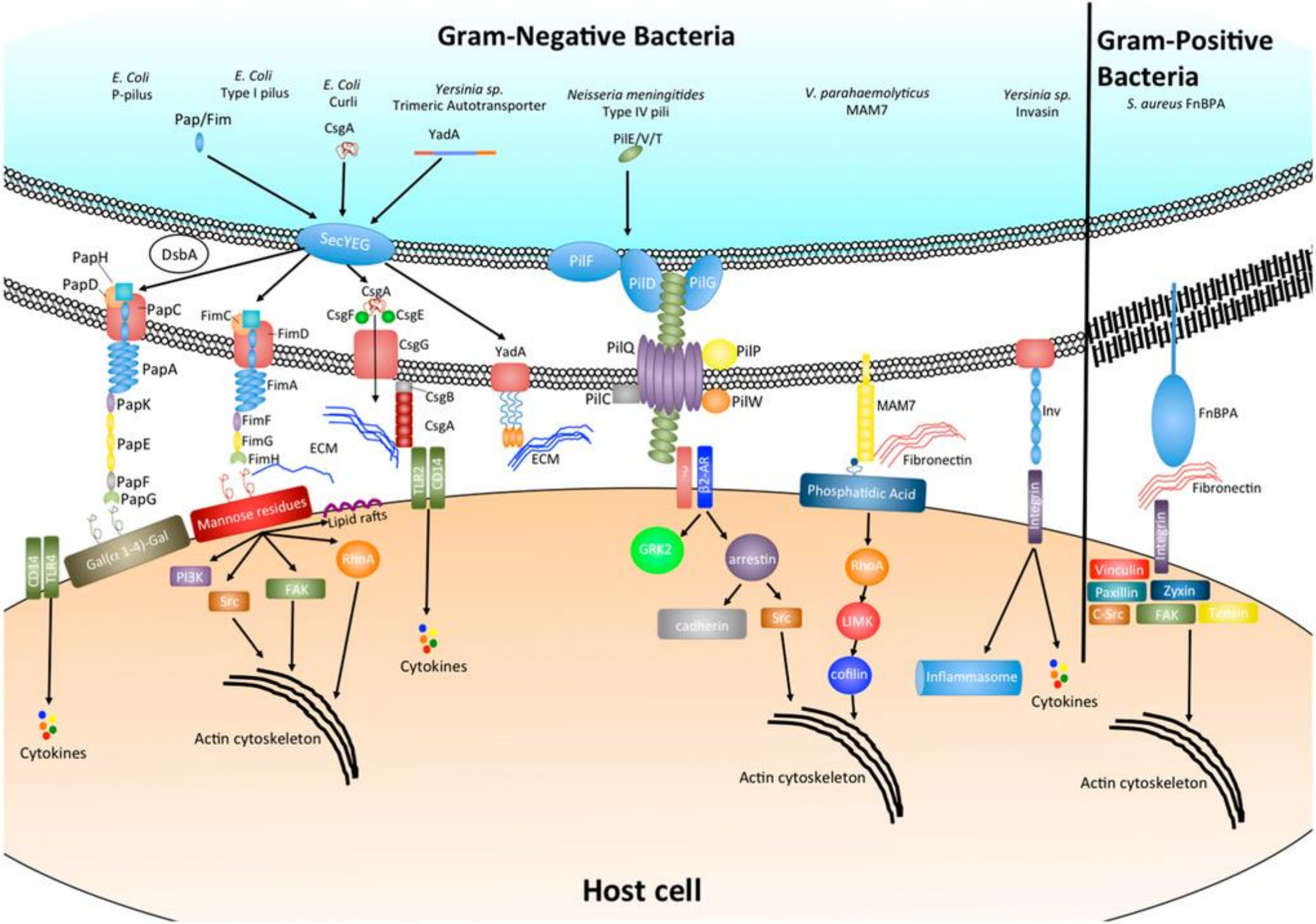
Fimbria is a short pilus that is used to attach the bacterium to a surface (attachment pilus)

Curli are a type of fimbriae (amyloid surface fibers.

Bacterial adherence structures are often very **target specific**

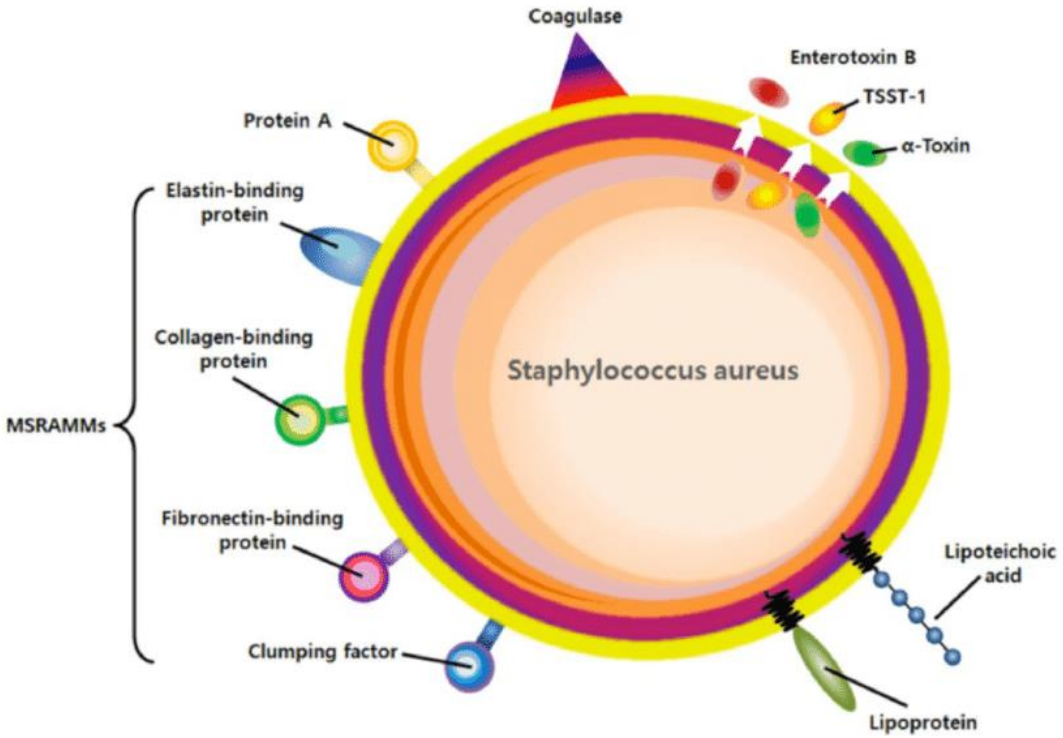
Adhesins enable bacteria to specifically recognize and **bind to a diverse spectrum of molecular motifs** on target surfaces, ranging from surface components of tissues or cells.

The ability of bacteria to **resist removal by hydrodynamic shear forces** is often critical since many surfaces in nature are submitted to strong flow forces.



The (apparently) redundant biofilm mechanisms

The Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM) in *S. aureus*

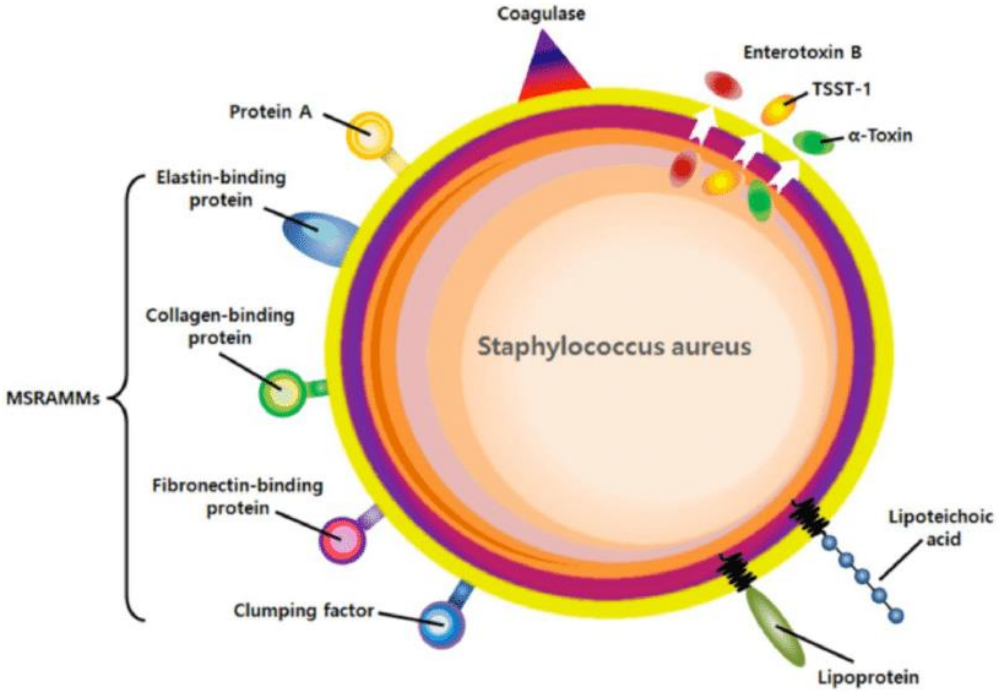


Cell wall-anchoring region

These environmentally regulated biofilm mechanisms are **niche-specific**

On the skin where **NaCl concentration is high** and **water availability is low**, production of the polysaccharide intercellular adhesin (PIA) may serve primarily to trap water with its role in intercellular adherence as a secondary function.

The (apparently) redundant biofilm mechanisms

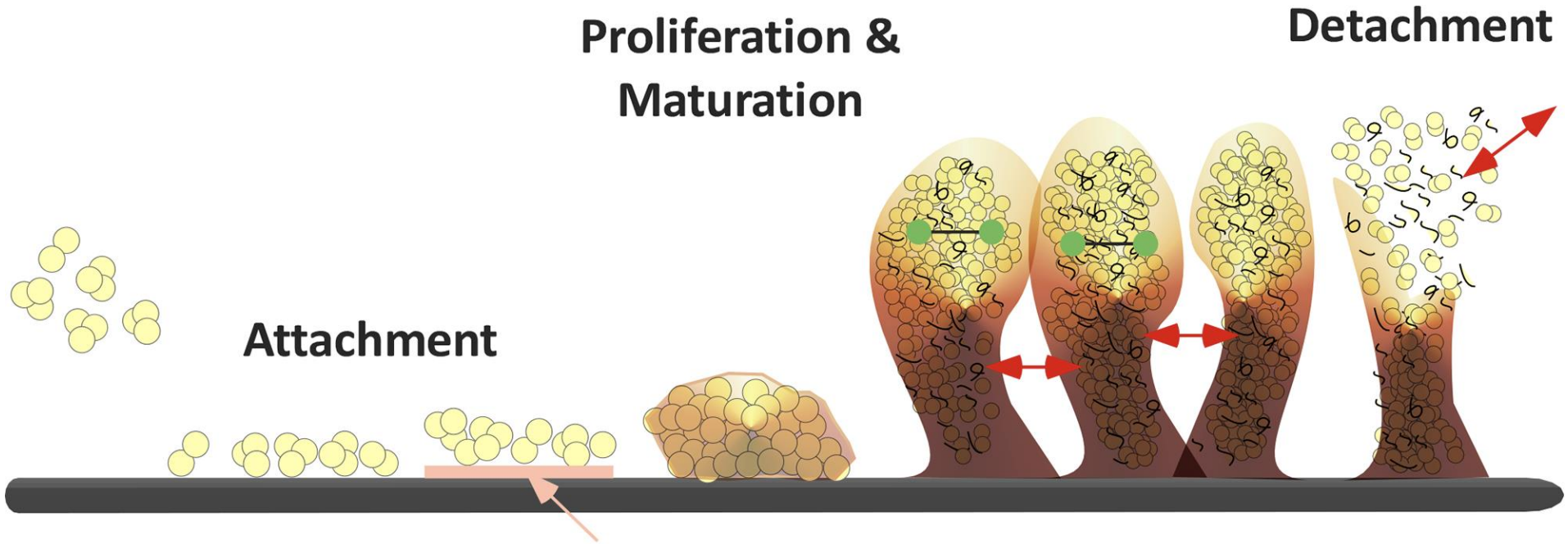


S. aureus has about 20 and *S. epidermidis* about 12 MSCRAMMs.

Prominent members are the fibrinogen- and fibronectin proteins, which include:

- clumping factors A and B (ClfA, ClfB),
- the serine/aspartate-rich (Sdr) protein family,
- fibronectin-binding proteins A and B (FnBPA, FnBPB).

THE MOLECULAR BASIS OF BIOFILM FORMATION
Staphylococcal biofilm development



to polymer surface
Surface hydrophobicity

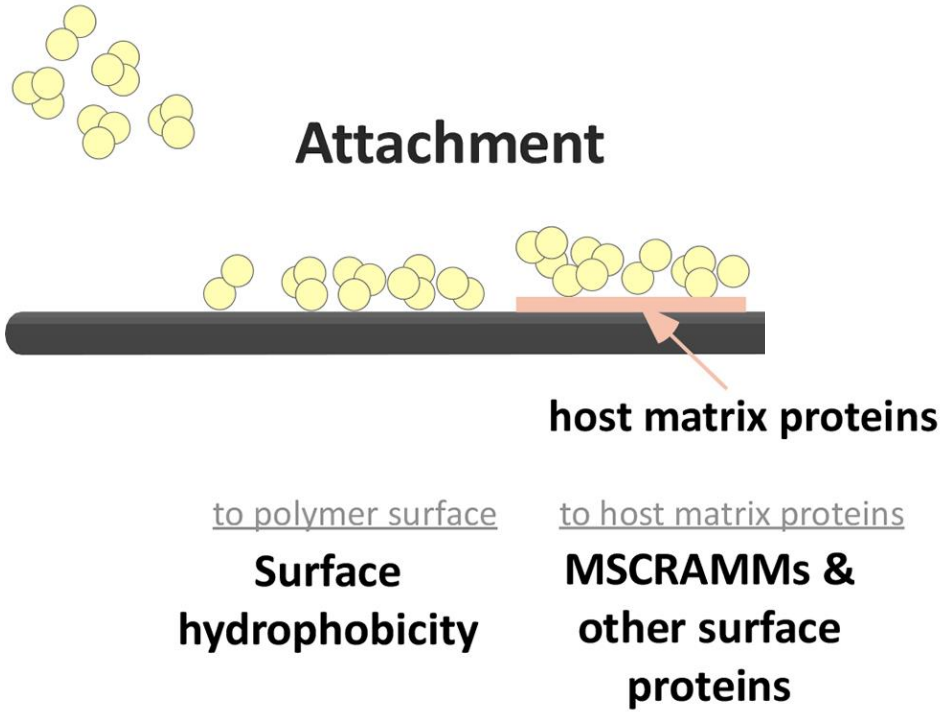
to host matrix proteins
MSCRAMMs & other surface proteins

MSCRAMMs (microbial surface components recognizing adhesive matrix molecules)

adhesive factors:
PIA
eDNA
Aap and other proteins

disruptive factors:
PSMs
Proteases
Nucleases

Attachment Phase



First stage of biofilm development

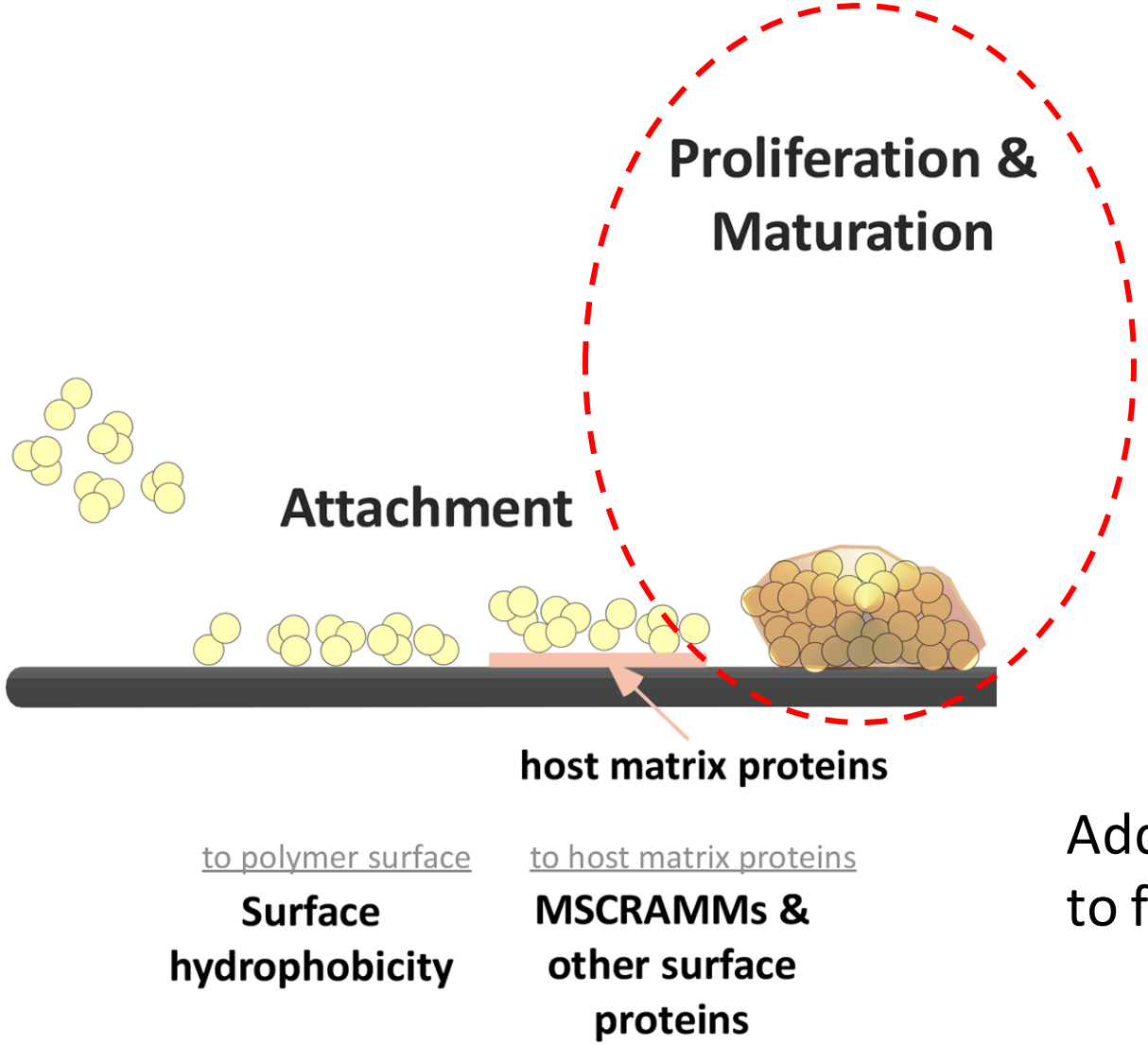
In contrast to motile organisms, staphylococci gain contact with a surface passively.

Indwelling devices (*in vivo)* rapidly become covered by host matrix material, (fibronectin, fibrinogen, vitronectin etc.).

Attachment to the device is facilitated by a series of specific staphylococcal surface proteins that interact with those human matrix proteins.

The most important family of such surface-expressed staphylococcal binding proteins is the **MSCRAMMs** (microbial surface components recognizing adhesive matrix molecules).

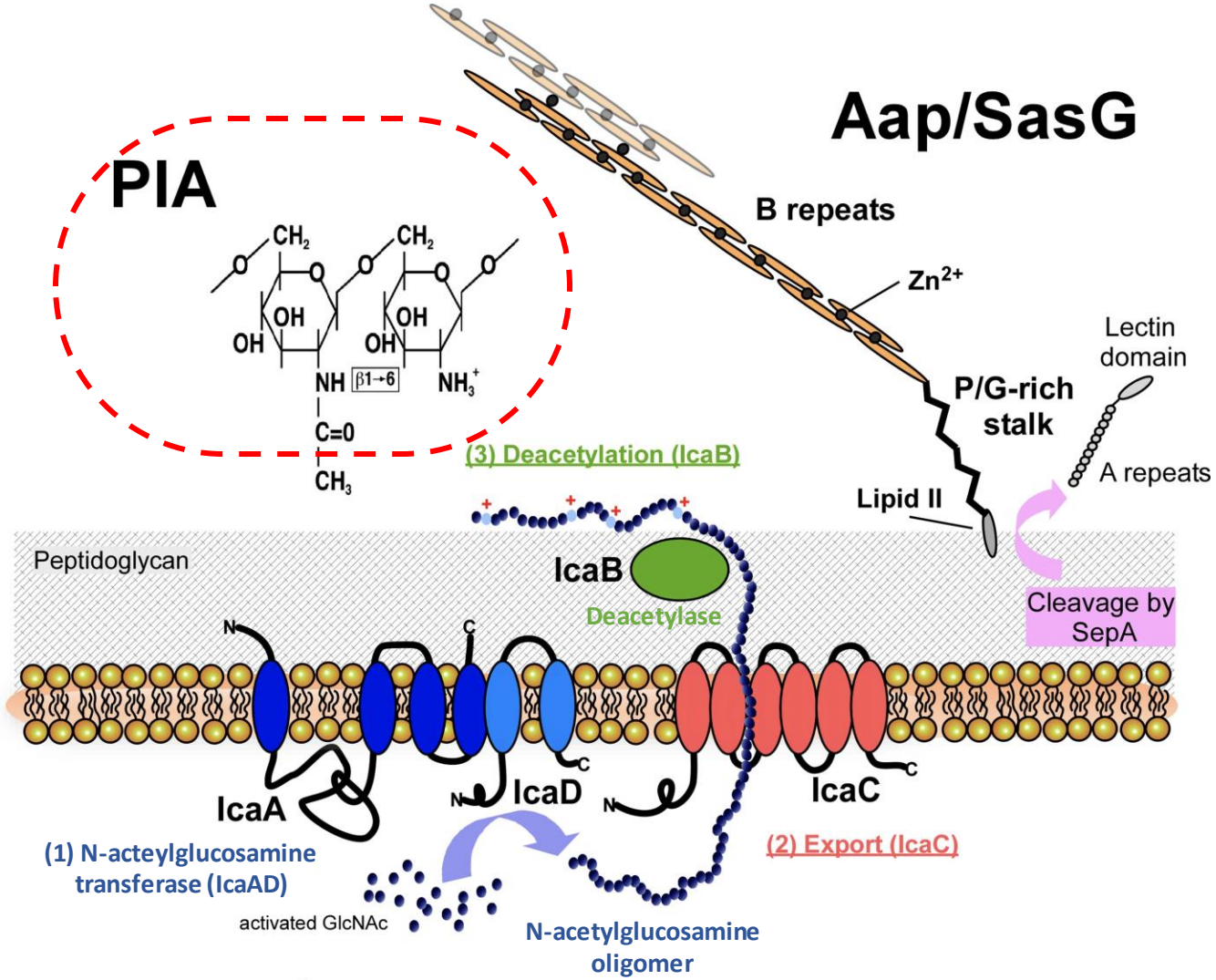
Proliferation and matrix formation



During the second stage of biofilm development, the microcolonies that have formed after attachment grow by proliferation.

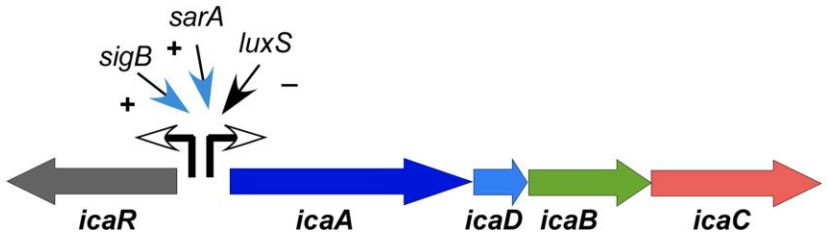
Additionally, cells secrete polymeric molecules to form the biofilm matrix.

Staphylococcal biofilm matrix components



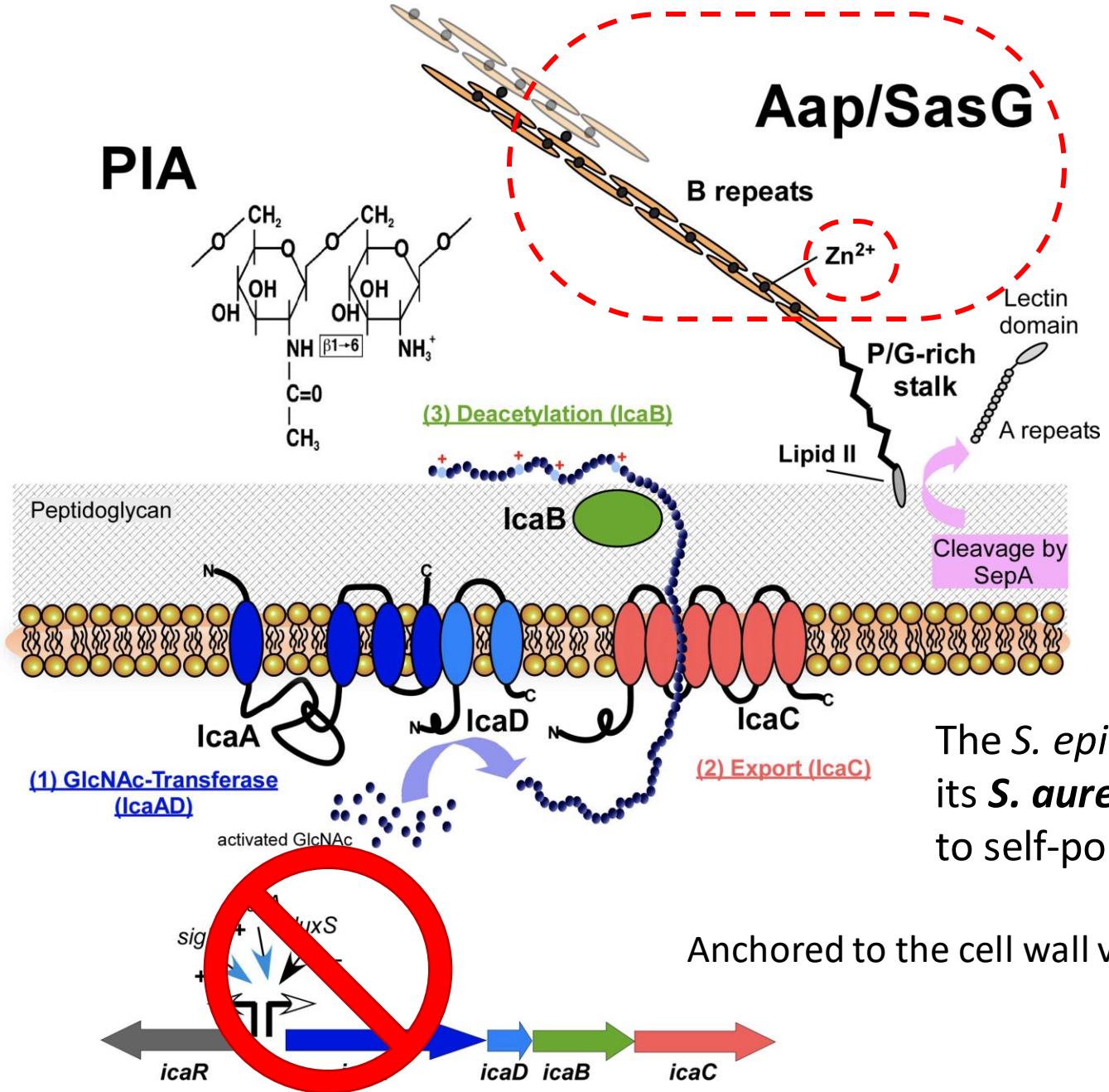
Staphylococci produce one main biofilm exopolysaccharide, which is called **polysaccharide intercellular adhesin (PIA)**, or according to its chemical composition, poly-Nacetylglucosamine (PNAG)

PIA biosynthesis is accomplished by the products of the ***ica* (intercellular adhesion) gene locus**, which comprises the *icaA, icaD, icaB*, and *icaC* genes and a divergently transcribed repressor, *icaR*



Alleviation of IcaR-mediated repression of *icaADBC* occurs **in response to environmental stimuli**

Staphylococcal biofilm matrix components



Biofilm formation can be accomplished by *S. epidermidis* or *S. aureus* isolates that do not harbor the *ica* locus

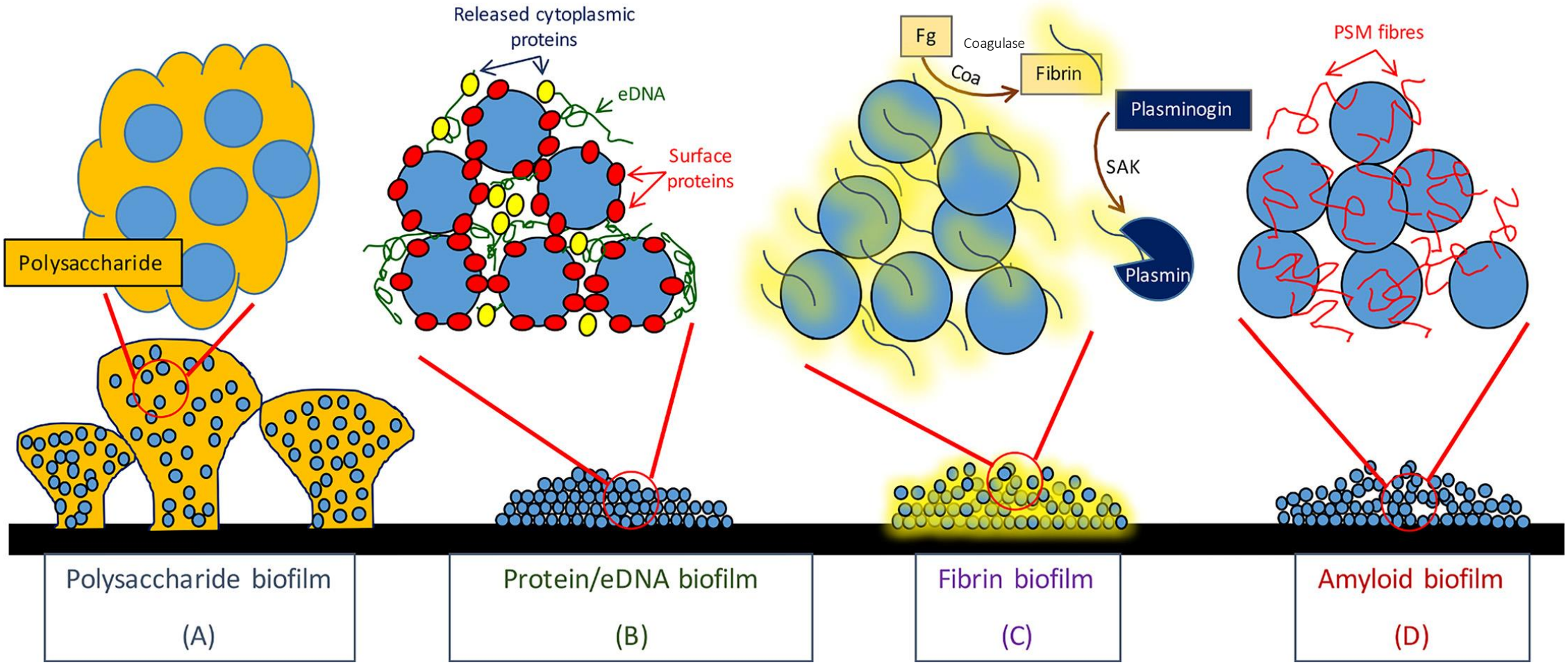
In *S. aureus* isolates, biofilm formation appears to be predominantly **protein-dependent** (amyloid-like fibril scaffolds)

The *S. epidermidis* **accumulation-associated protein (Aap)** and its ***S. aureus* homologue, SasG**, stand out due to their capacity to self-polymerize and form fibrils that interconnect cells

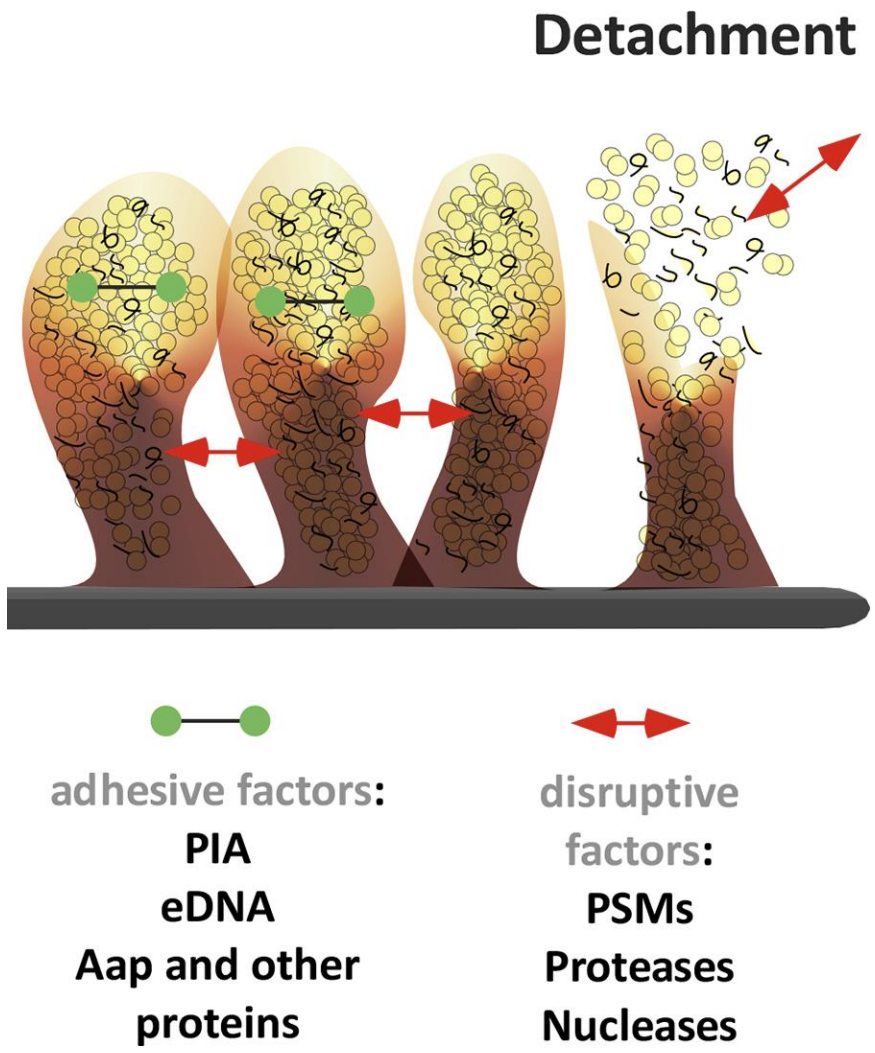
Anchored to the cell wall via sortase-catalyzed covalent linkage to lipid II.

The redundant mechanisms of *S. aureus* biofilm formation

"to stick to surfaces at all costs"



Structuring and Detachment



Biofilms do not grow as undifferentiated “bricks” but have a characteristic **three-dimensional** structure (“**mushroom**”-like, with fluid-filled **channels** between towers)

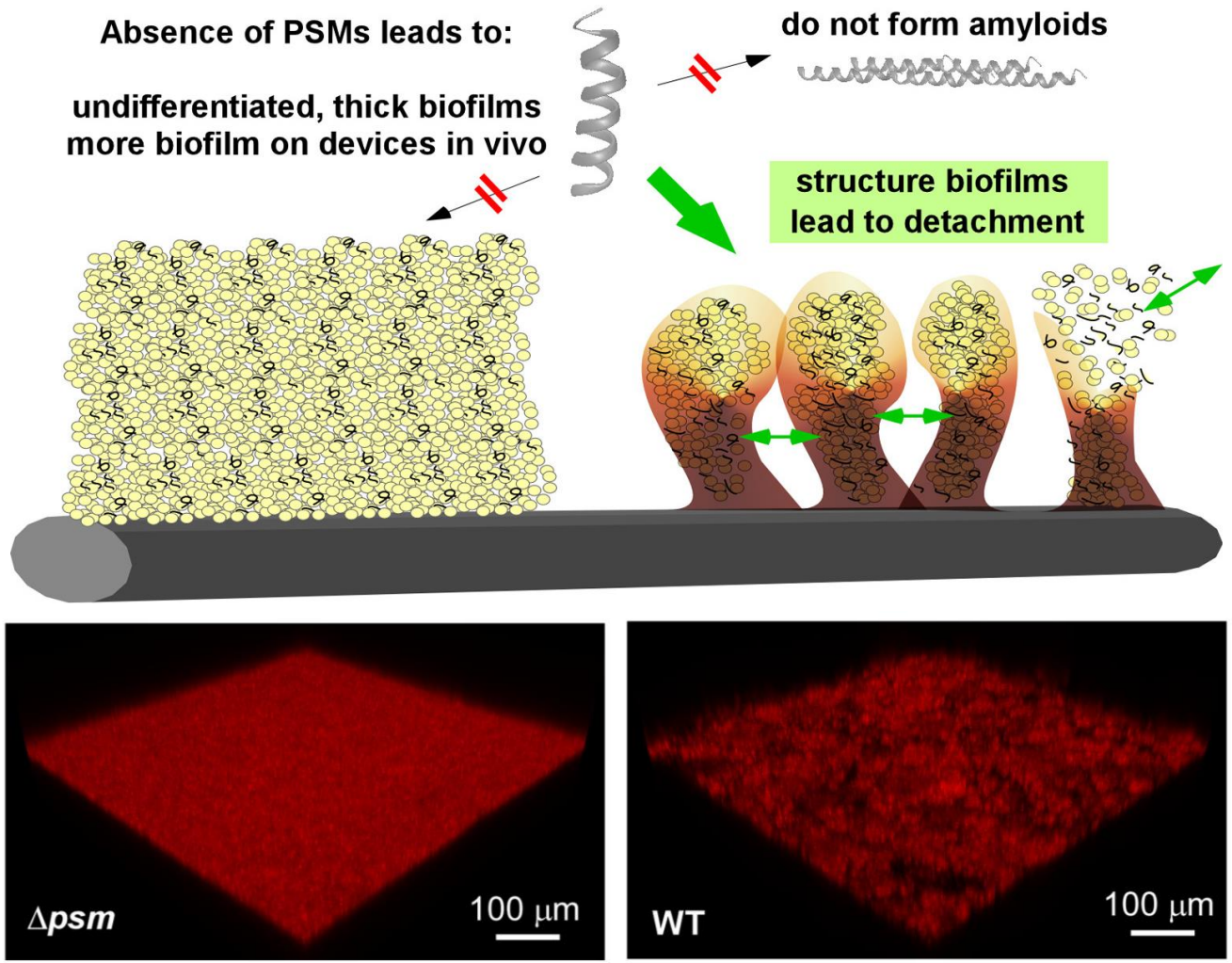
The second maturation stage of development comprises **disruptive forces** mediated by enzymes that degrade biofilm polymers, such as **nucleases** and **proteases**, and **surfactant-like molecules**, such as the staphylococcal phenol-soluble modulins (**PSMs**).

At high concentrations, **PSM peptides mediate lysis** of red blood cells, osteoblasts and leukocytes

Proteases are the most important biofilm-degrading enzymes

In the biofilm matrix eDNA is subject to degradation by **nucleases**

Role of PSM in *Staphylococcus epidermidis* biofilm



PSMs, are able to disrupt cellular interactions within biofilms, thereby loosening up the sticky biofilm agglomerations and introducing channels in the biofilm structure.

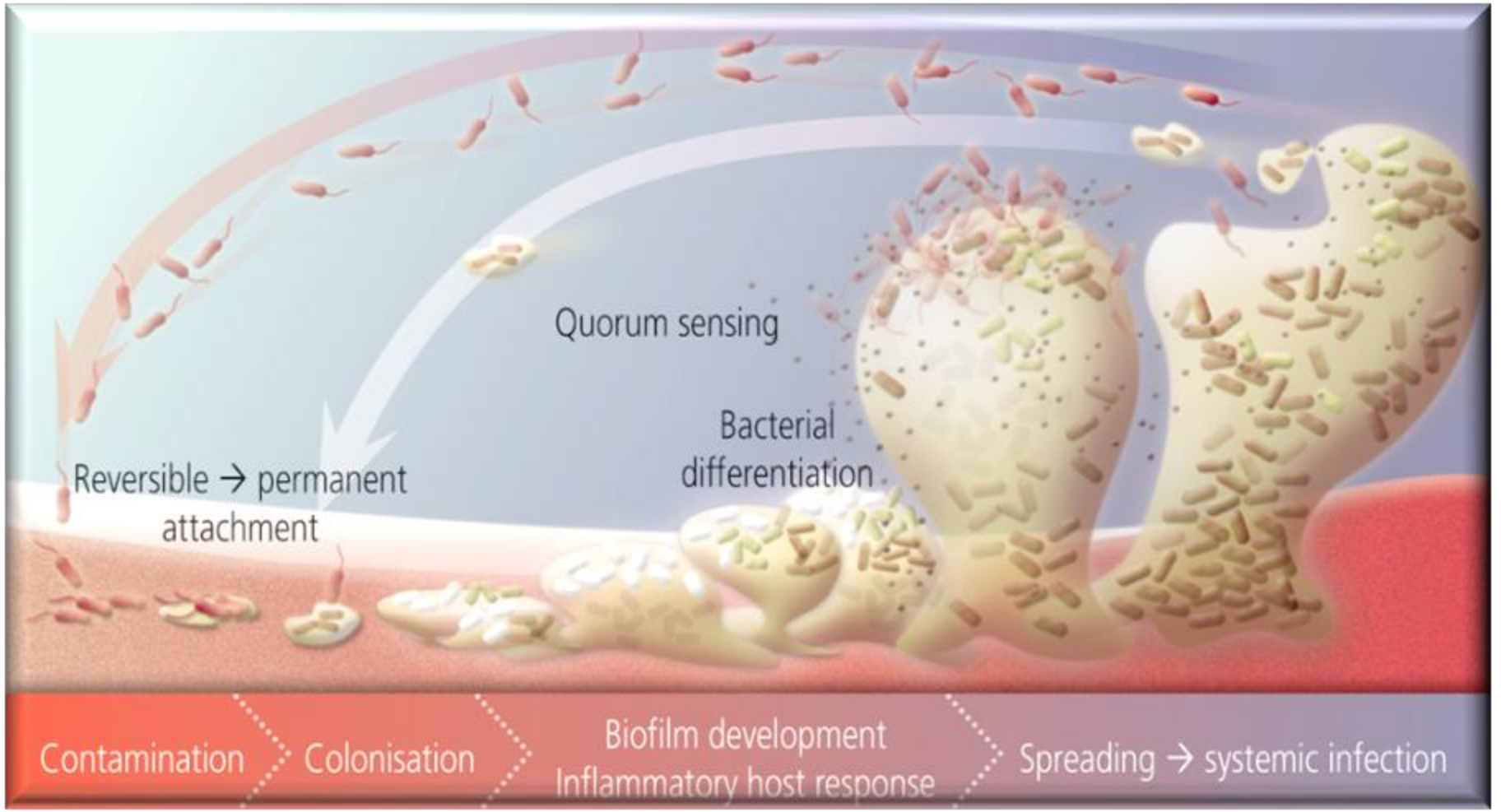
Such channels are vital components of biofilms, as they enable nutrients to be delivered to deeper biofilm layers, keeping all cells in the biofilm alive.

Non-uniform secretion of PSMs among biofilm cells is necessary to form channels

Upon strong production of PSMs at a given location in the biofilm, channels form; when this happens at a **high rate**, entire biofilm clusters may **detach**.

Social evolution in biofilms: life cycle

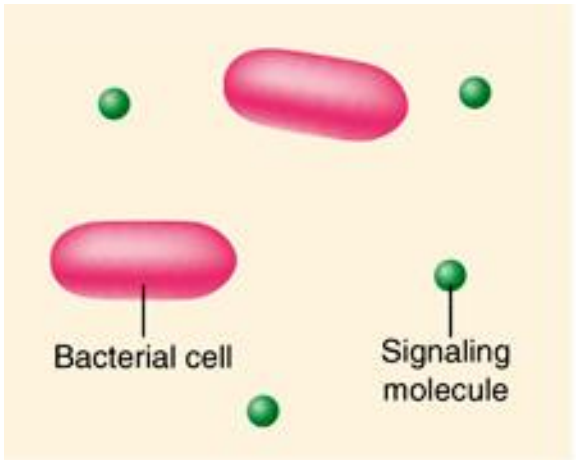
Biofilm formation enables single-cell organisms to assume a temporary multicellular lifestyle, in which “group behaviour” facilitates survival in adverse environments



Quorum Sensing the bacterial talk

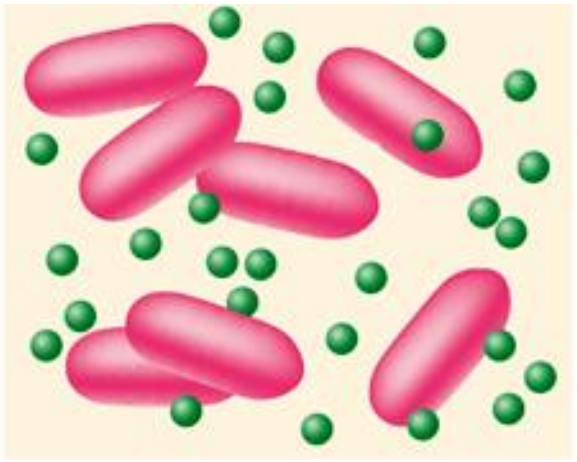
Quorum: in politics, this is the number of votes that must be cast for an election or referendum to be valid.

Low cell Density



Individual Behaviors

High cell Density

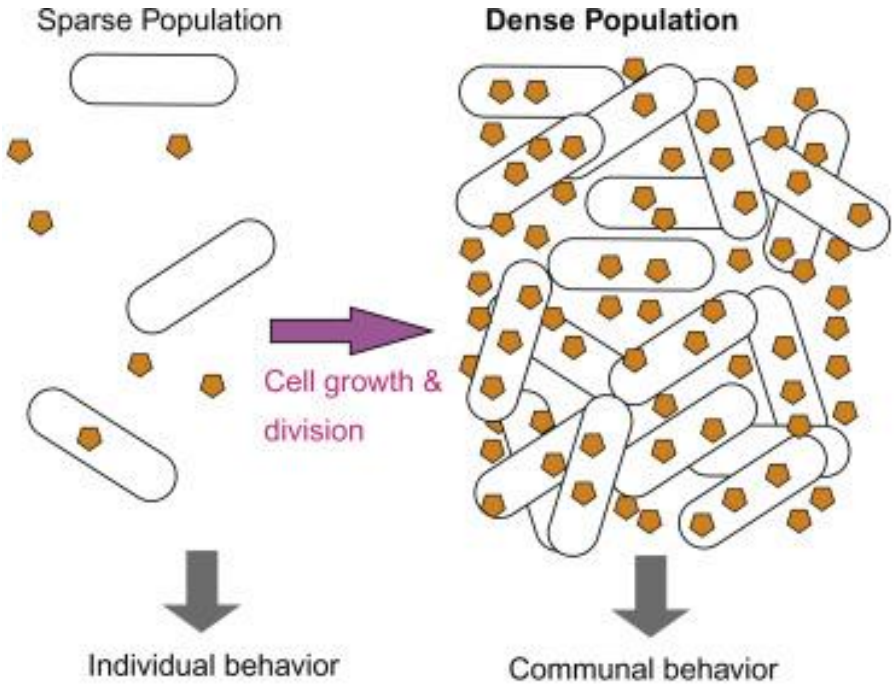


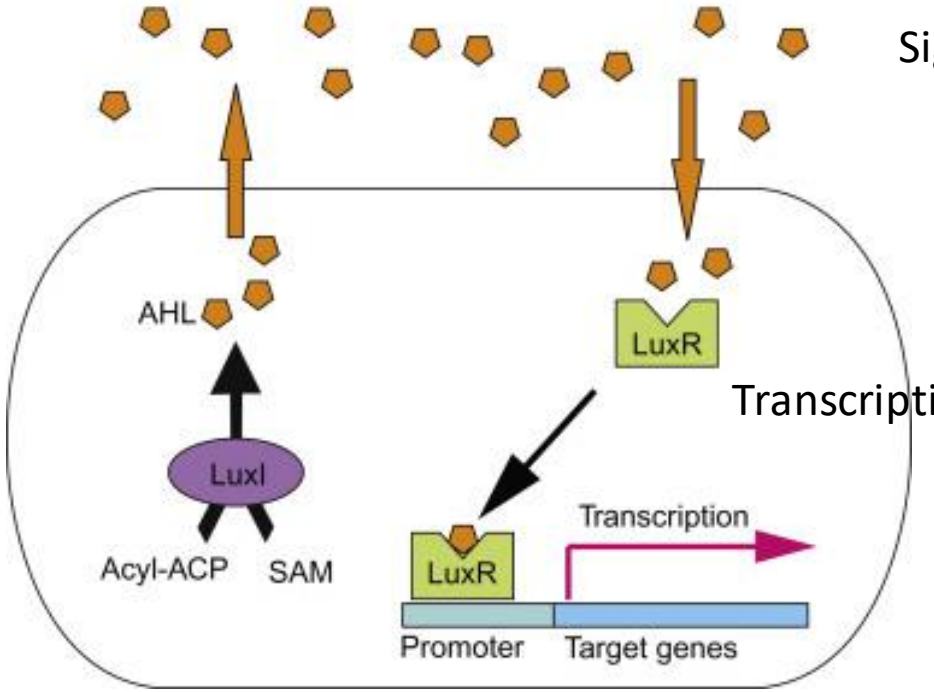
Group Behaviors

1. Cell to cell communication
2. Density dependent
3. Requires signalling molecules
4. Influences gene expression and bacterial behaviour

QS controls:
biofilm, exoenzymes, membrane vesicles, siderophores, induction of sporulation, swarming motility, and competence for horizontal gene transfer

Low cell concentration High cell concentration





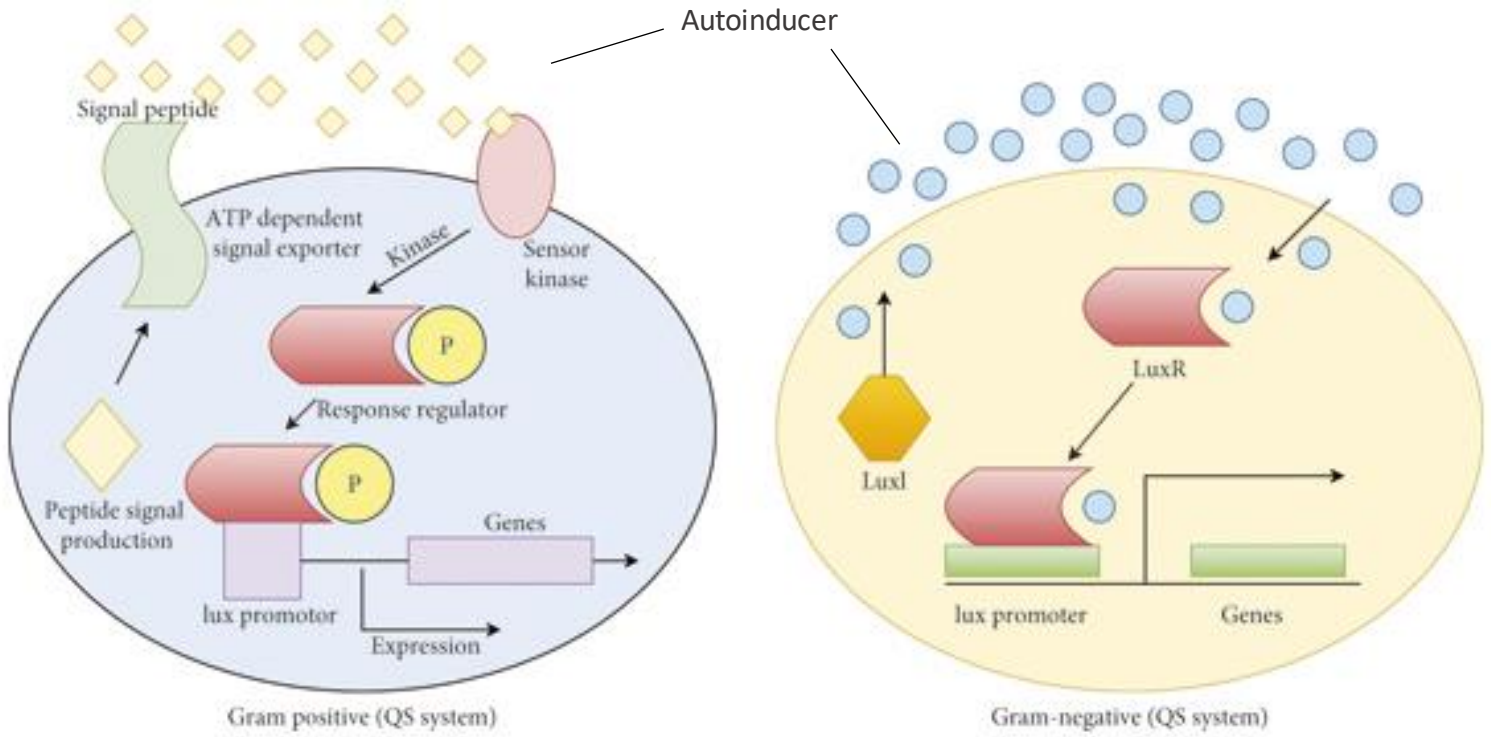
Signal molecule

Transcriptional activator

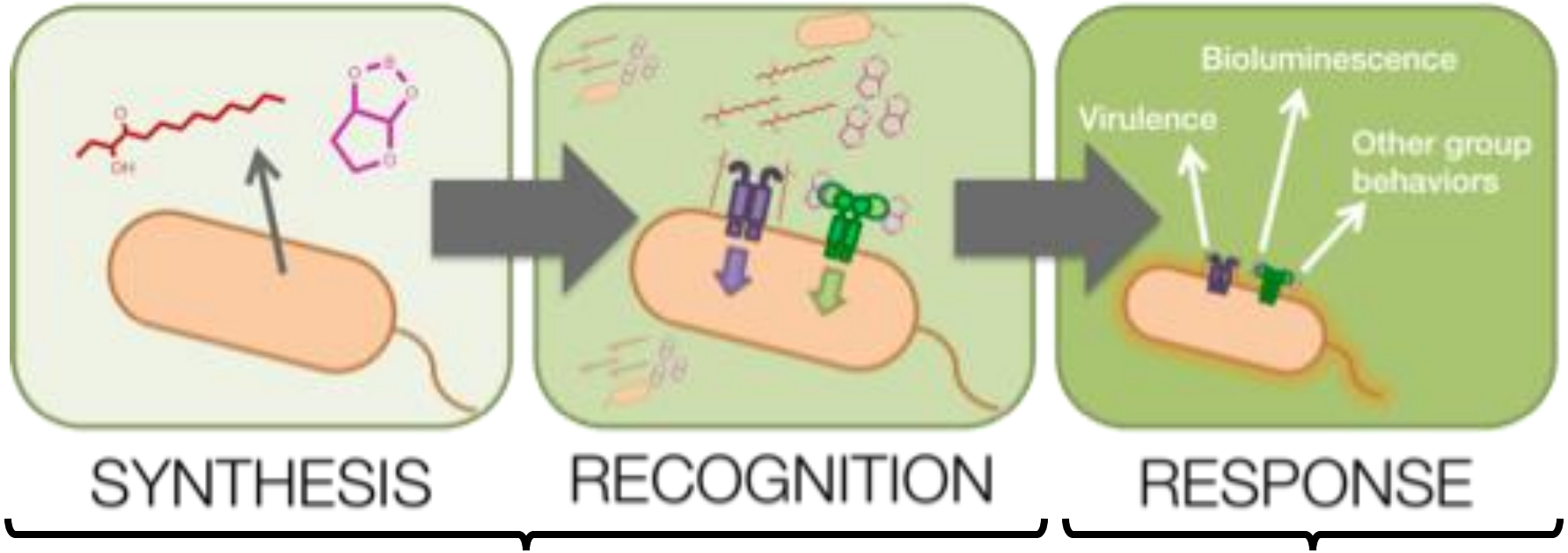
Target gene

QS: How bacteria can coordinate activity and synchronize their response to external signals

- Signaling molecules (Autoinducer
- Transcriptional activator
- Target genes



QS: three step approach



Signal molecules

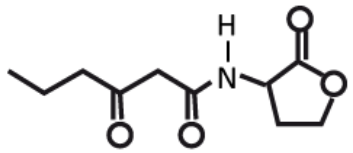
Alteration in gene expression – Biofilm formation

Bacterial QS relies on:
(1) networks of autoinducers,
(2) autoinducer synthases,
(3) partner autoinducer receptors
(4) downstream signal transduction components that convert the information contained in autoinducers into changes in gene expression.

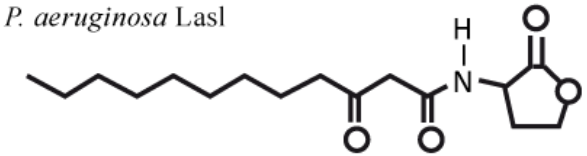
The languages of bacteria

A Acyl-Homoserine Lactone Autoinducers

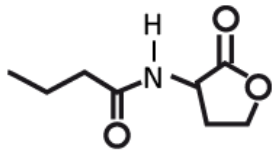
V. fischeri LuxI



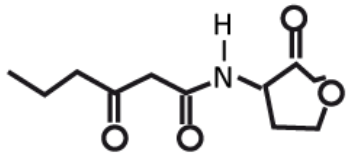
P. aeruginosa LasI



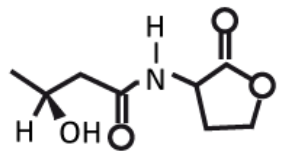
P. aeruginosa RhlI



P. aeruginosa EsaI



V. harveyil LuxM



B Oligopeptide Autoinducers

B. subtilis/ComX — ADPITRO*WGD

B. subtilis/CSF — ERGMT

S. aurous 'subgroup 1 — YSTCDFIM, B—C=o

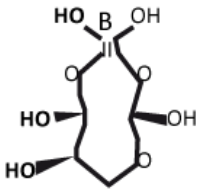
S. aurous 'subgroup 2 — GVNACSSLF, S—C=o

S. aurous 'subgroup 3 — YINCDFILL, S—C=o

S. aurous 'subgroup 4 — YSTCYFIM, S—C=o

C AI-2

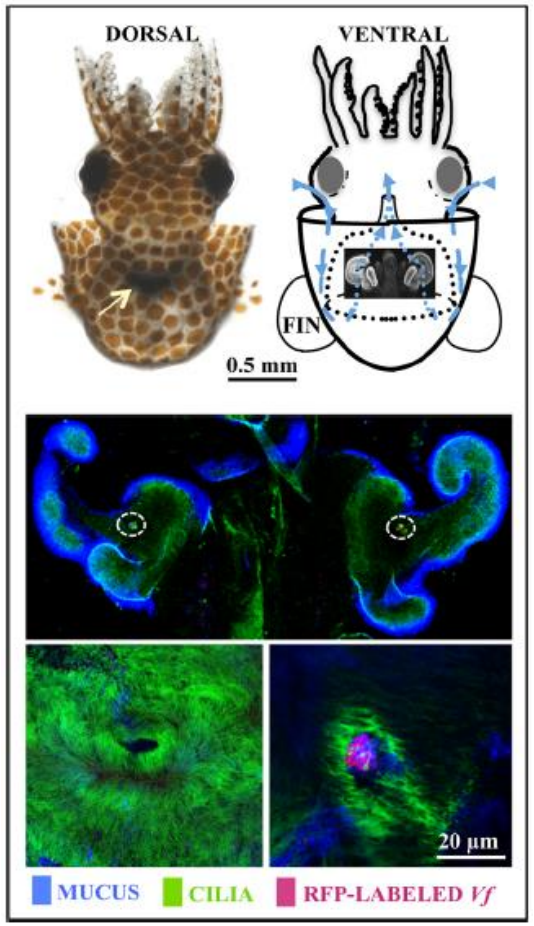
V. harveyil LuxS



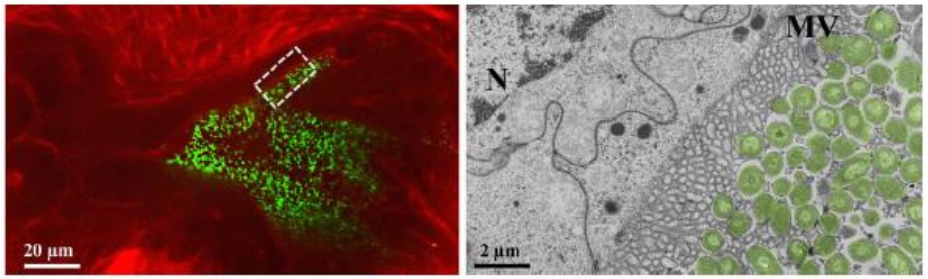
Vibrio fischeri and bioluminescence

In 1970, the first QS mechanism was observed in *V. fischeri*

The Hawaiian squid *Euprymna scolopes*



The nascent light organ (black) can be seen through the body wall (white arrow) of the living juvenile animal

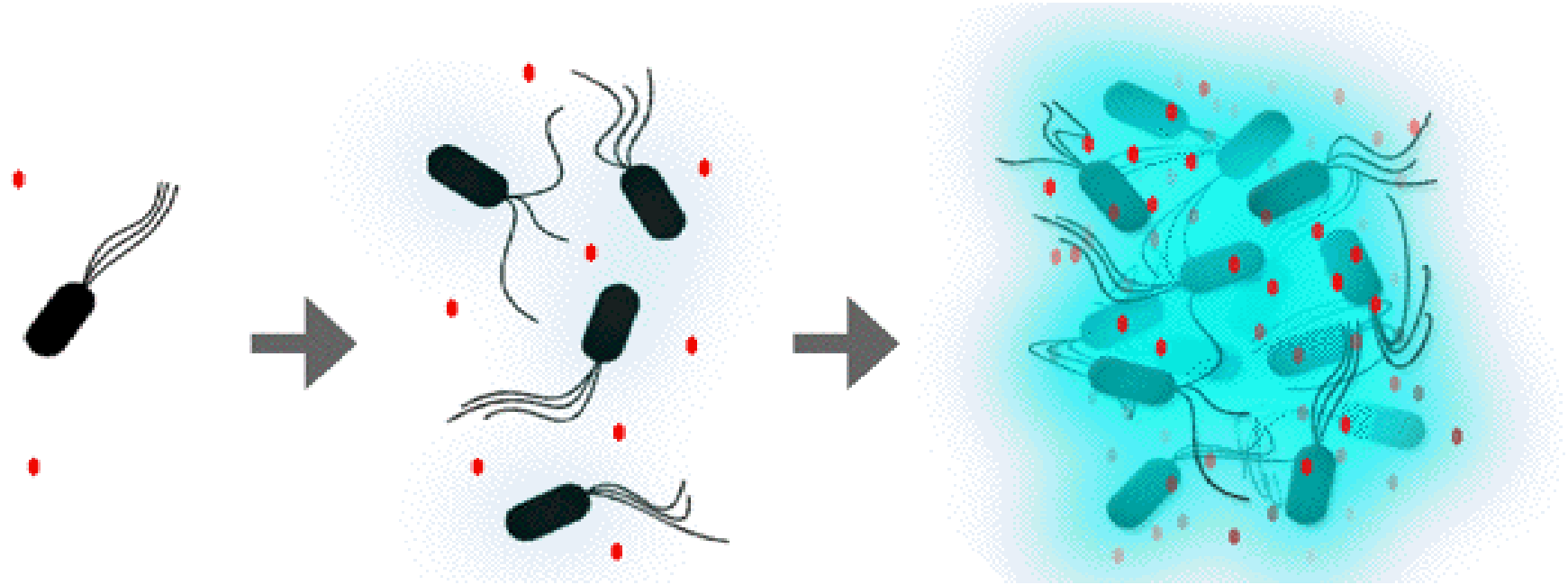


V. fischeri cells in a host crypt and associating with microvilli along the apical surfaces of host epithelia

V. fischeri

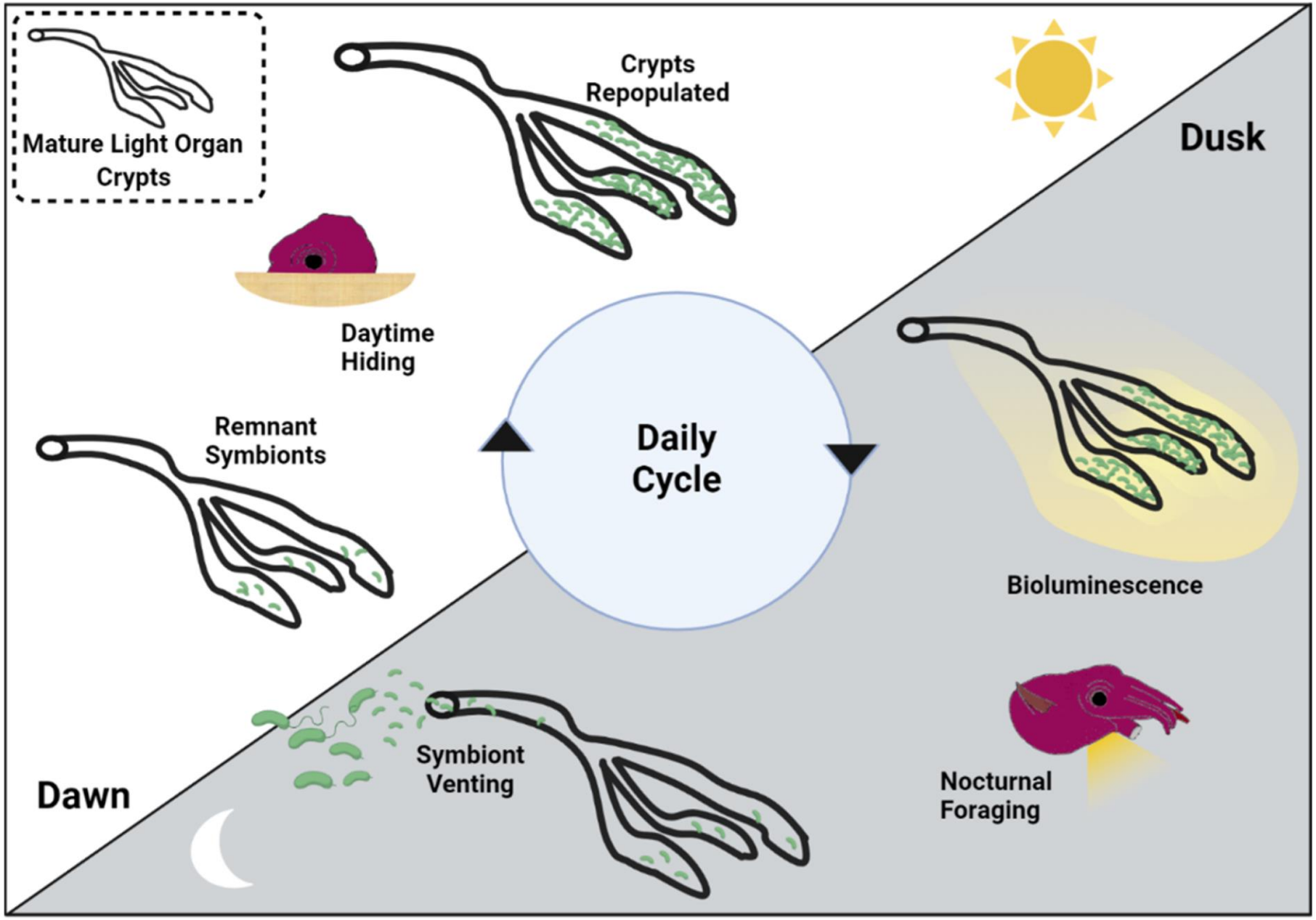
V. fischeri cells in the ocean colonize the light organs of juvenile squid and fish

Ciliated cells within the animals' photophores selectively promote the growth of the *V. fischeri* cells and actively reject any competitors.

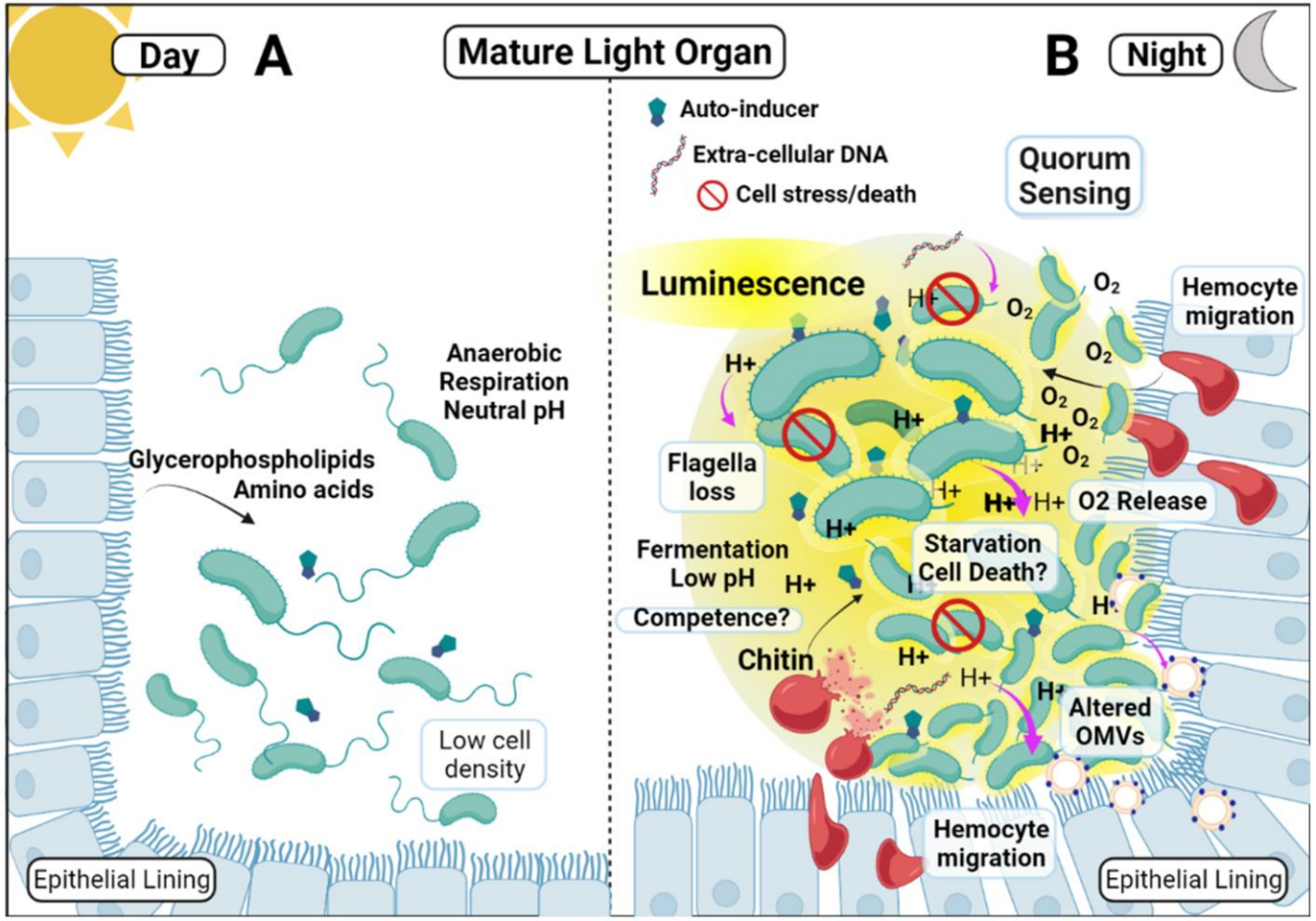


The circadian rhythm controls light expression

The life-cycle of *V. fischeri*



Nocturnal acidification of the mature light organ as a central cue coordinating many of the regulatory signal networks that maintain the symbiosis



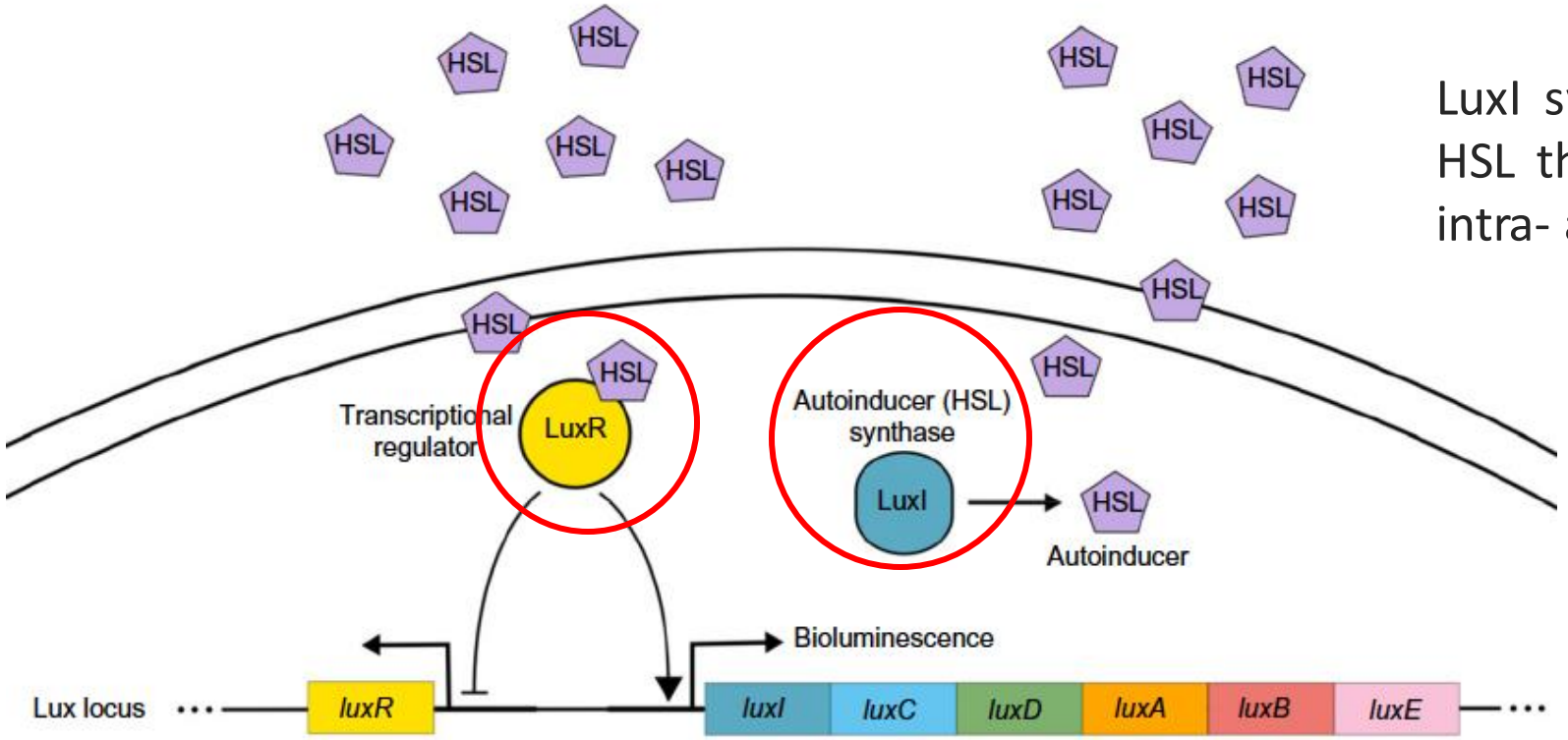
(A) Host-provided nutrients are metabolized through anaerobic respiration with no lowering of matrix pH. Flagella may be present, having been induced just prior to the **dawn venting**.

(B) Acidification-cued interactions present at the end of the night.
Fermentation of **host-provided chitin** lowers the matrix pH.

Flagella loss

Quorum sensing in *V. fischeri*

Acetyl homoserine lactones (HSL)



LuxI synthesizes the signaling molecule (AI) HSL that can passively diffuse between the intra- and extracellular environment

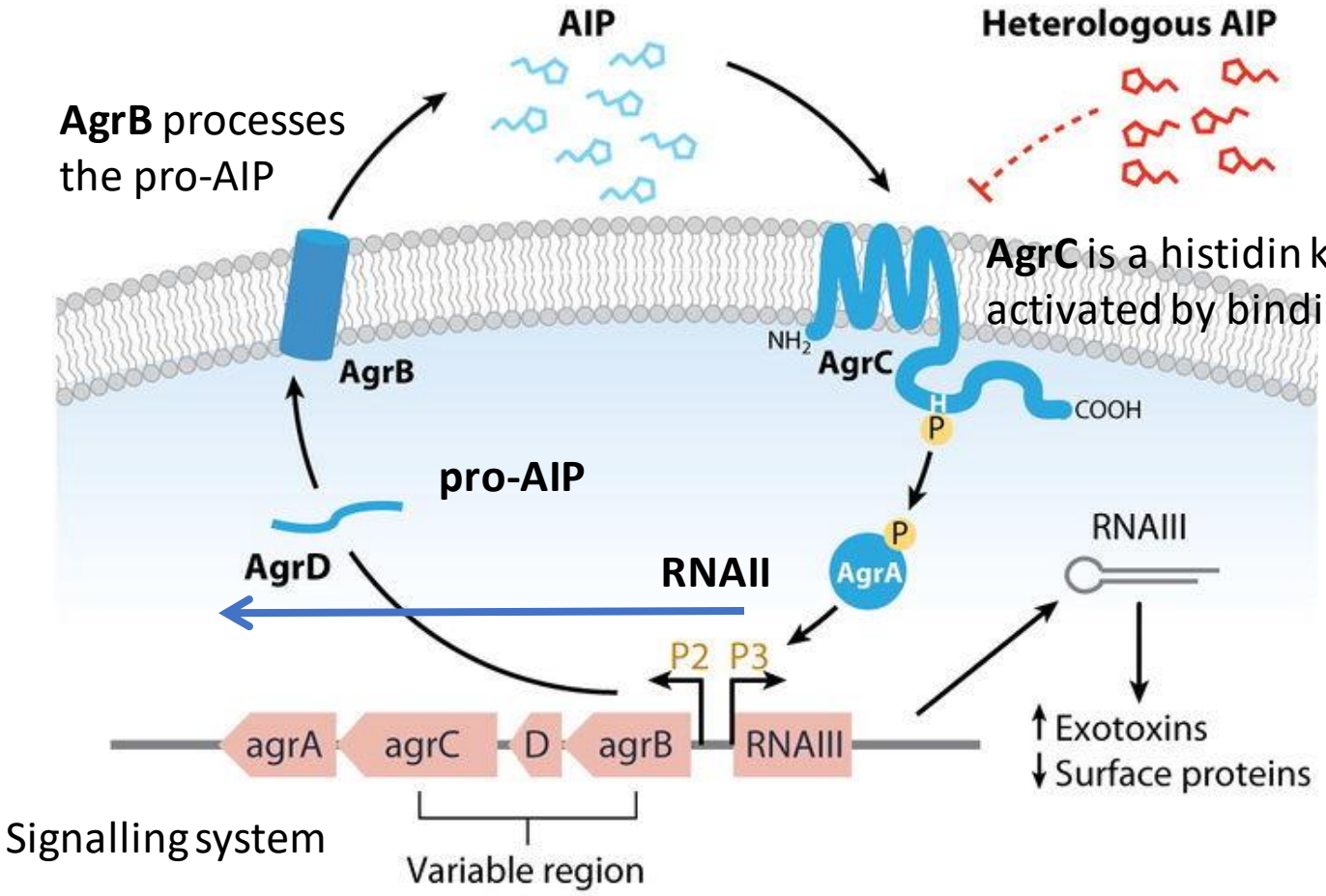
When a concentration threshold is reached, HSL binds to the intracellular transcriptional regulator LuxR.

Luciferase operon

The LuxR–HSL complex not only activates the *luxICDABE* operon but also represses the transcription of *luxR* by binding to the *luxR* promoter

S. aureus accessory gene regulatory (Agr) system

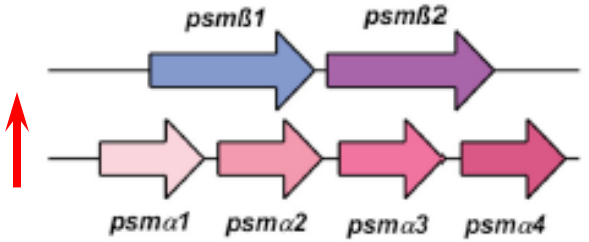
In Gram+ AIP must be actively transported through their peptidoglycan cell wall using an ATP-binding cassette (ABC) transporter system.



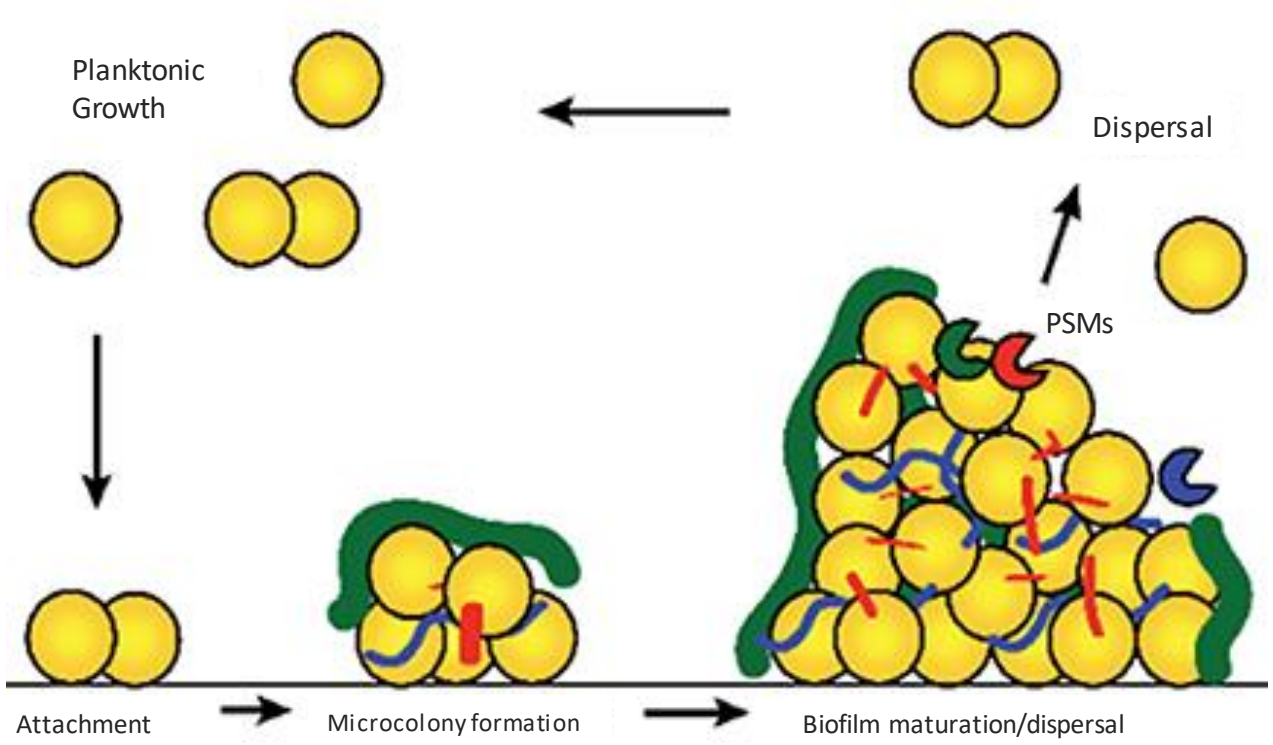
Agr contributes to *S. aureus* pathogenicity in several infection types

AgrC is a histidin kinase that is activated by binding AIP

Phenol Soluble Modulins (PSMs)



Biofilm dispersal strategy utilized by *S. aureus*

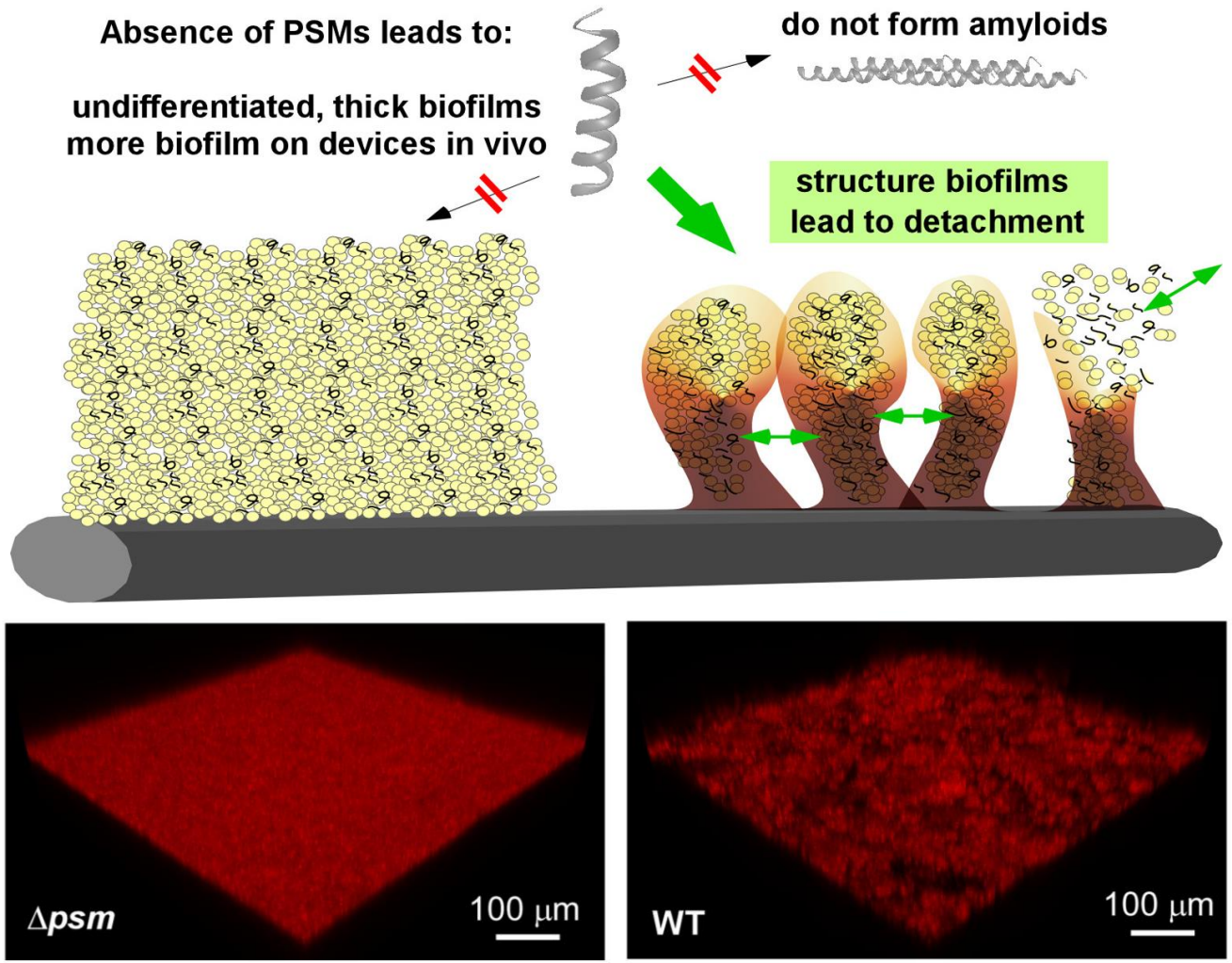


The Phenol Soluble Modulins (PSMs), have the potential to destroy immune cells (cytolytic activity), thereby contributing to the immune evasion capacity of *S. aureus*.

PSMs degrade the extracellular polymeric matrix.

PSMs allow the detachment of biofilm clusters *in vitro* and dissemination from biofilms on indwelling devices *in vivo.*

Role of PSM in *Staphylococcus epidermidis* biofilm



PSMs, are able to disrupt cellular interactions within biofilms, thereby loosening up the sticky biofilm agglomerations and introducing channels in the biofilm structure.

Such channels are vital components of biofilms, as they enable nutrients to be delivered to deeper biofilm layers, keeping all cells in the biofilm alive.

Non-uniform secretion of PSMs among biofilm cells is necessary to form channels

Upon strong production of PSMs at a given location in the biofilm, channels form; when this happens at a **high rate**, entire biofilm clusters may **detach**.

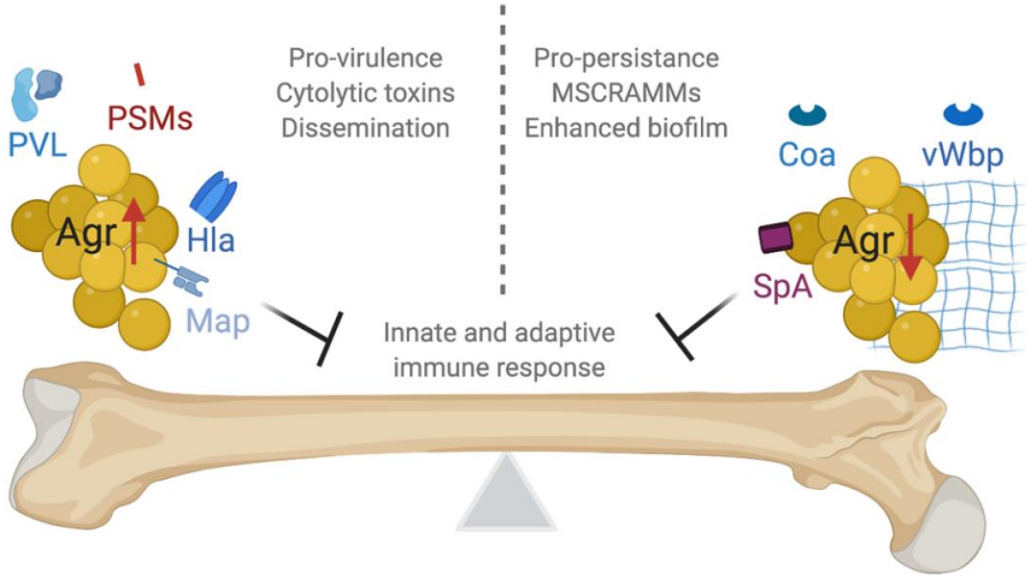
The Agr System has paradoxical effects during infection

Agr controls expression of toxins that increase *S. aureus* pathogenicity and disease severity; however, inactivating agr mutations often occur in *S. aureus* clinical isolates.

Agr activation results in repression of many microbial surface components (MSCRAMMs) that facilitate bacterial adherence. Agr also regulates production of proteases (PMSs) that degrade adhesion proteins including MSCRAMMs.

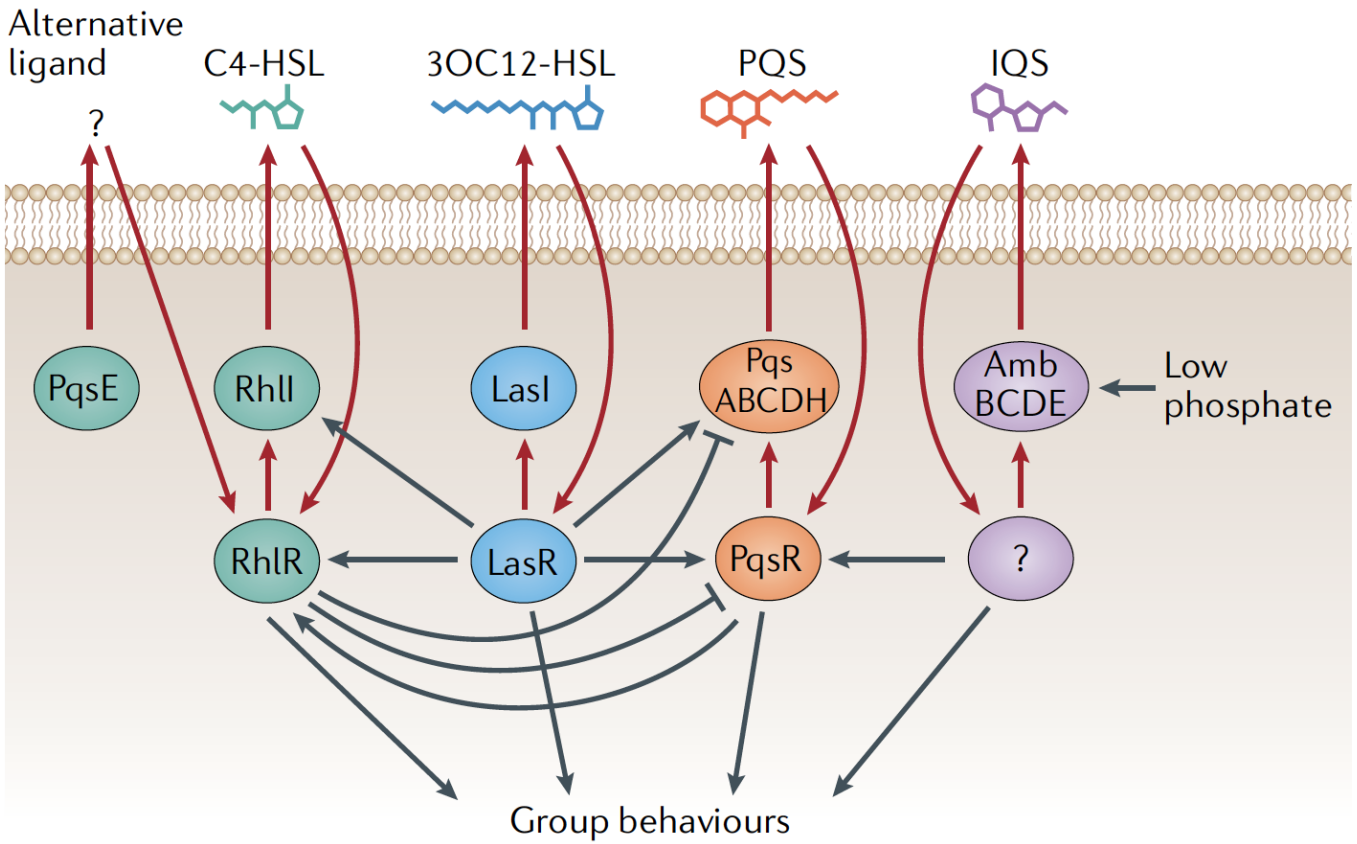
Agr-negative strains exhibit a fitness **advantage under antibiotic stress** and have been associated with greater rates of mortality and duration of bacteremia.

Agr expressing *S. aureus* strains **can disseminate** from medical devices and distant sites to bone

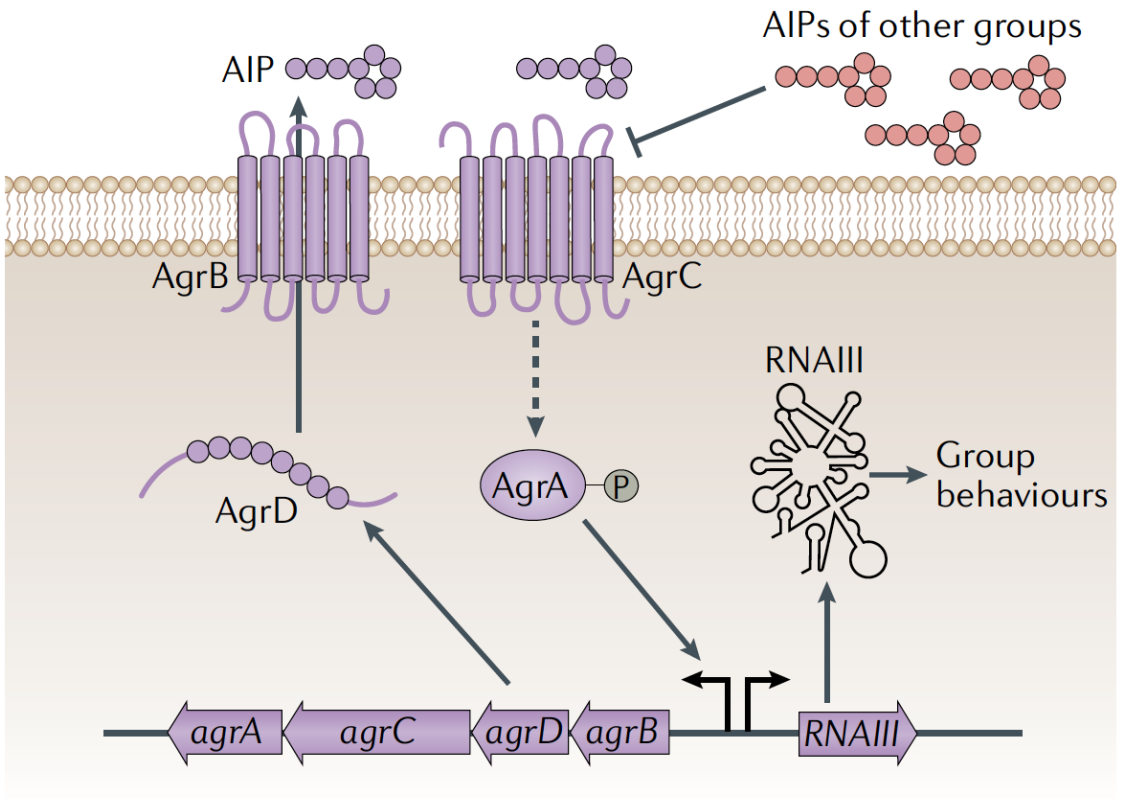


[Butrico 2020]

Pseudomonas aeruginosa



Staphylococcus aureus



Why is so difficult to treat biofilm-growing microorganisms?

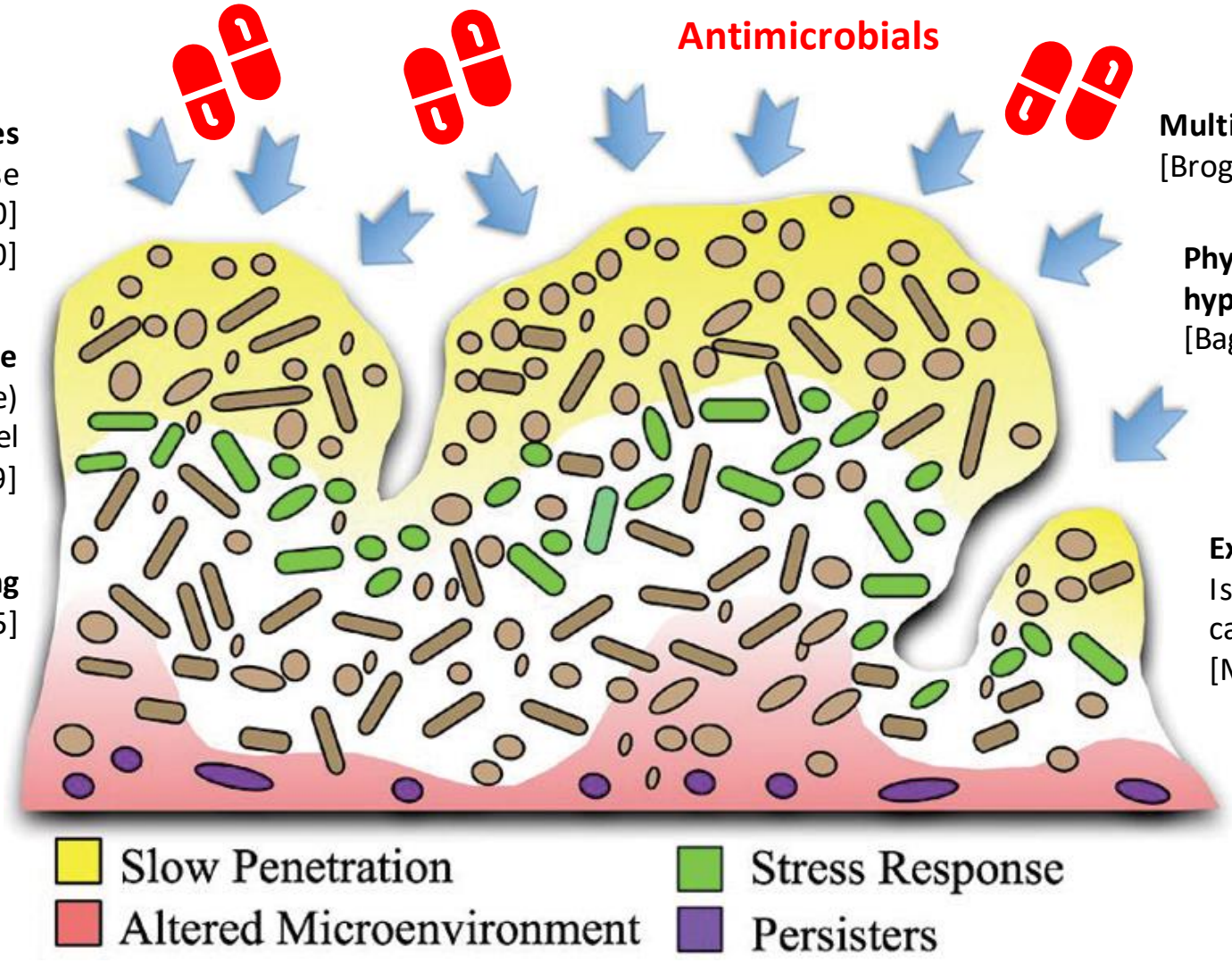
Bacteria living in biofilms can be up to 1,000 times more tolerant to antibacterial compounds than their planktonic counterparts

Antibiotic-modifying enzymes
AmpC β-lactamase
[Anderl et al. 2000]
[Bagge et al. 2000]

Exopolysaccharide
(D-glucose, D-mannose and L-rhamnose) and the electrostatic sequestration model
[Byrd et al. 2009]

Quorum sensing
[Bjarnsholt et al. 2005]

'Persister cells'
[Broun, Liu and Lewis 2000]



Multispecies interactions
[Brogden et al. 2005]

Physiological heterogeneity, hypoxia and reduced growth rate
[Bagge et al. 2004]

Horizontal gene transfer
[Savage et al. 2013]

Extracellular DNA
Is an anionic macromolecule, it can chelate cations
[Mulcahy et al. 2008]

Distinguishing between resistance, tolerance and persistence

Resistance

The genetic ability to counteract antimicrobial treatments.
Resistance is quantified by the minimum inhibitory concentration (MIC)

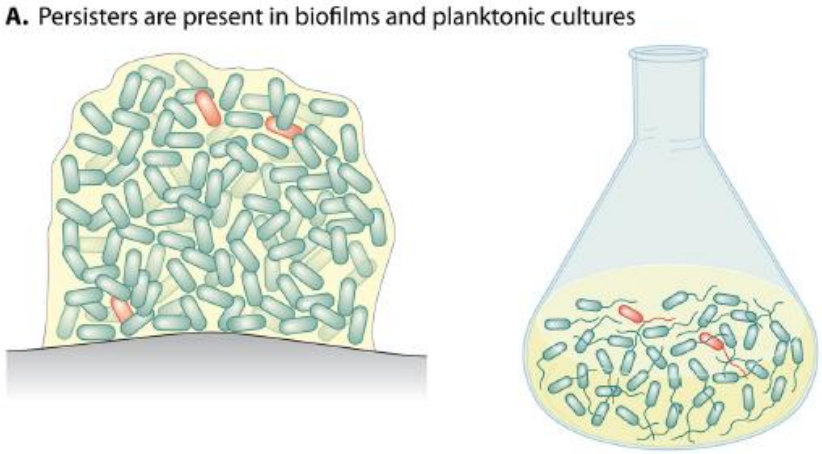
Tolerance

The ability to evade antimicrobial treatments of genetically susceptible microorganisms

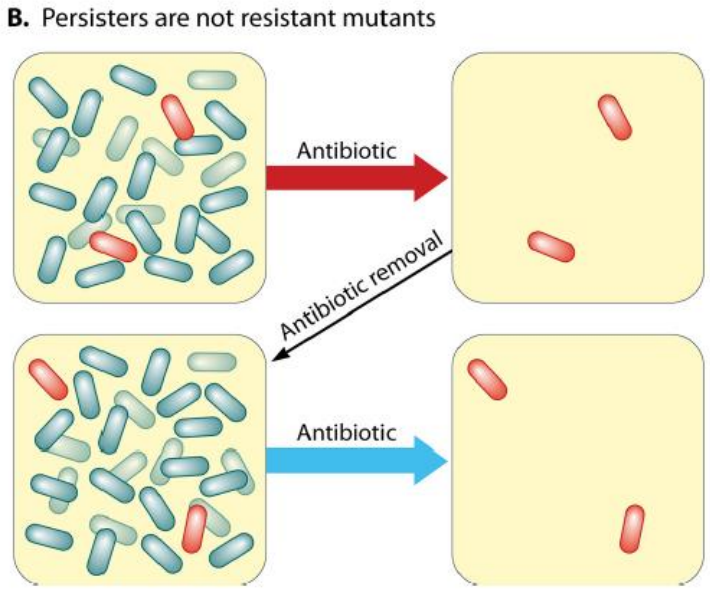
Persistence

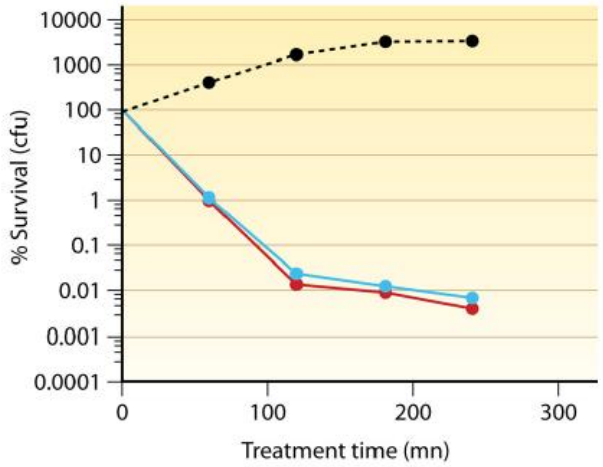
Prolongs the duration of treatment that bacteria can sustain only for a subpopulation

Bacterial Persister Cells



Persisters are present under planktonic and biofilm conditions and account for only a small subset of the whole population (0.001% to 0.1%).





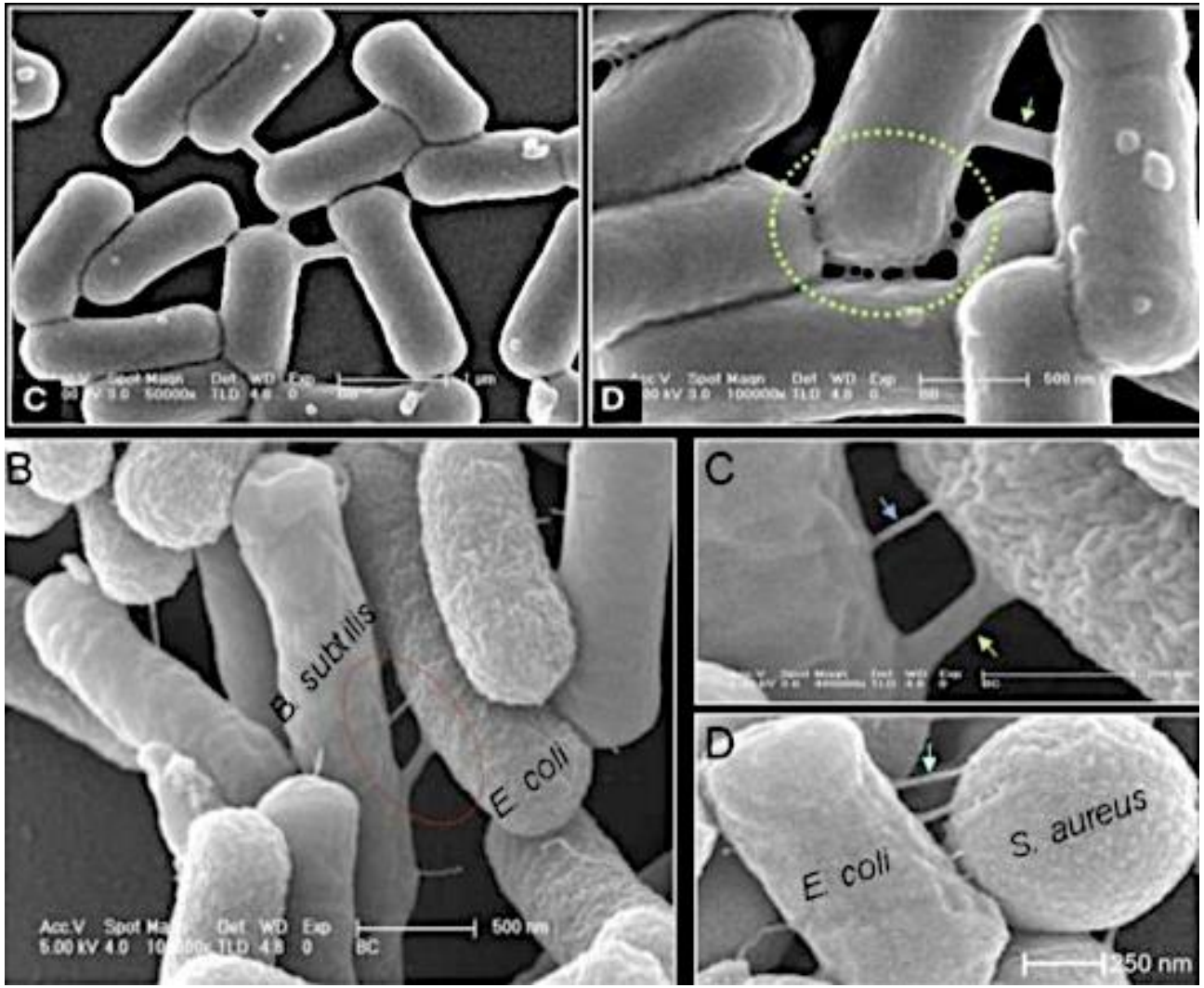
The reduced growth rates of persister cells in biofilm are the major reasons for the reduced susceptibility of biofilms to antibiotics

Biofilm production and multidrug resistance (MDR)

Patients suffering from biofilm-related infections are also exposed to nosocomial microorganisms present in their health care environment and selected by repeated antibiotic treatments.

Treatment of biofilm-related infections is difficult, not only due to biofilm recalcitrance toward antibiotics but also due to potential infection by MDR carrying resistance genes

Horizontal gene transfer within biofilm

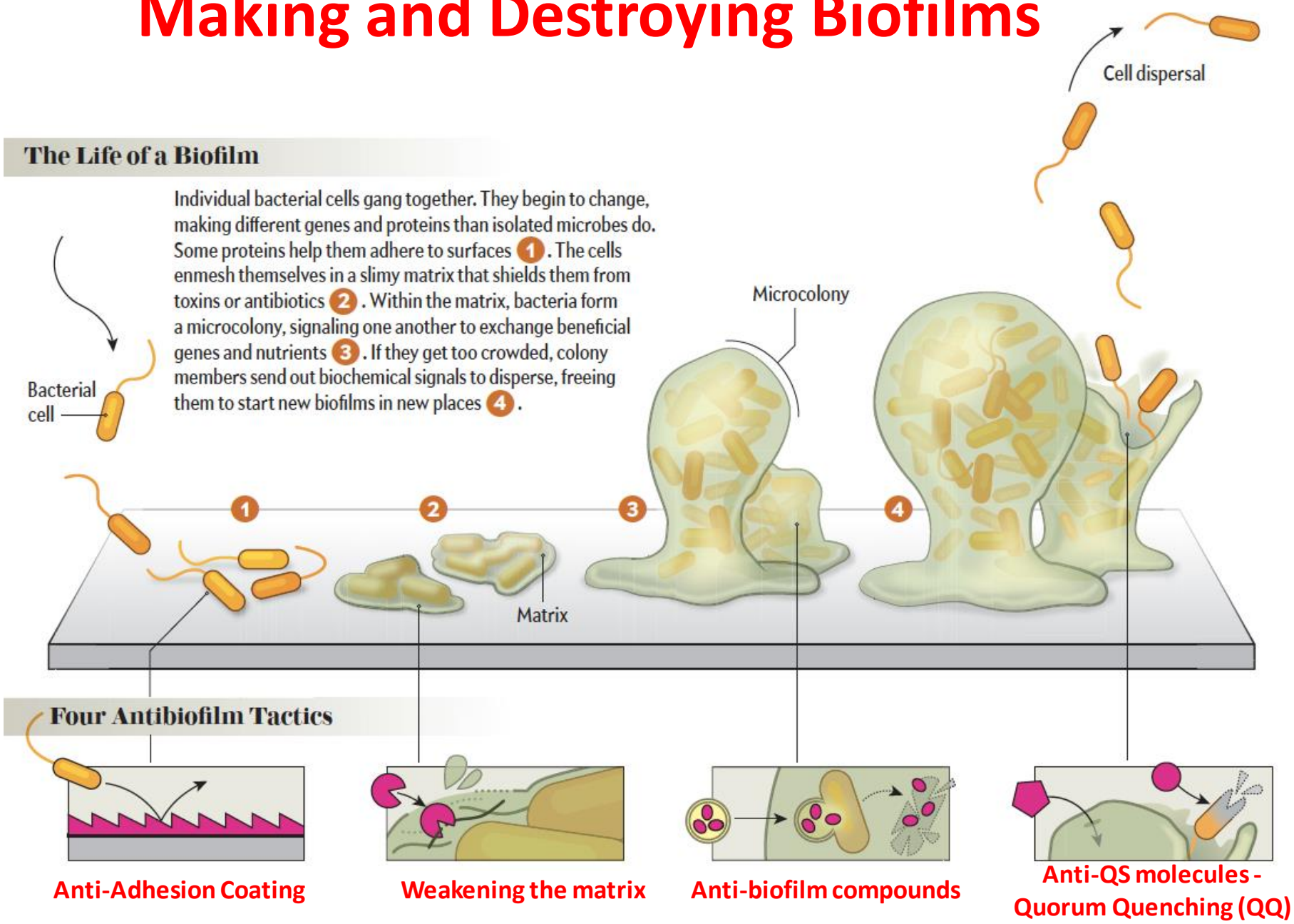


Horizontal gene transfer: conjugation, transformation, transduction are increased in biofilms

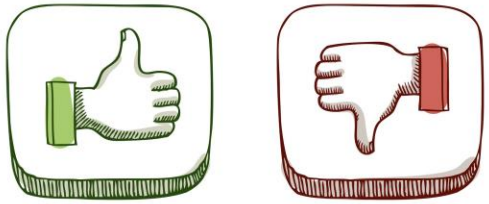
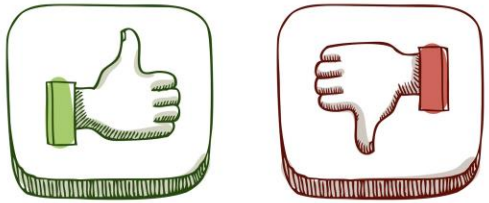
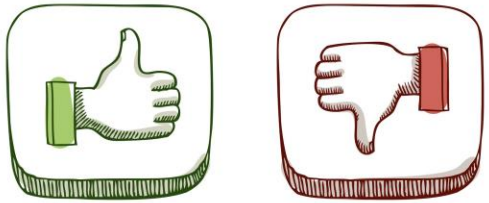
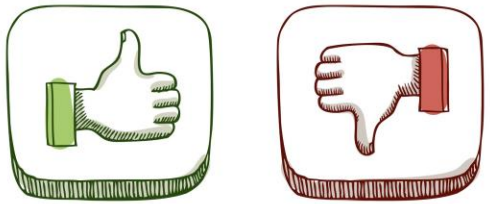
The biofilm lifestyle also increases plasmid stability and the range of mobile genetic elements

Which treatment works best against biofilms?

Making and Destroying Biofilms

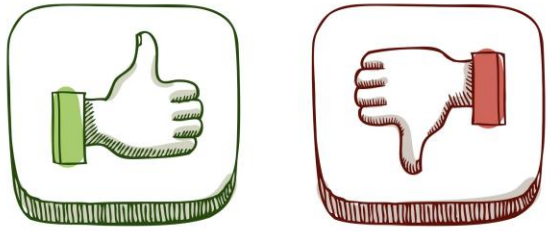


Bactericidal and Anti-Adhesion Coating

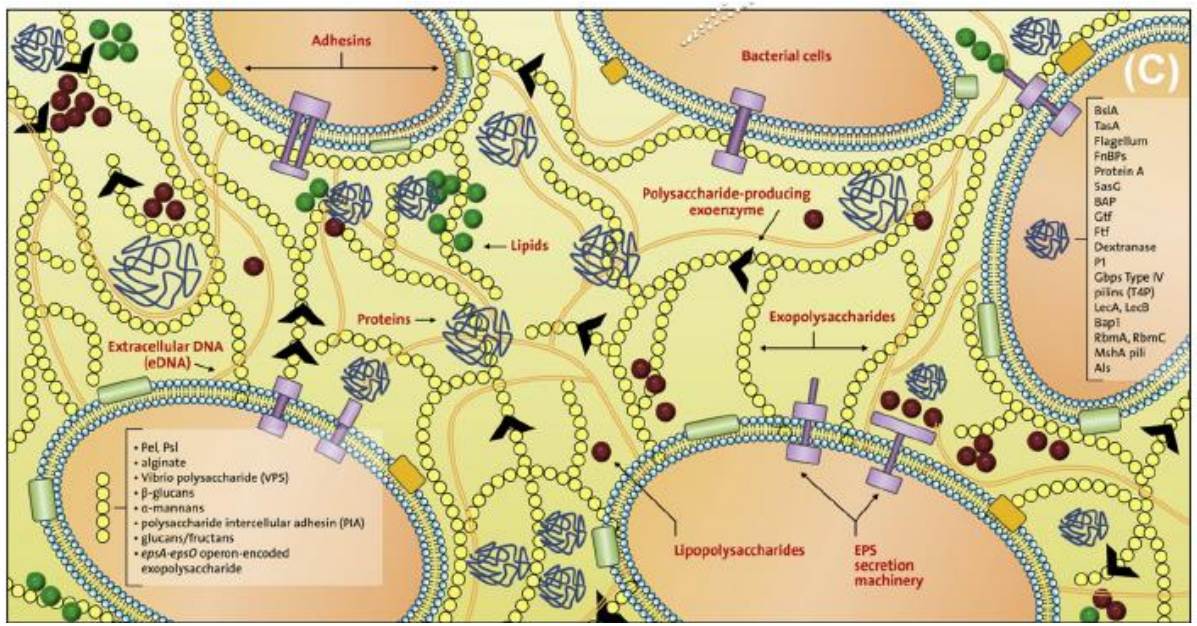
Silver coated biomaterials		• Increased risk of thrombosis • Toxicity
Vancomycin or Gentamicin on titanium implant		• Increased antibiotic resistance
Chlorhexidine–silver sulfadiazine or minocycline–rifampicin CVCs reduce colonization and CRBSI		• The cost effectiveness of these catheters • Increased antibiotic resistance
Surface roughness of biomaterials or increased hydrophobicity		• The cost effectiveness

Weakening the matrix

Proteinase and DNAse

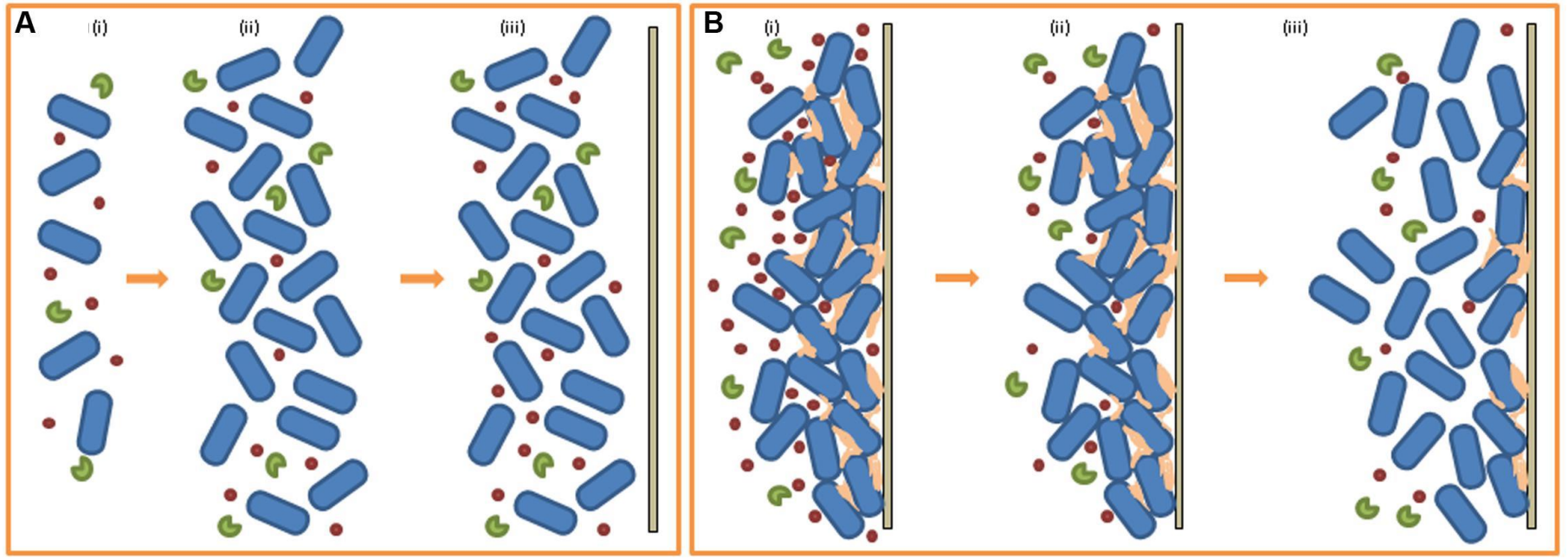


Highly toxic compounds



Anti-Quorum sensing strategies

Quorum quenching (QQ) is the process of preventing QS by disrupting the signaling



Since QS regulates many virulence traits, there is a belief that inhibition of QS activity (QQ) will reduce pathogenicity and promoting microbial eradication

Antibiotics as QS inhibitors

In the 1980s it was recognized that treatment of diffuse panbronchiolitis (a biofilm-associated disease of the lung) with **macrolides**, such as **erythromycin**, was beneficial in long-term disease prognosis and survival

Similar findings were reported for cystic fibrosis patients infected with *P. aeruginosa*, where improved lung function in children was seen following six to 15 months of **azithromycin** treatment

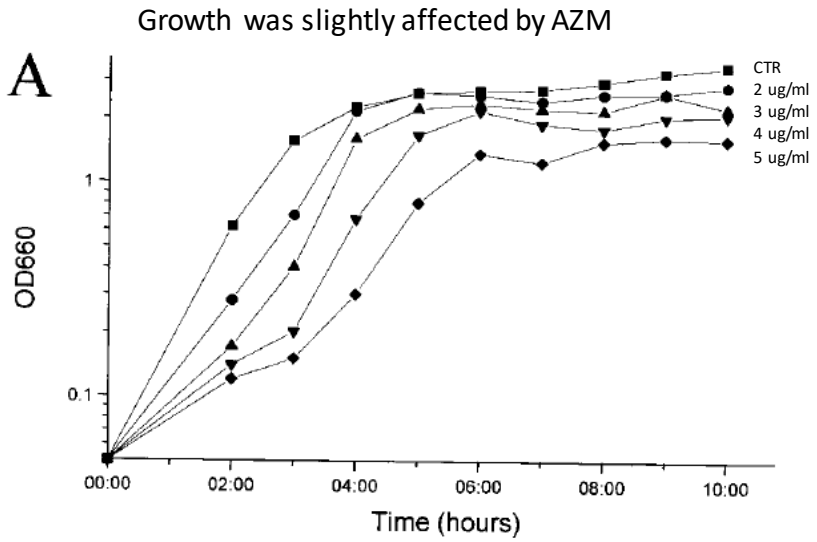
Erythromycin treatment reduced HSL (Autoinducer) production of more than 70% in PAO1 treated with subinhibitory drug concentrations

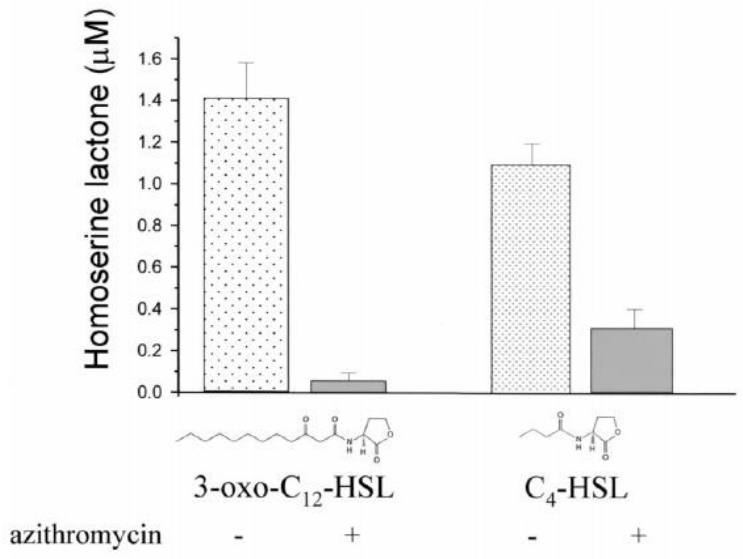
Cephalosporin (ceftazidime) and a fluoroquinolone (ciprofloxacin) were also able to inhibit HSL production in *P. aeruginosa*

Azithromycin (AZM) Inhibits QS in *P. aeruginosa*

AZM is a macrolides that binds to the 23S rRNA in the 50S ribosomal subunit, blocking the peptide exit channel

AZM improved the clinical outcome of CF patients infected with *P. aeruginosa*.
The highest clinically-achievable concentration of AZM was below the MIC for *P. aeruginosa*, raising the question of why AZM exhibits therapeutic activity



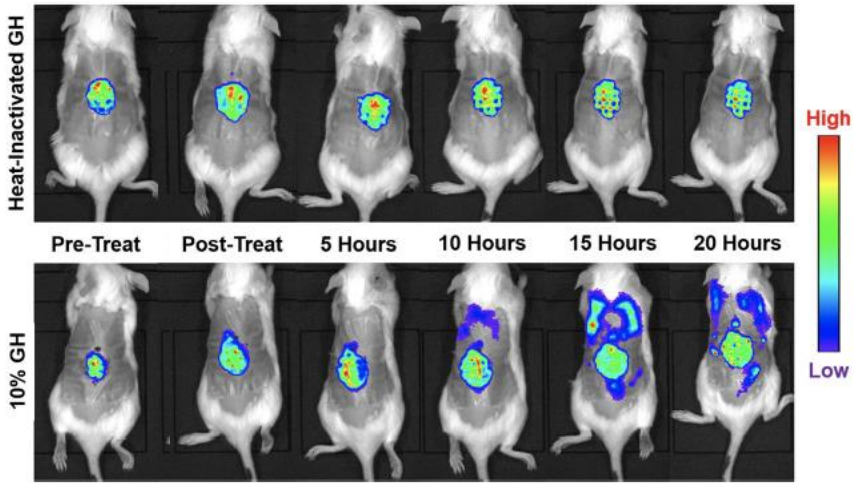


Structurally-unrelated antibiotics, including the β-lactam, ceftazidime and the fluoroquinolone, ciprofloxacin also strongly impinge upon QS

[Tateda 2001]

The consequences of biofilm dispersal on the host

Agents that can degrade biofilms are being pursued for clinical applications

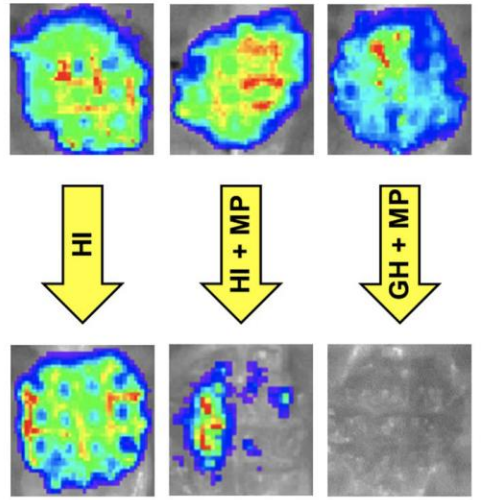


In vivo dispersal triggered by **glycoside hydrolase (GH)** therapy.

Treatment of 48-hour-old mouse chronic wounds, infected with bioluminescent ***P. aeruginosa***, with 10% GH, or heat-inactivated control, resulted in dispersal and systemic spread of the infection.

Clear localization of bacteria in other organs can be seen in the treated group.

1. GH disperse biofilms *in vivo*, but **cause rapid septicaemia**
2. Dispersal-mediated septicaemia is dependent upon swimming-motility
3. Antibiotics protect against dispersal-mediated septicaemia, and are potentiated by concurrent GH therapy



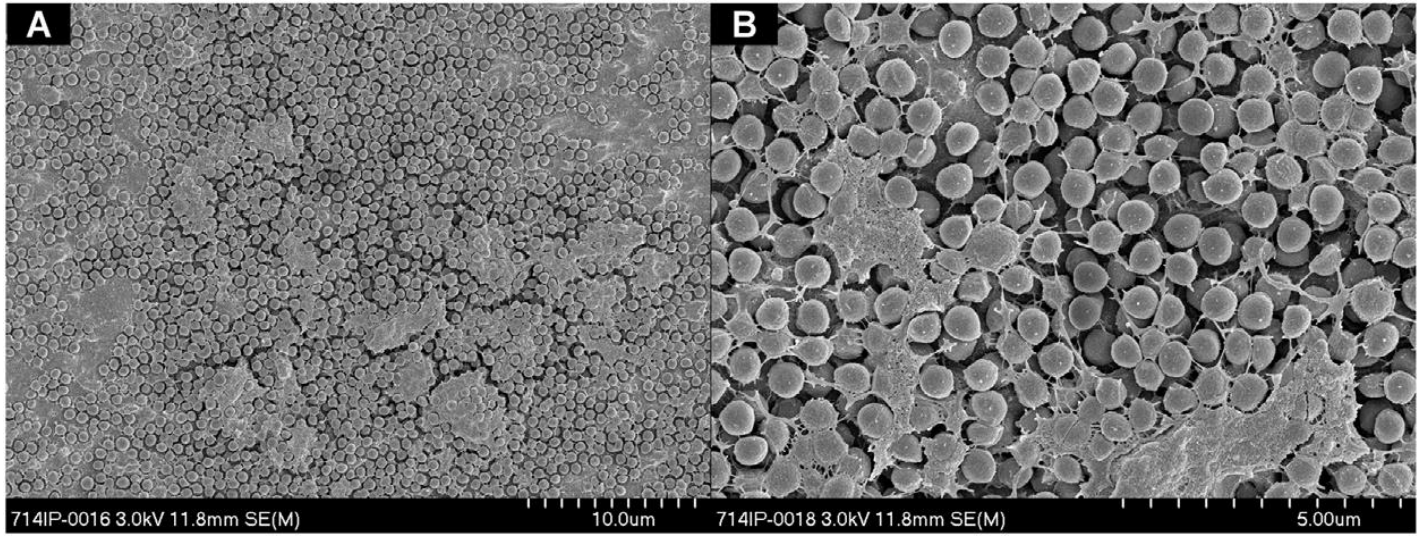
Meropenem (MP)

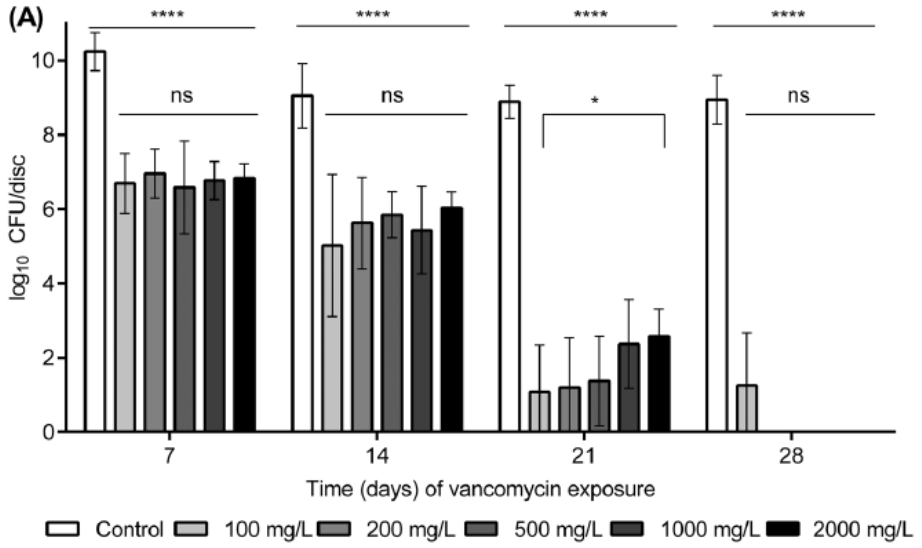
[Fleming & Rumbaugh, 2018]

Vancomycin activity against *S. aureus* biofilm

Vancomycin exposures at 15 mg/L may not be adequate in eradicating biofilm-producing *S. aureus*. Alternative treatments or combination therapy should be explored to optimize outcomes in biofilm-associated infections.

[Rose and Poppens, 2008]





S. aureus biofilm eradication from **medical implants** was possible by vancomycin alone at concentrations **higher than 100 mg/L** for extended periods. The required concentrations are not achievable by systemic therapy, and the duration required is not achieved by currently available local antibiotic delivery vehicles.

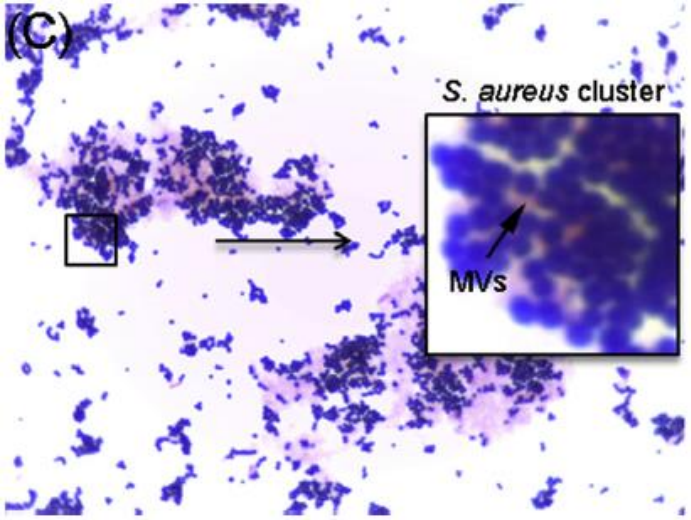
[Post et al, 2017]

Vancomycin-induced biofilm formation by methicillin-resistant *S. aureus*

Chronic wound infections caused by *S. aureus* are largely associated with biofilm formation

Table 1
Minimum inhibitory concentration of antibiotics against *S. aureus* planktonic and biofilm cells.

Antibiotic (class)	ATCC 29213		BWMR22	
	Planktonic	Biofilm	Planktonic	Biofilm
Erythromycin (macrolide)	0.5 (S)	16 (R)	4 (S)	16 (R)
Gentamycin (aminoglycoside)	0.25 (S)	8 (I)	32 (R)	128 (R)
Levofloxacin (quinolone)	0.25 (S)	8 (R)	8 (R)	64 (R)
Oxacillin (beta-lactam)	0.25 (S)	4 (R)	4 (R)	16 (R)
Tetracycline (tetracycline)	0.25 (S)	8 (I)	4 (S)	32 (R)
Vancomycin (glycopeptides)	0.5 (S)	4 (I)	2 (S)	8 (R)



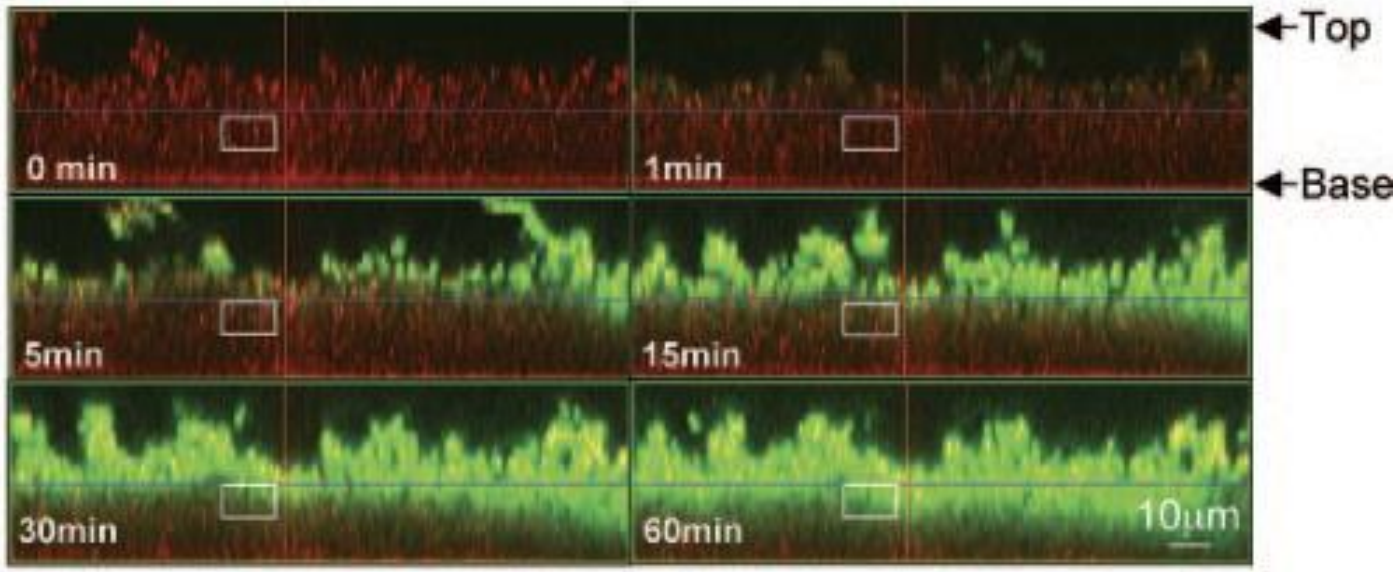
crystal violet

The membrane vesicle (MVs) derived from *S. aureus* mediate the surface adhesion and intercellular aggregation during biofilm formation. The production of MV can be induced as a stress response of *S. aureus* to vancomycin.

[He et al, 2017]

Diffusion of vancomycin into biofilms of *S. aureus*

- **Vancomycin binds to free-floating bacteria in water within 5 min.**
- **Vancomycin binds to cells within the deepest layers of a biofilm after 1h**

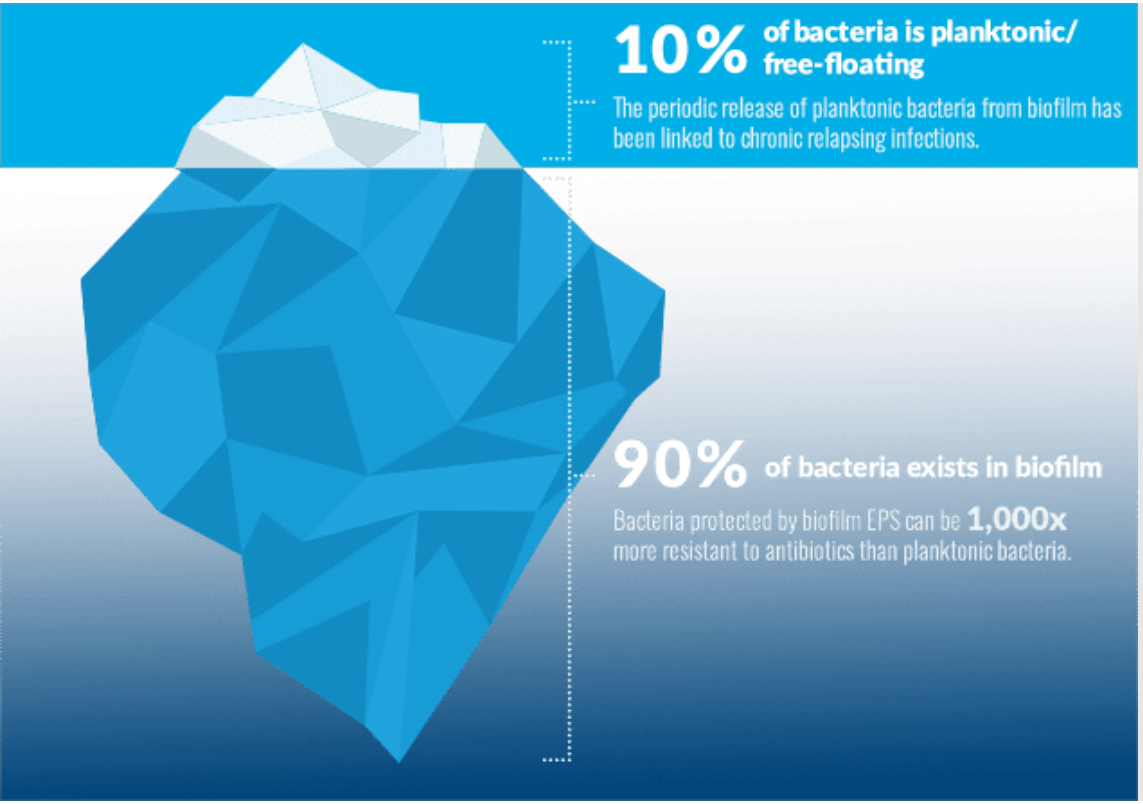


This gradual exposure may allow the biofilm bacteria to undergo stress-induced metabolic or transcriptional changes that increase resistance to the antibiotic.

[Jefferson et al, 2005]

The impact of biofilm in Healthcare-associated Infections (HAI)

Biofilms kill as many people as cancer does and fight off antibiotics



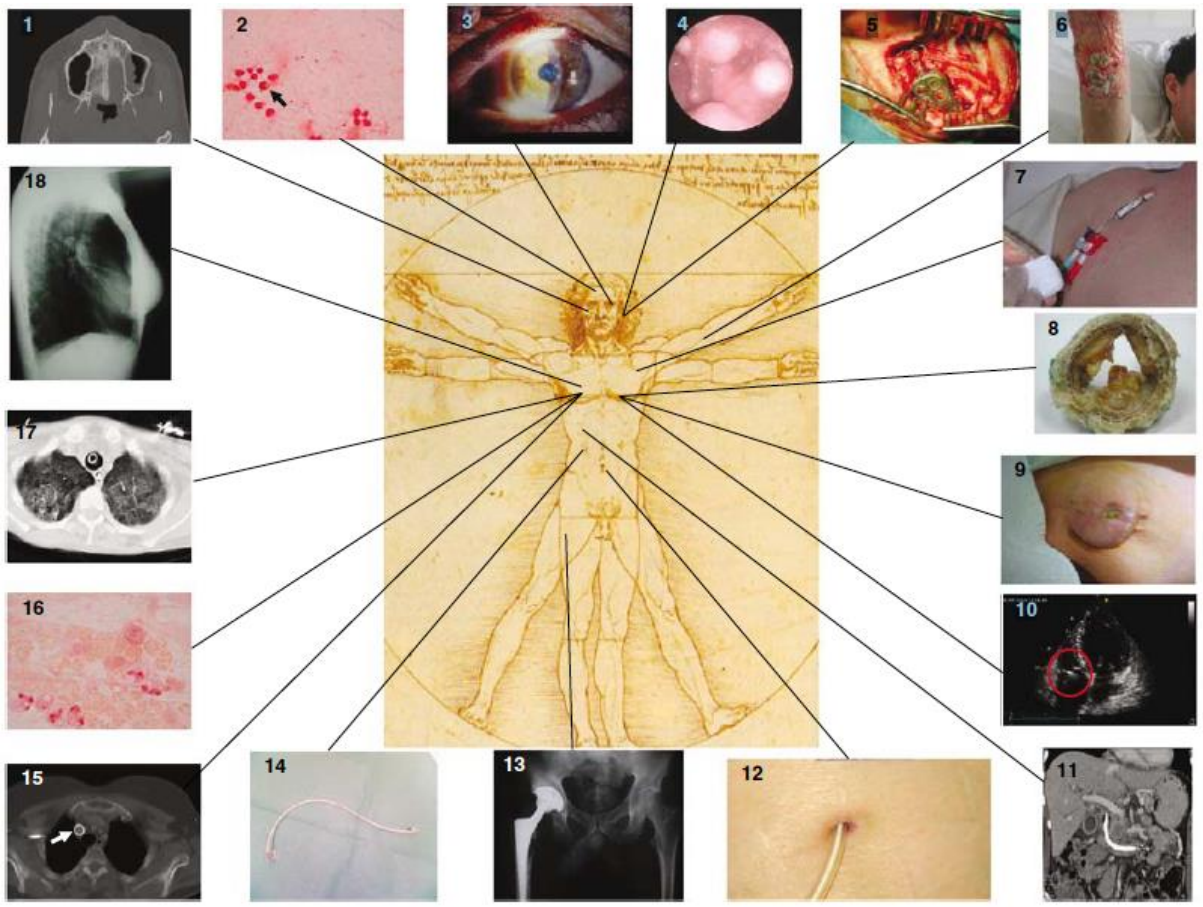
More than 70-80% of bacterial infections currently treated in hospitals are caused by biofilms

Biofilms cause over 2 million infections annually, resulting in US$11B in additional costs

The problem is that our strategies to combat bacterial infections are geared toward individual bacteria, not biofilms.

[Centers for Disease Control and Prevention Report, 2012]

Clinical significance of biofilm-based infections



- **Biofilm-associated tissue infections**
  chronic rhinosinusitis
  odontogenic infections
  intestinal infections
  heart valves
  infected pressure ulcers

- **Medical device-related infections:**
  orthopedic implants
  biliary stents
  vascular catheters
  urinary catheters

Clinical and laboratory indications for diagnosis of biofilm infections

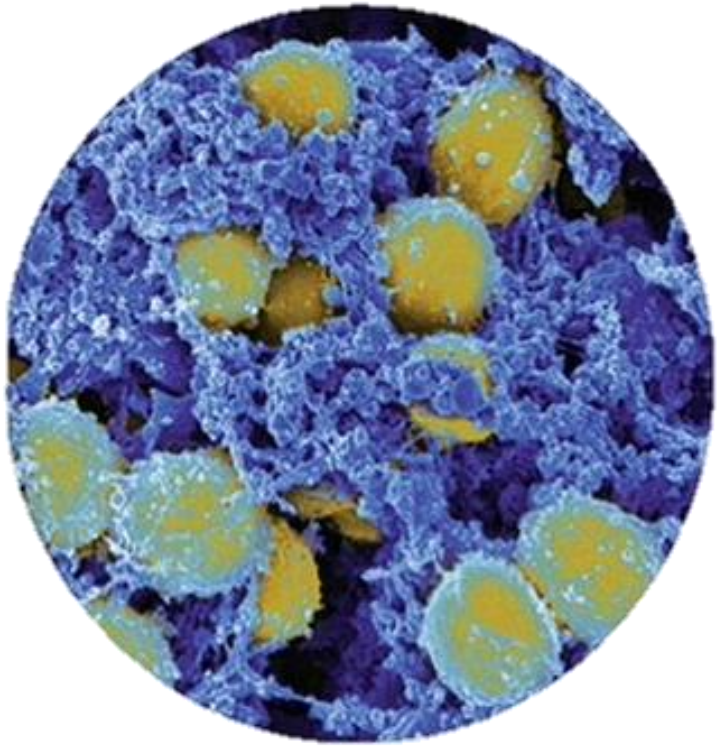
- Clinical signs of infection e.g. the classical but frequently low-grade inflammatory reactions
- Medical history of biofilm-predisposing condition (e.g. implanted medical device, cystic fibrosis)
- Persisting infection lasting >7 days (this is unspecific, and other reasons are frequent such as resistance to the antibiotics used)
- Failure of antibiotic treatment and recurrence of the infection (particularly if evidence is provided that the same organism is responsible on multiple time points)—typing of the pathogen
- Documented evidence/history of antibiotic failure
- Systemic signs and symptoms of infection that resolve with antibiotic therapy, only to recur after therapy has ceased.
- Microscopic evidence from fluid/tissue samples obtained from the focus of the suspected infection
- Positive culture/non-culture-based techniques (PCR) of fluid or tissue sample

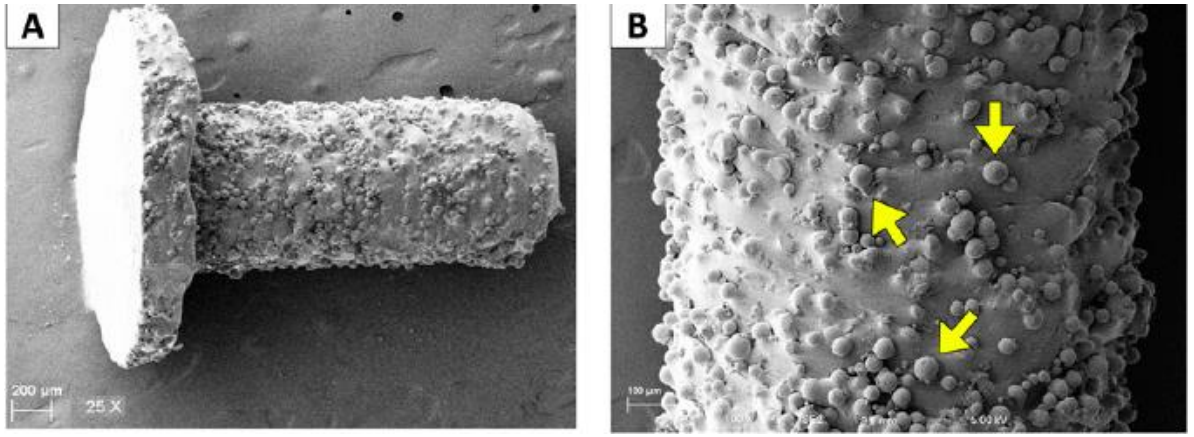
[ESCMID guideline - Høiby et al. 2015]

The clinical impact of *staphylococcal* biofilm

75% of osteomyelitis are caused by staphylococci.

S. aureus is the most common pathogen and over 50% of cases are caused by MRSA strains.





Biofilm on titanium implant

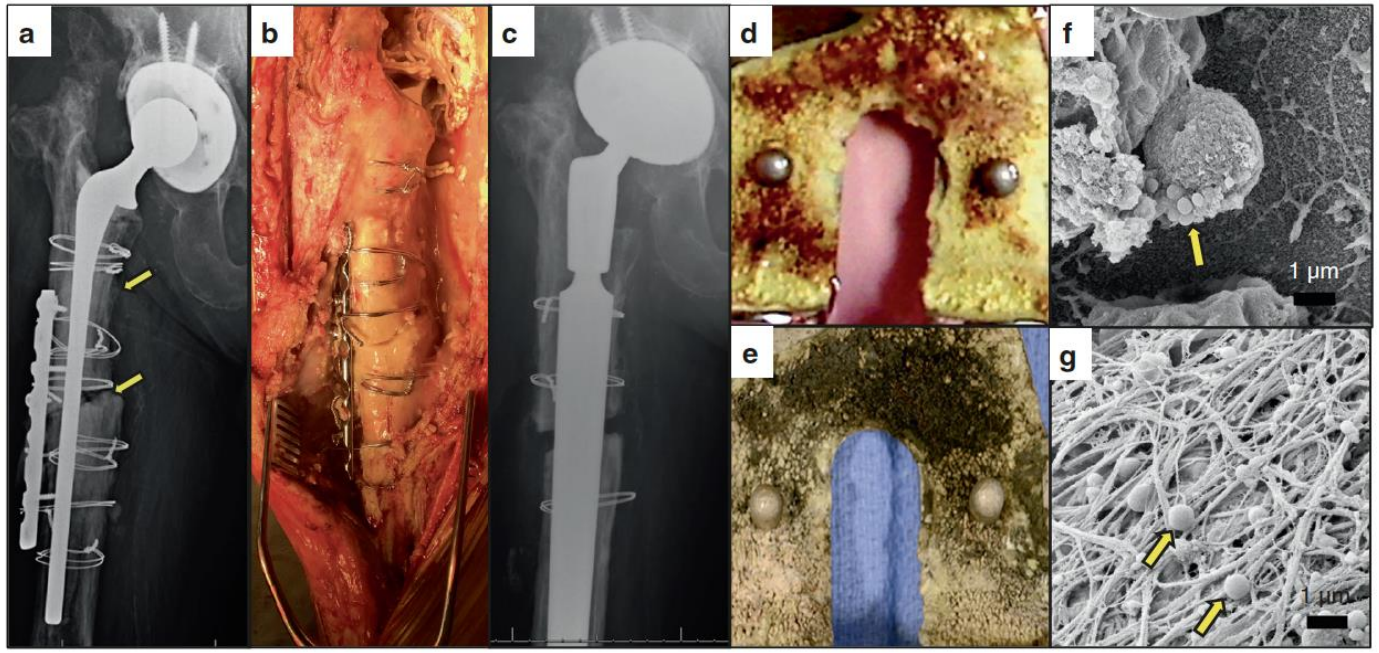
Biofilm in prosthetic joint infection

PJIs account for up to 12% of the indications for revision hip arthroplasty, and 22% for revision knee arthroplasty

The **lack of systemic inflammation** in chronic PJI may indicate biofilm-associated infection.

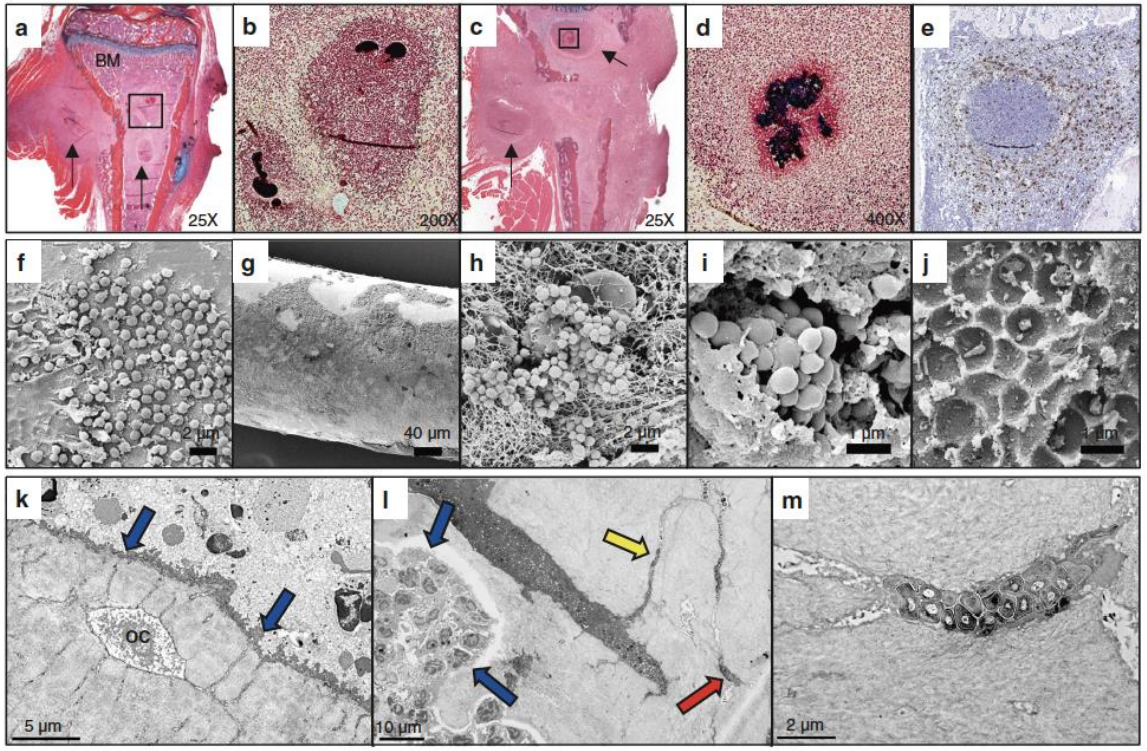
Most biofilm species **escape detection** by conventional culture-based methods.

A **large proportion of culture-negative infections** may be misdiagnosed as aseptic loosening and fail to receive appropriate treatment.



Orthopaedic implant-associated infections

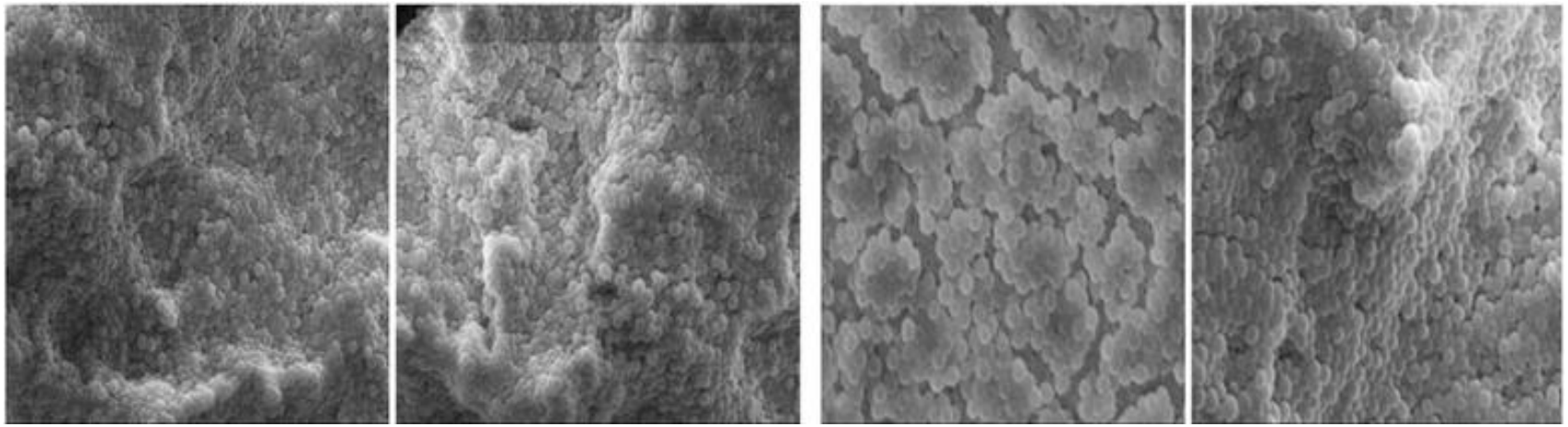
Implant coatings and **antimicrobial therapies to combat osteomyelitis**



- Chemical modifications
- Silver as an antimicrobial
- Antibiotics (Gentamicin is the most studied, vancomycin, fosfomycin, doxycycline, minocycline, rifampin, colistin, daptomycin, and cefoxitin.
- Antimicrobial peptides (AMPs),
- Biofilm dispersal agents

Biofilm chronic rhinosinusitis

The **lack of an effective and durable response to antibiotic therapies** in patients affected by chronic rhinosinusitis has suggested the possible involvement of microbial biofilms.

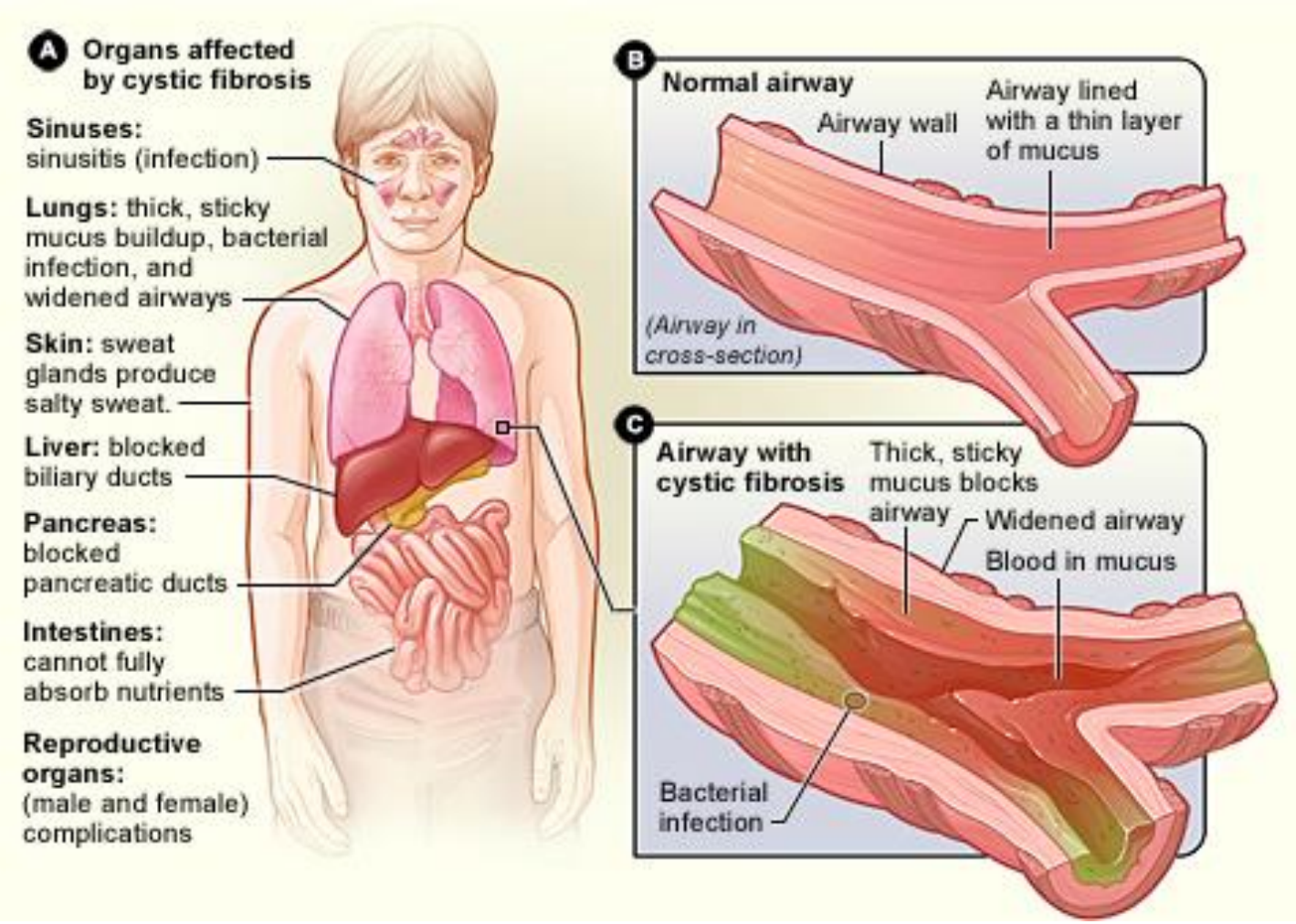


In 2006, Sanderson et al. by FISH of biopsy specimens identified the presence of microbial biofilms: *Haemophilus influenzae* has been found in 80% of these samples, and, in smaller percentages, *Streptococcus pneumoniae* and *Staphylococcus aureus* were also identified.

Staphylococcus aureus, S epidermidis and *Pseudomonas aeruginosa* accounted for the majority of the bacterial isolates. *Aspergillus flavus*, was the commonest amongst the fungi. 45 % of the 40 bacterial isolates and 50 % of the *A. flavus* isolates were found to be biofilm producers.

[Nayak et al, 2016]

P. aeruginosa Biofilm Lung Infection in Cystic Fibrosis (CF)



CF is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), a membrane anion channel.

The vast majority of individuals with CF will eventually become chronically infected with biofilm-growing *P. aeruginosa*.

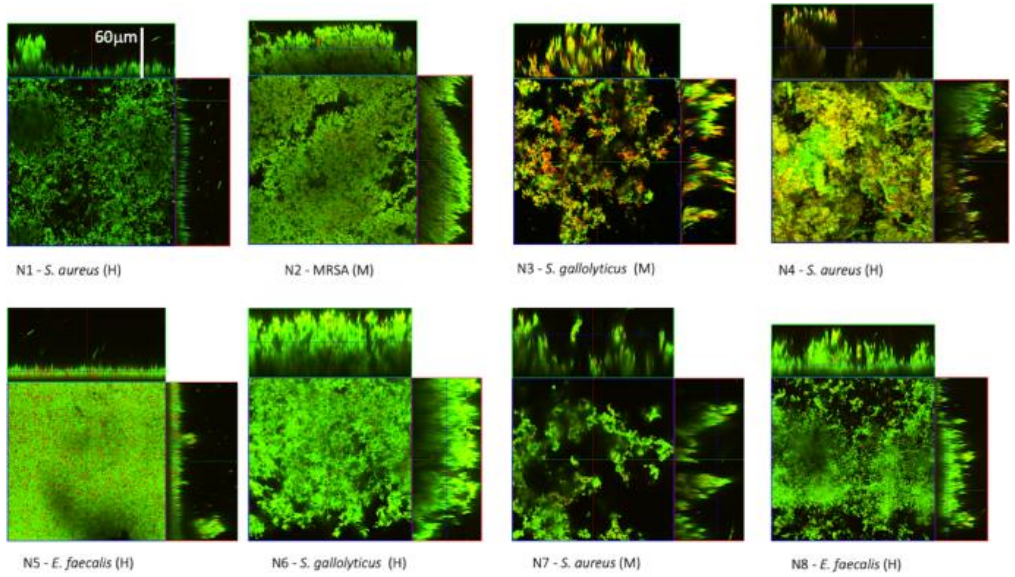
Persistence of CF airway infections is associated with non-resolving inflammation, accelerated lung disease, and earlier mortality

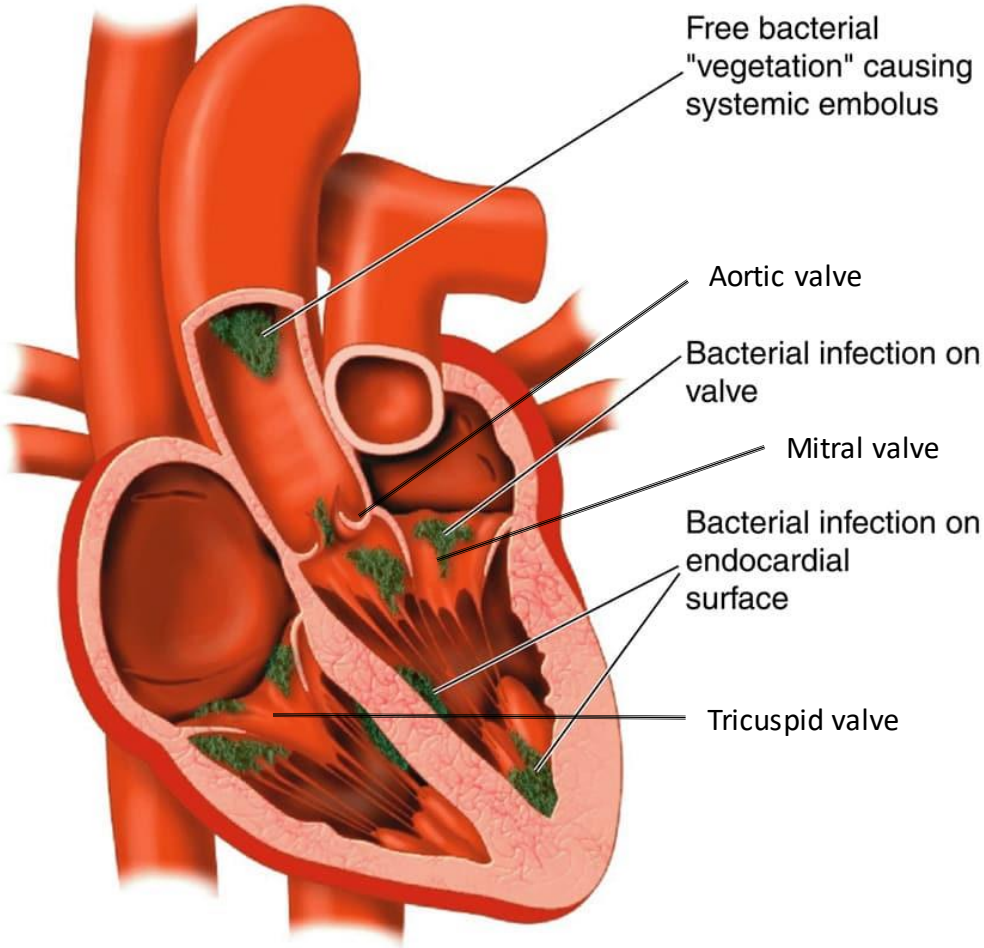
Infective endocarditis (IE)

IE is an infection of the endocardium, which is the inner lining of the heart chambers and heart valves.

Mortality rates of 20–25%

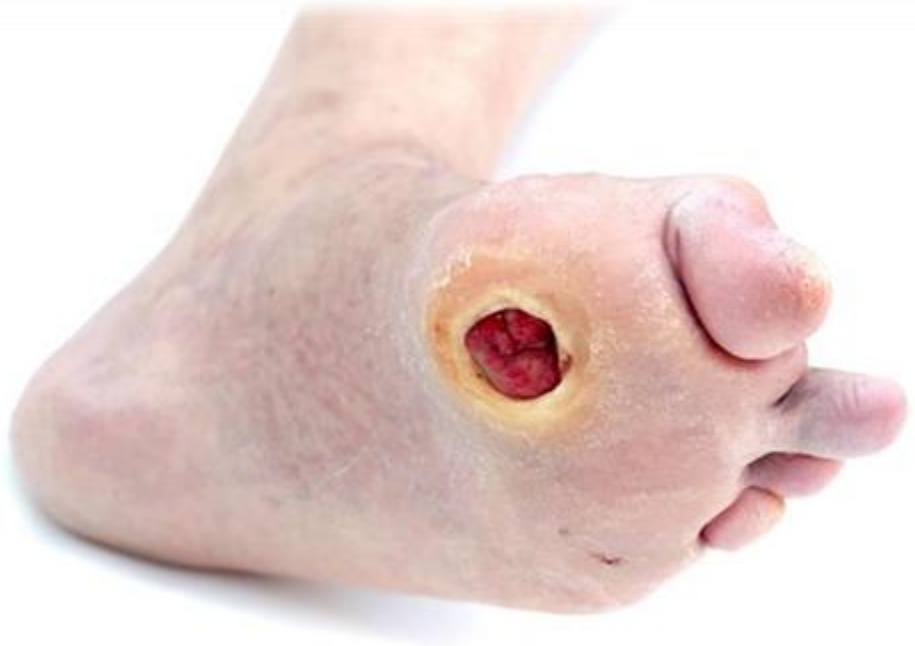
The echocardiogram allows the direct visualization of the endocarditic vegetations





Wounds are an ideal environment for bacterial growth

2-6% of the adult population suffer from chronic/non-healing wounds
Ulcers precede 85% of all amputations. Diabetic ulcer is the reason for 70% of all lower limb amputations.

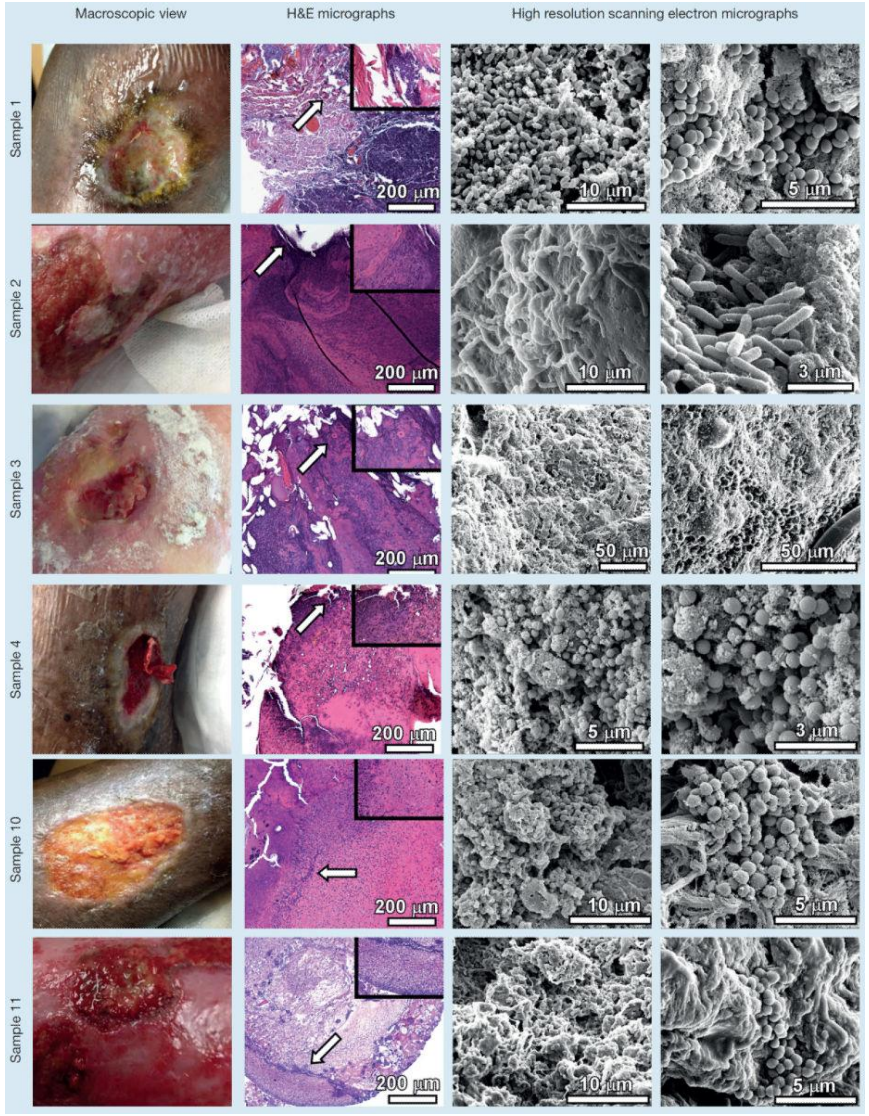


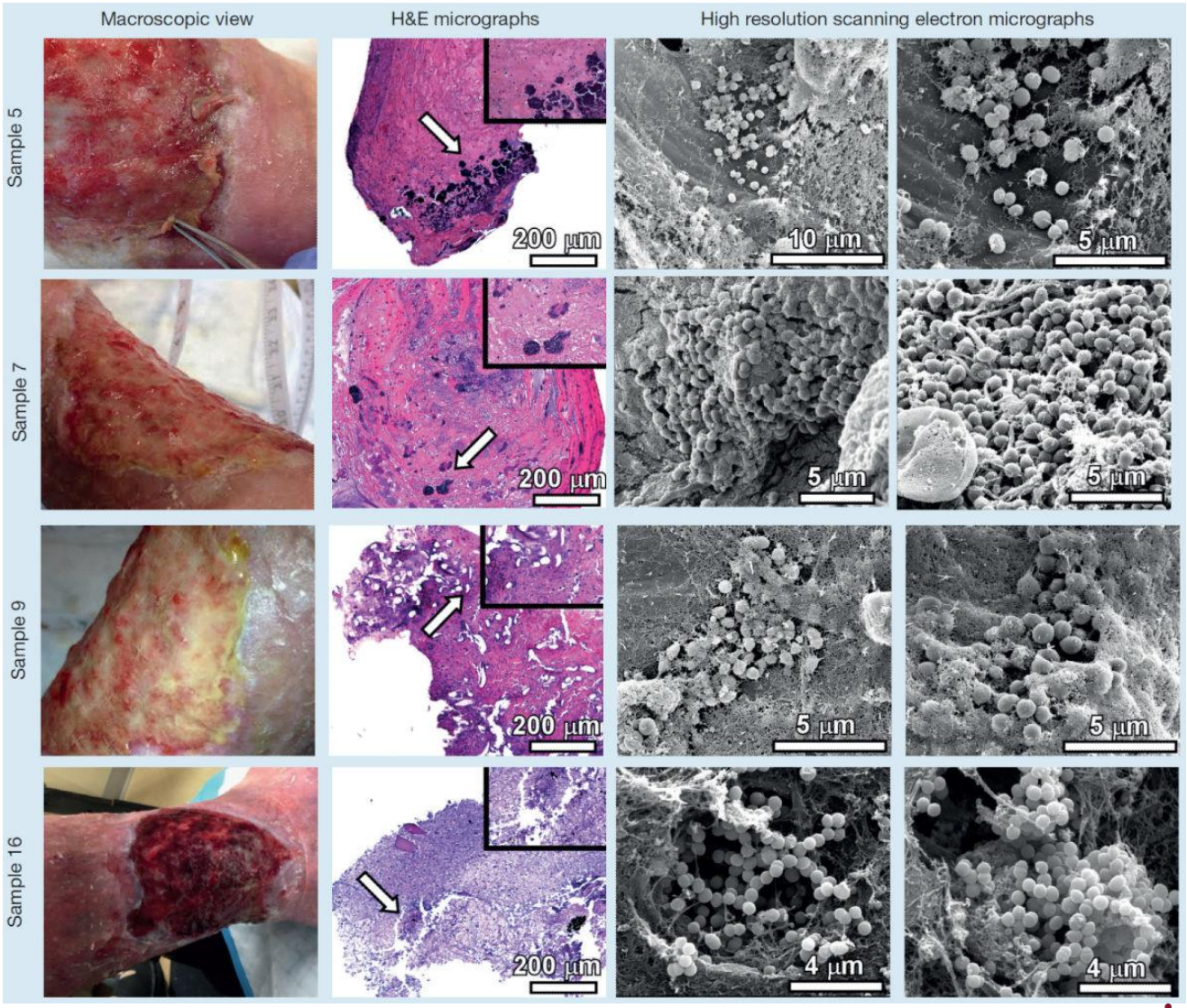
All wounds are contaminated with microorganisms that are part of the skin microflora

Wound infection depends on the pathogenicity and virulence of the microorganisms and on the immune competency of the host

Wound healing occurs in the presence of bacteria

In non-healing wounds the microbiome exists in a biofilm state



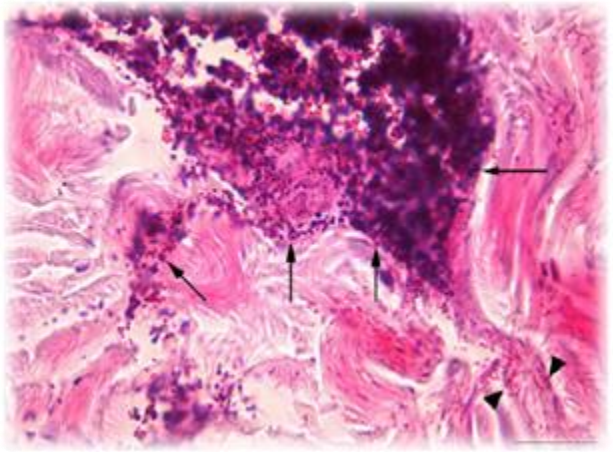


[Hurlow et al, 2016]

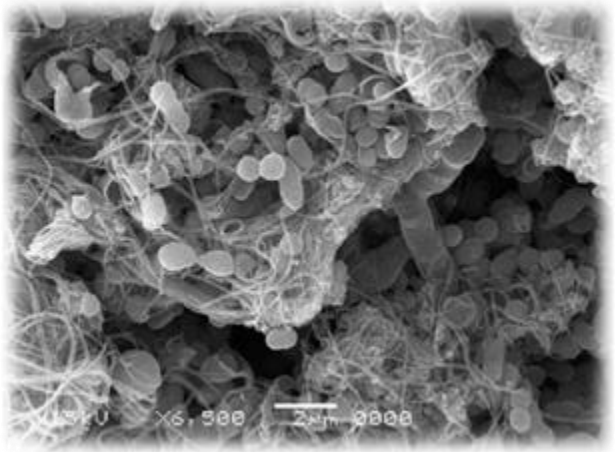
In non-healing wounds the microbiome exists in a biofilm state

Bacteria in deep and poorly oxygenated wounds are strongly associated with a virulent metabolism, producing capsular and extracellular polysaccharides

Slow or non-healing wounds were enriched in biofilm-related functional genes, compared to wounds that achieve closure by 12 weeks



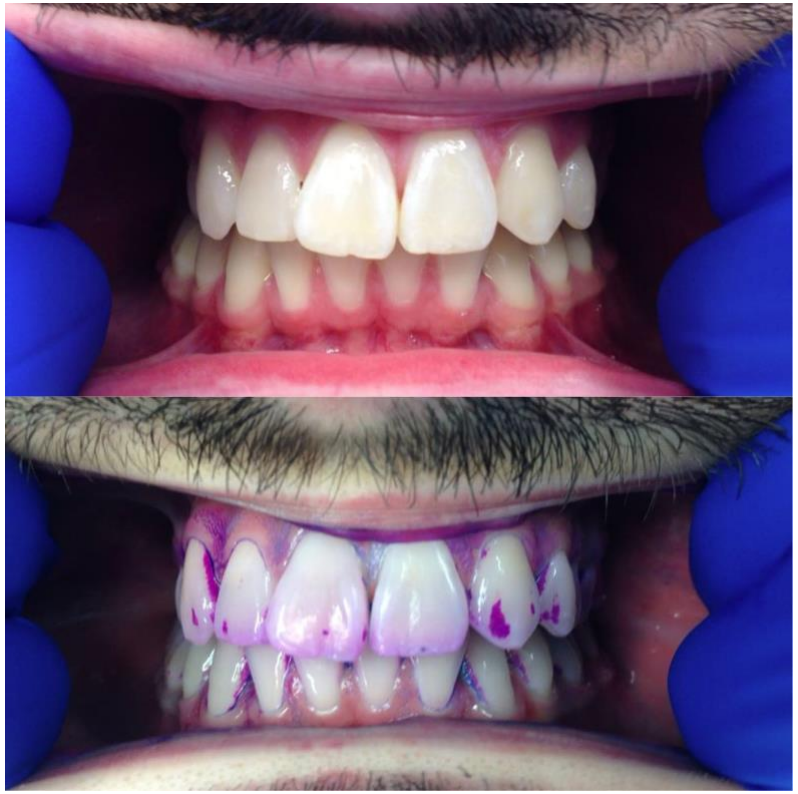
Gram positive cocci and Gram negative rods within the deeper collagen



Higher magnification of the biofilm amongst collagen fibrils

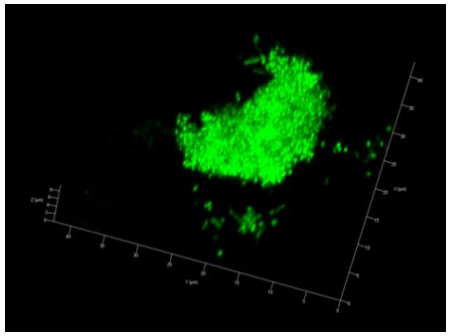
[Kalan et al., 2019]

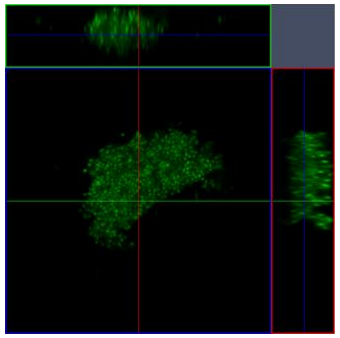
Biofilms in dental caries and periodontitis



Disclosing gel - before and after

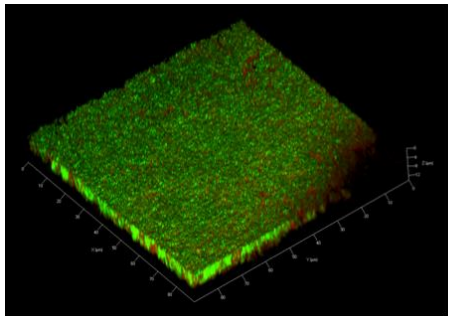
Biofilm on dental implants

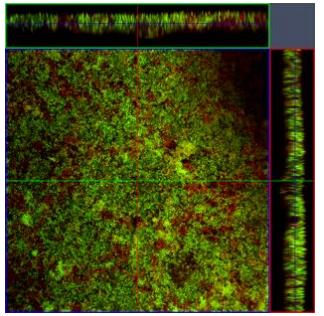




8 µm

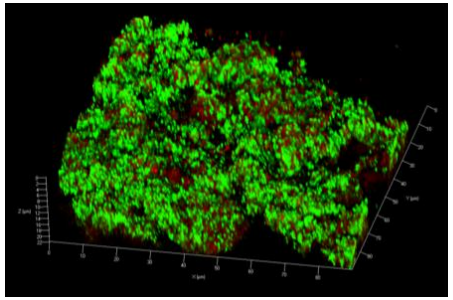
Chlorhexidine

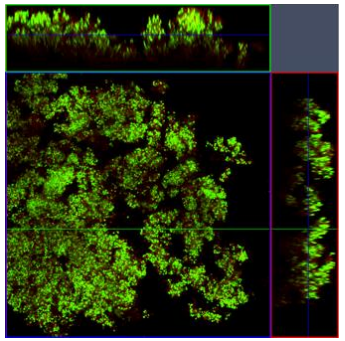




14 µm

Untreated 4 days

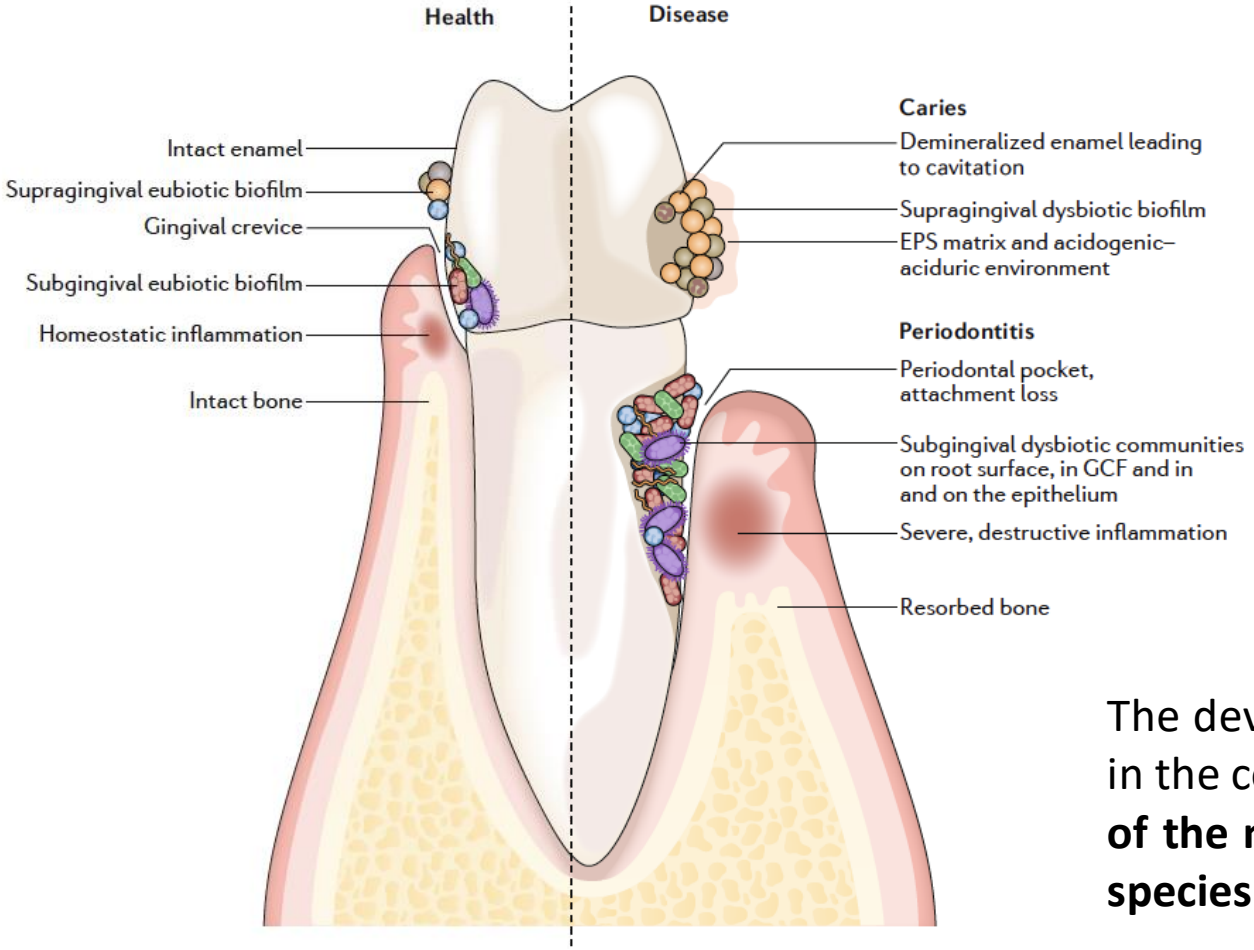




22 µm

Untreated 7 days

Biofilms in dental caries and periodontitis



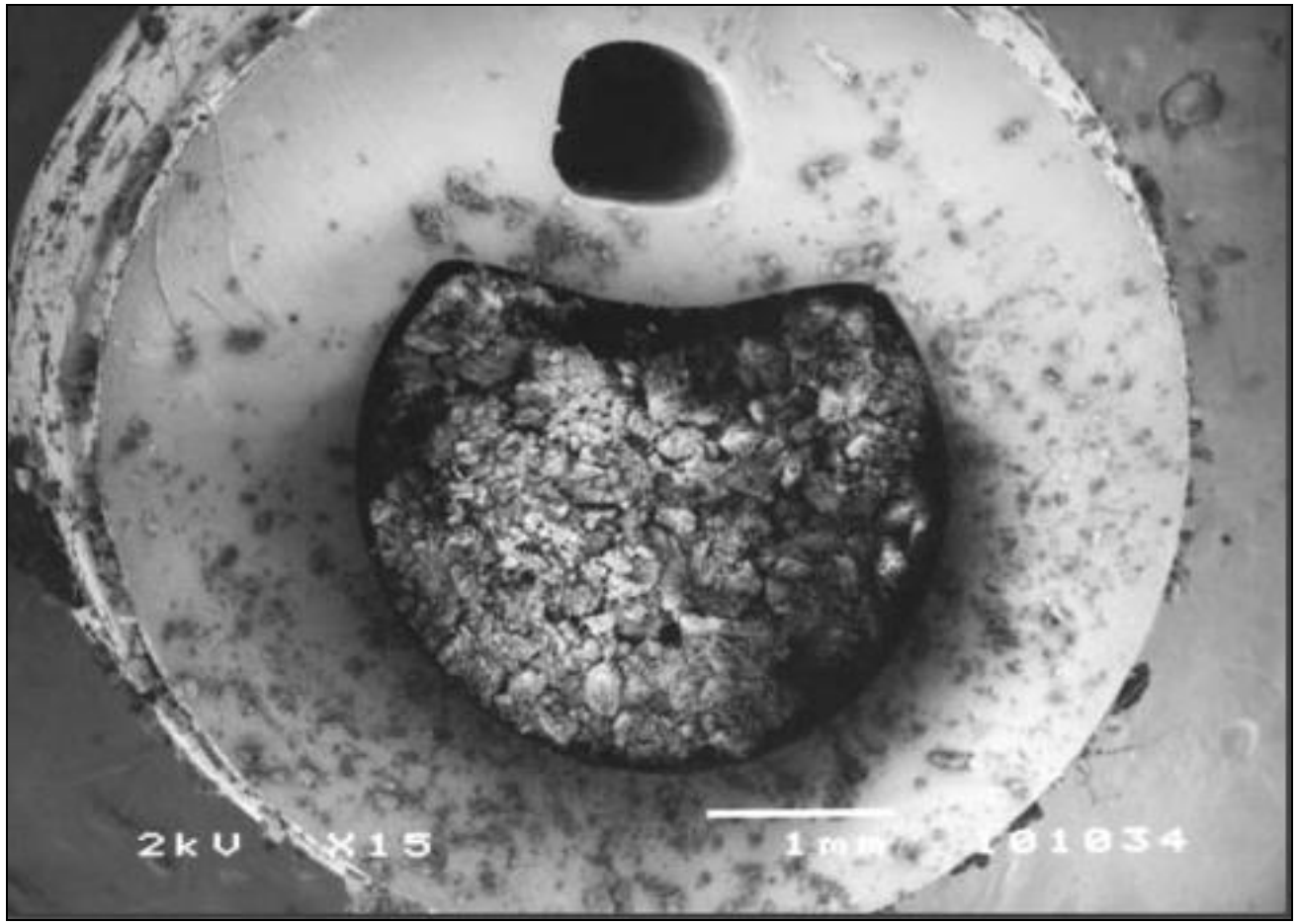
Dental biofilm + long time periods without removal of the biofilm + overexposure to dietary carbohydrates + low pH = increase in biofilm mass and in cariogenic bacteria in the biofilm leading to plaque formation.

When more cariogenic bacteria are present in the plaque the pH remains at or below pH5.5 for longer periods of time resulting in demineralization of tooth enamel (Caries).

The development of **periodontitis** is accompanied by profound shifts in the composition of subgingival communities (**increases in diversity of the microbiome**), with the emergence of different **gram-negative species** (destructive host response).

[Curtis 2020]

Catheters-associated urinary tract infections



Catheter lumen obstruction by urease-producing bacteria (*Proteus* and *Klebsiella*)

[Jacobsen et al, 2008]

Infections associated with vascular catheters

Indwelling devices are usually associated with microbial biofilms and eventually lead to catheter-related bloodstream infections (CRBSIs).

The mortality rate of CRBSIs is 12–25 %.

Aseptic care and antibiotic-impregnated catheters (like minocycline/rifampin, chlorhexidine), preventive locks can be proposed in some cases.

When the diagnosis of CRBSI is suspected on clinical symptoms, it requires a microbiological confirmation by **paired blood** cultures in order to avoid unnecessary catheter removal.

Antibiotic lock technique (ALT) can be used as an attempt to eradicate biofilm formed on the inside lumen of the catheter.

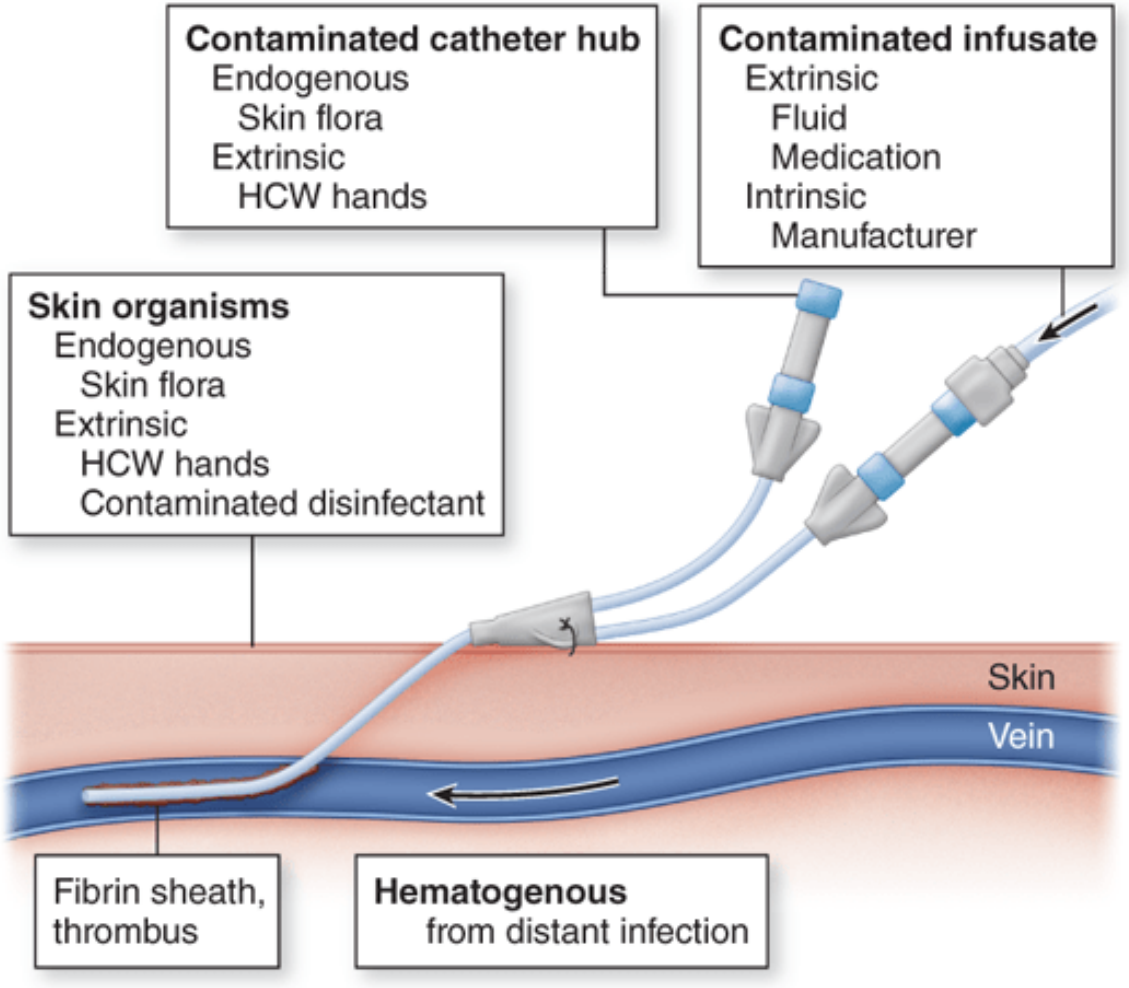
Sources of infections

Extraluminal contamination

- Patient's own skin micro flora
- Microorganism transferred by the hands of Health Care Worker
- Contaminated entry port, catheter tip prior or during insertion
- Contaminated disinfectant solution
- Invading wound

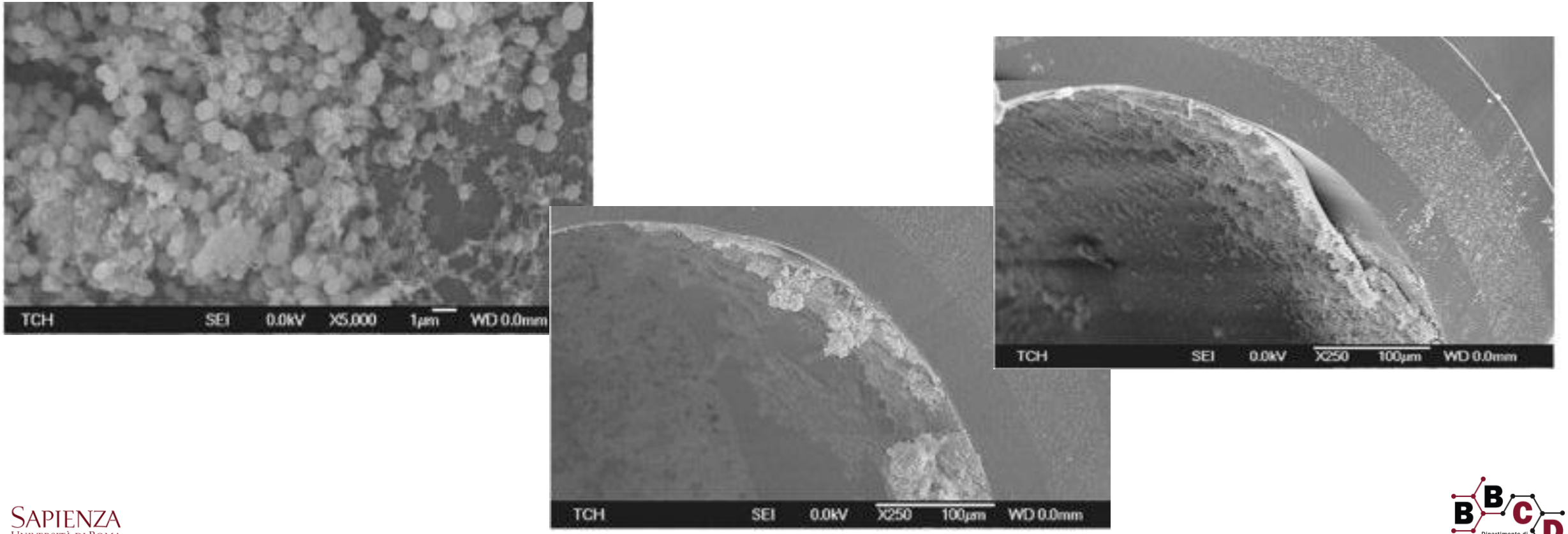
Intraluminal contamination

- Contaminated infusate (fluid, medication)
- Infection from distant focus

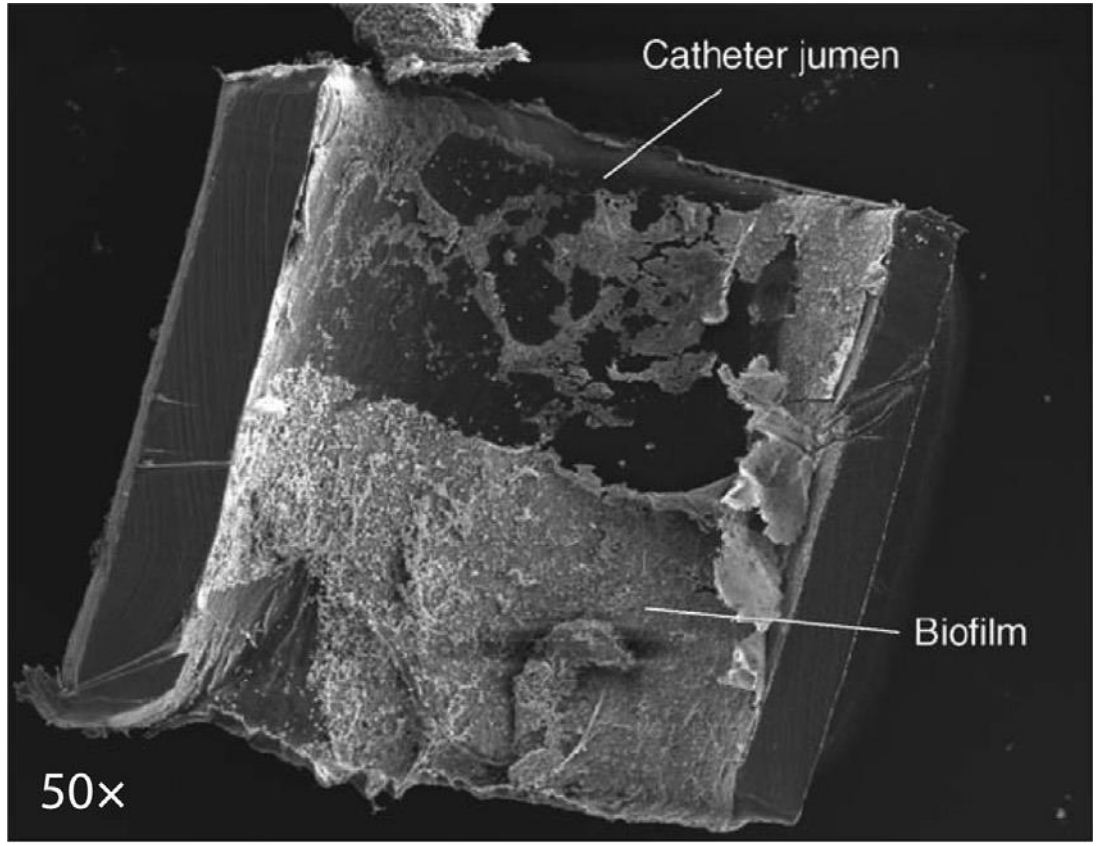


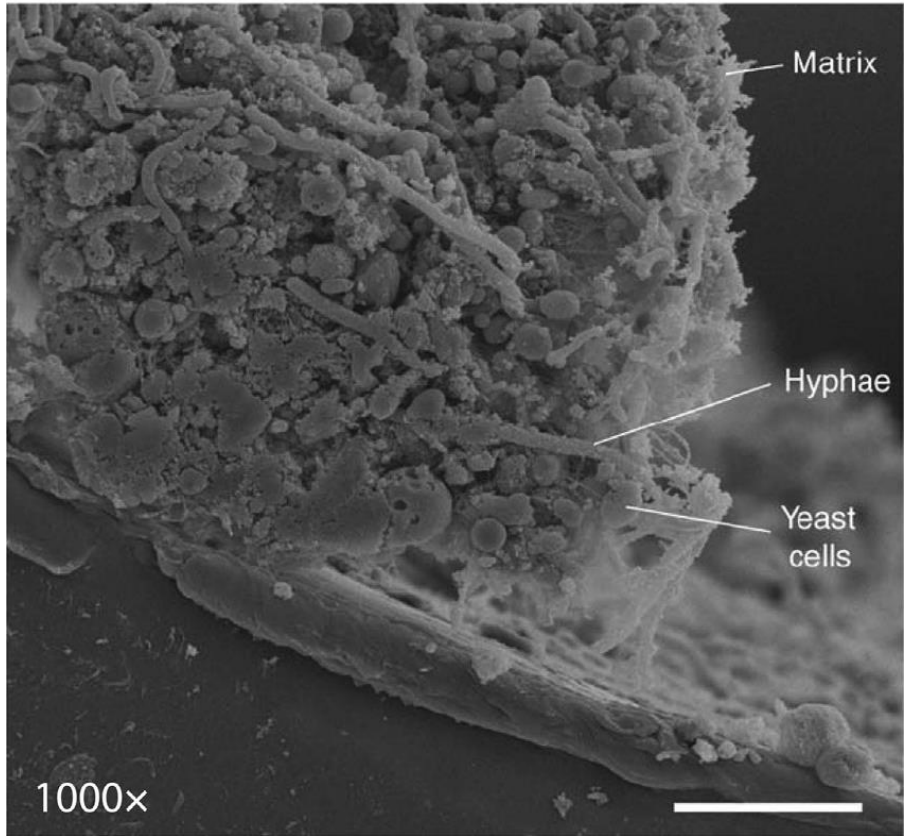
Infections associated with intravascular catheters

Within a few hours of catheter insertion proteins, derived from the host, condition both the external and internal surfaces of the catheter. Organic coatings are composed of fibrin, fibronectin, thrombospondin and laminin which are known to affect the adhesion of Gram positive bacteria



Scanning electron micrographs of a *Candida albicans* biofilm developed in vivo on a catheter lumen surface





Quantitative Tip Cultures

Removed the catheter aseptically after local disinfection of the insertion site. Avoid contact of the tip with the skin.

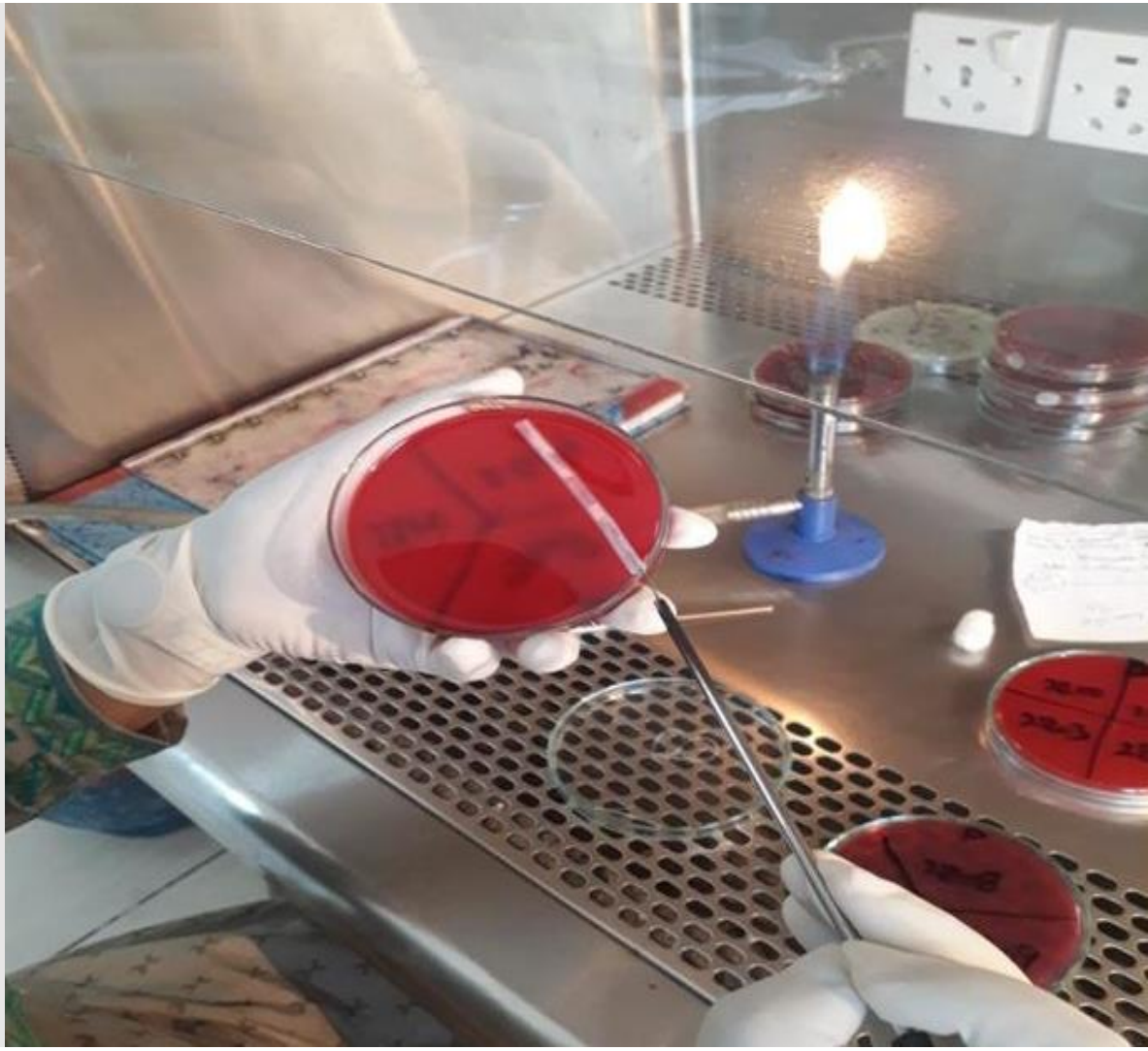
Drip 1 mL of sterile water in the catheter and vortex for one minute

Plate 0.1 mL of the suspension over the whole surface of a blood agar plate.

Incubated at 37°C for five days .

Identify and count the colonies of each species.

Corrected the counts for the initial 1/10 dilution. Quantitative culture results are reported as CFU/ml.



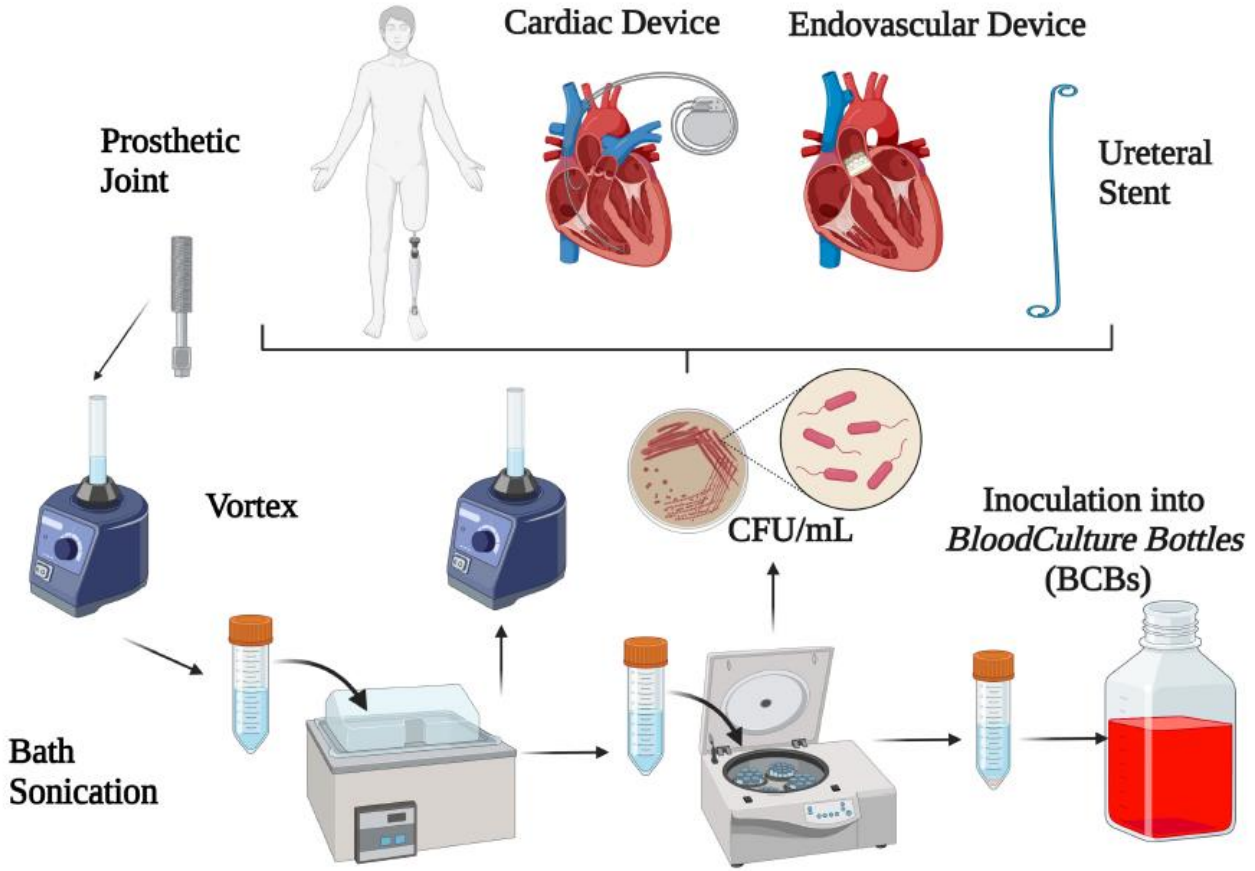
[Brun-Buisson et al., 1987]

Sonication

Sonication is based on applying long-wave ultrasounds (~20 kHz) to detach sessile microorganisms embedded in biofilm.

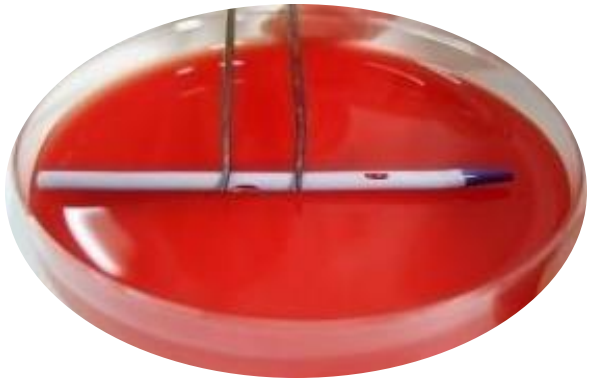
Ultrasound waves radiate through a liquid media, releasing a high amount of energy on the surface of the foreign body, dislodging bacteria from the device.

The most widely used protocol: 1-min or 5-min duration of sonication at the power of 0.22 - 0.04 W/cm2

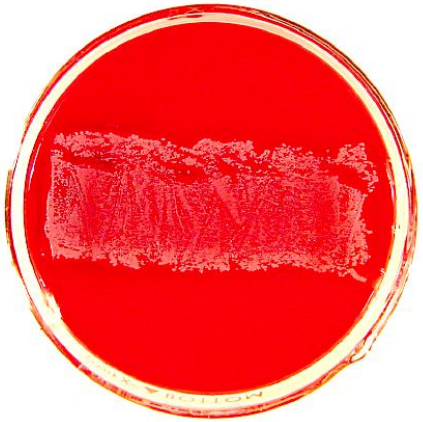


Maki's Roll Plate
Semiquantitative method

Rolling the external surface of a catheter tip back and forth on the surface of a blood agar plate at least three times and then incubating the plate for 72 h at 37°C



Positive



Growth >15 CFU

At least 15 CFU from a 5-cm segment of catheter tip define a colonization (45–84% sensitivity; 85%specificity).

Negative

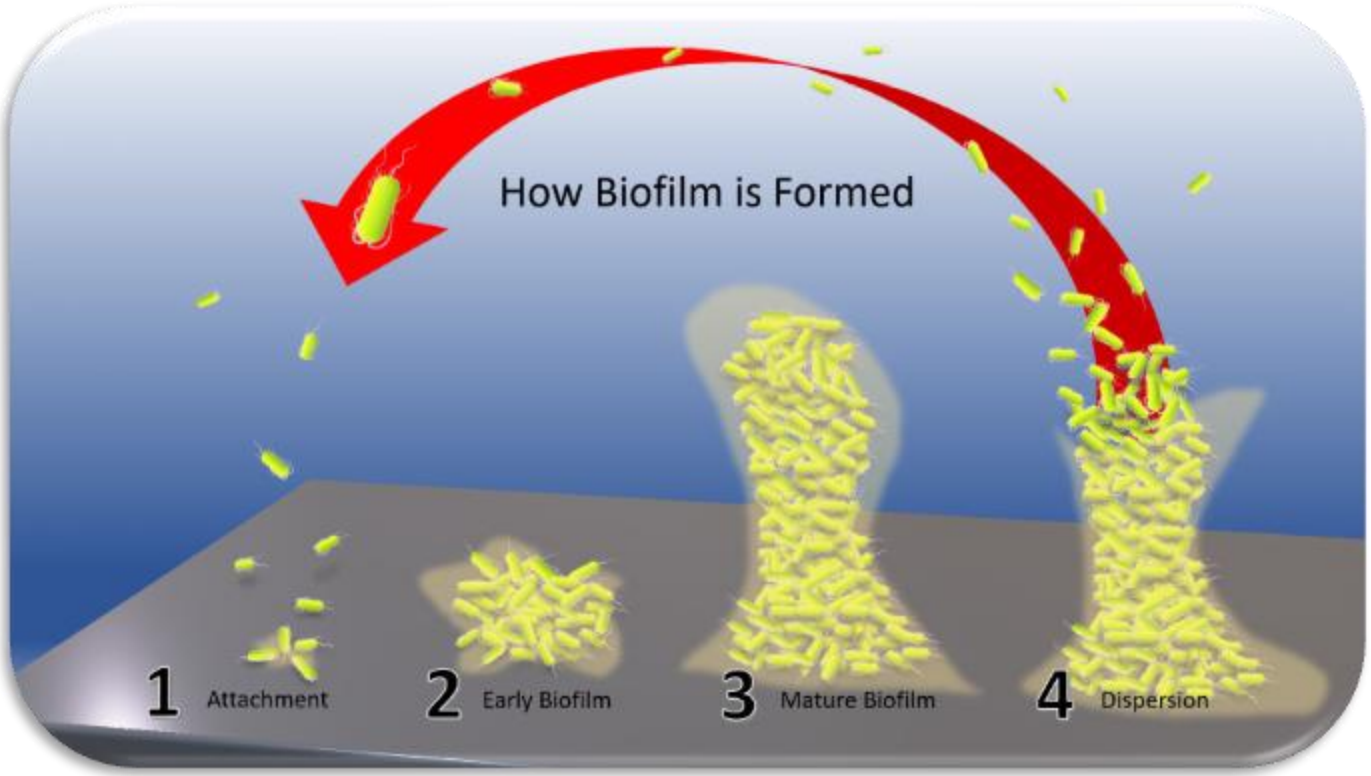
Growth <15 CFU

WHY Biofilm MATTERS

Biofilm antimicrobial susceptibility testing

An unmet clinical need

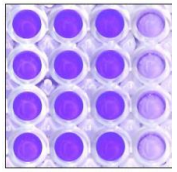
Antibiograms are performed on planktonic cells and do not take into account biofilm production



Which methods should be used in clinical practice to detect biofilms?

Current assays to test biofilm production

Colorimetric tests
[Christensen et al 1985; Pettit et al 2005]

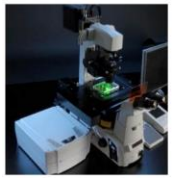


Biofilm in flow cells
[Sternberg et al, 2006]

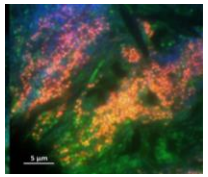
Phenotypic tests
[Freeman et al. 1989]

Static biofilm
[Ceri et al, 1999]

Microscopy systems
[Benoit et al 2010]



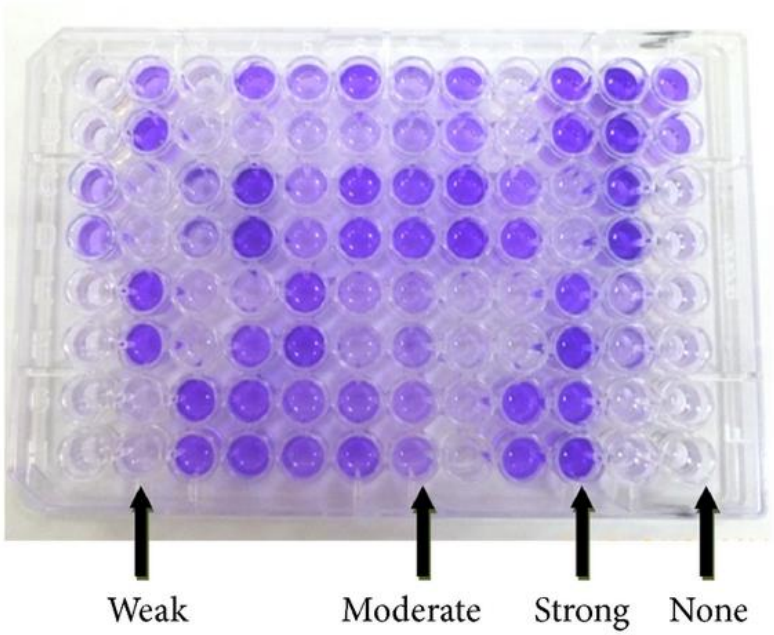
***In-vivo* biofilm**
[Thurnheer et al, 2004]



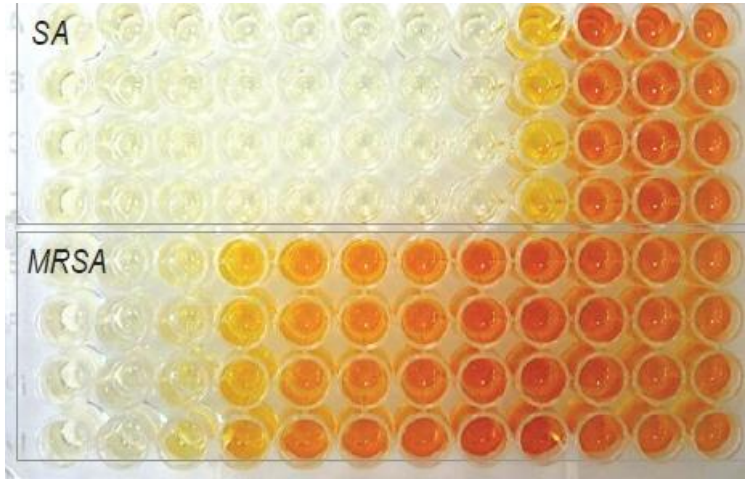
Time consuming, labour intensive, highly variable, low accuracy and low cost-effectiveness

Colorimetric assays

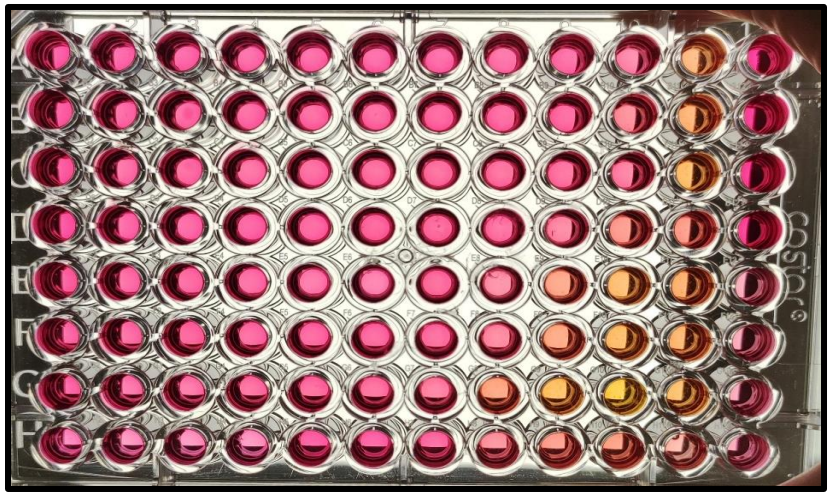
Crystal violet assays



XTT assays

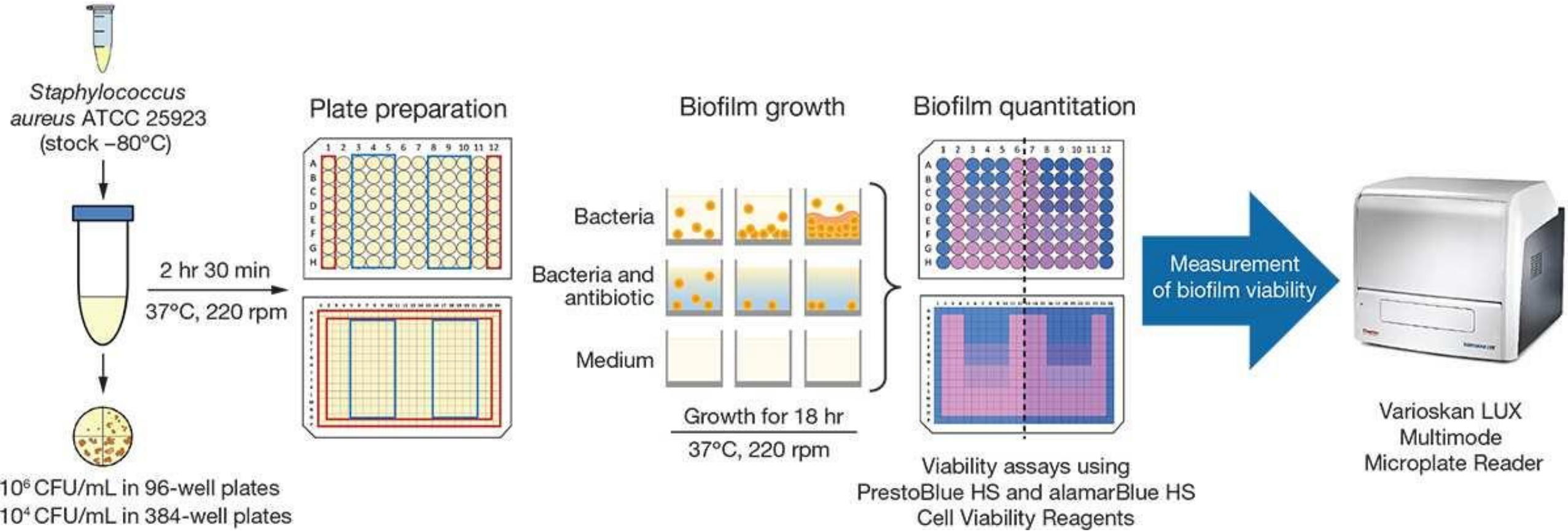


Phenol red assays



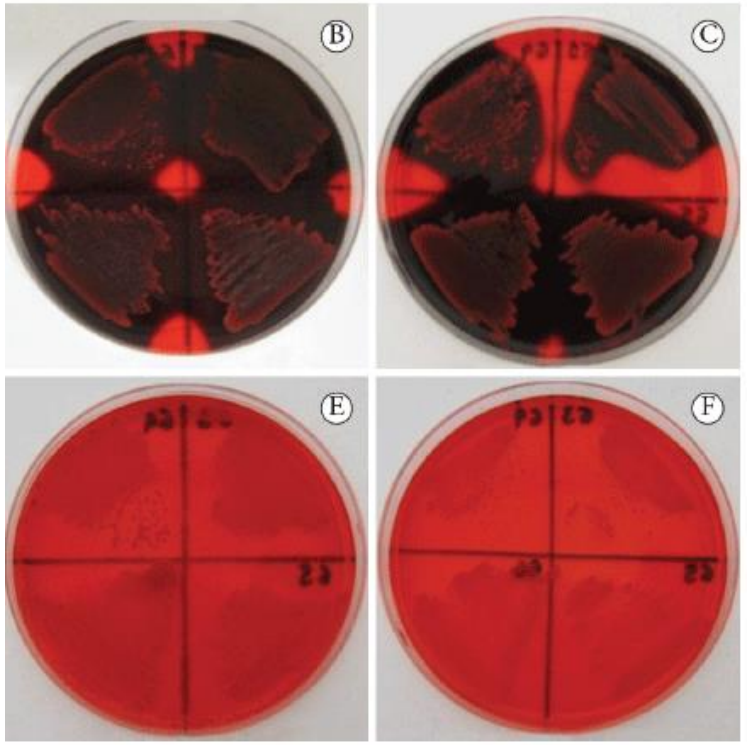
OD values discriminate between: strong-, moderate-, weak-biofilm producers and non-producer strains.

Kinetics of biofilm formation



Phenotypic tests: Congo Red Agar

Poly-N-acetylglucosamine (PNAG) surface carbohydrate, a major component of staphylococcal biofilms often correlates with the appearance of black colonies on Congo red agar

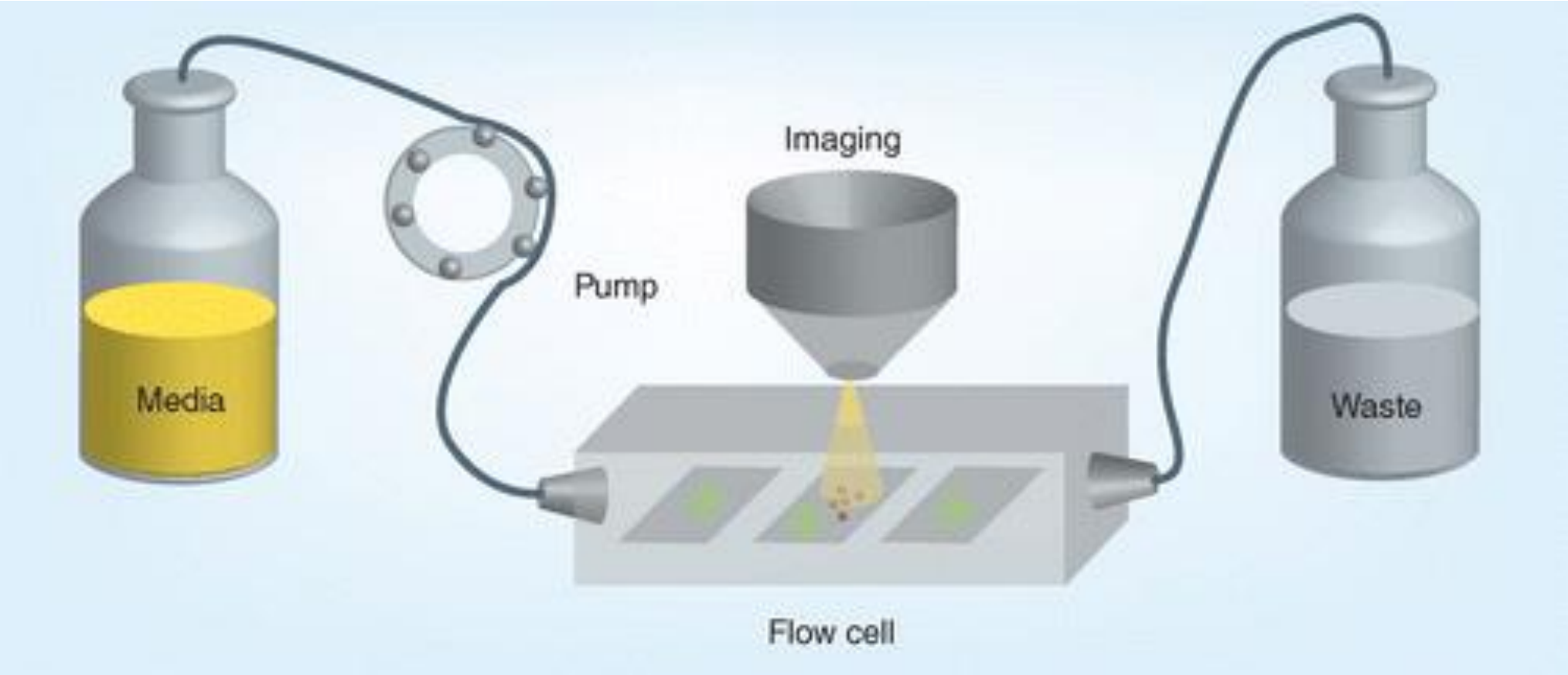


Congo red is a dye that can be used as a pH indicator, due to a color change from blue/black to red at pH 3.0 e 5.2

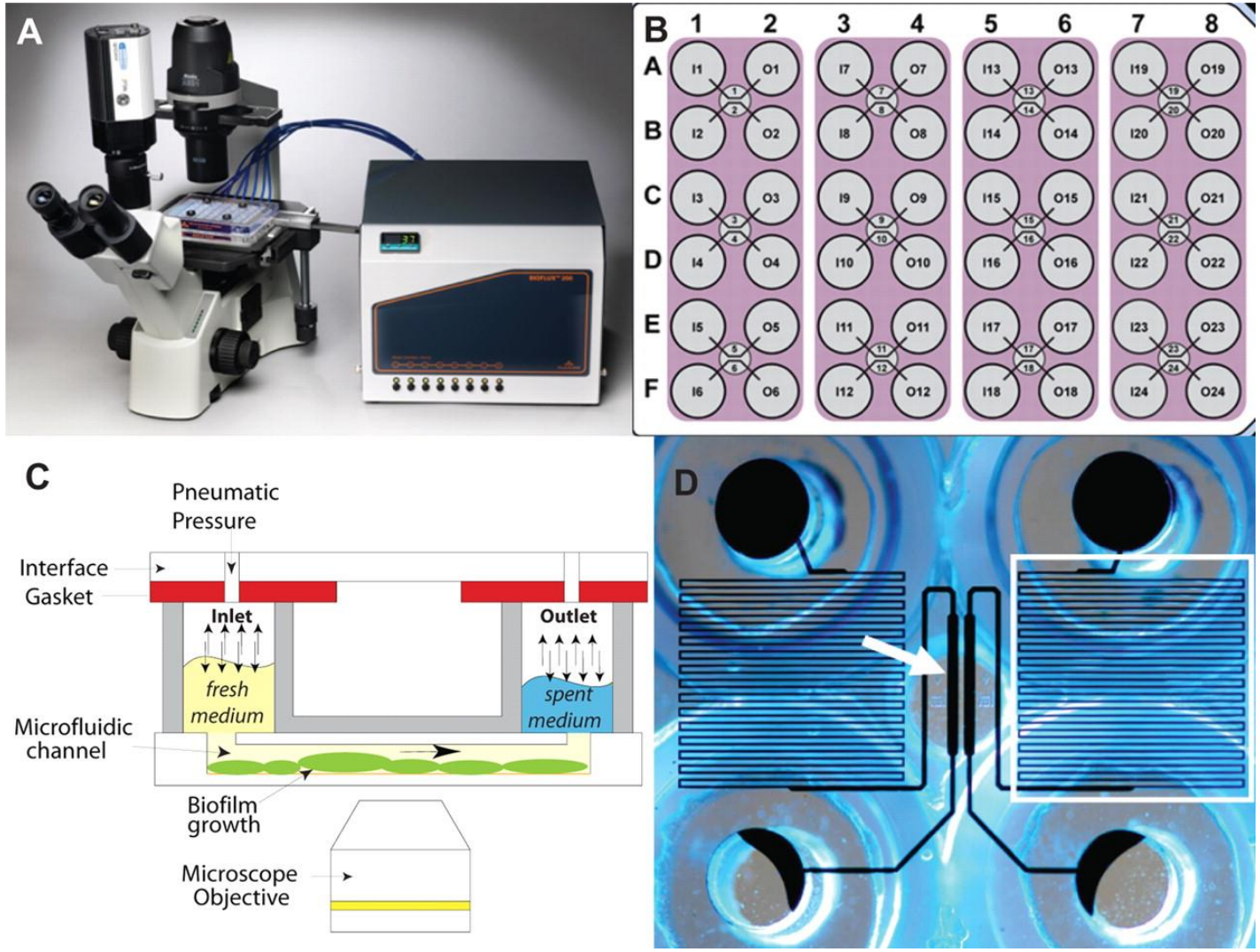
Direct identification of slime-forming strains (black) and non-slime-forming strains (red)

Low accuracy, but it is cheap and easy to perform

Drip Flow Biofilm Reactor



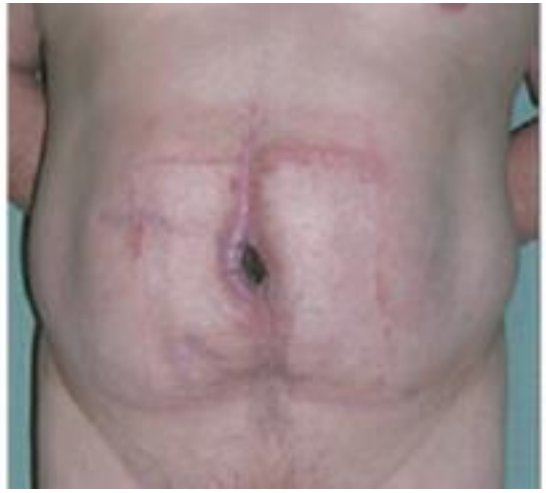
Microscopic systems: Bioflux

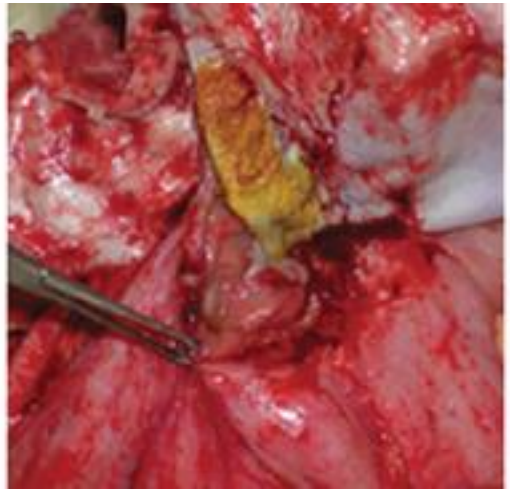


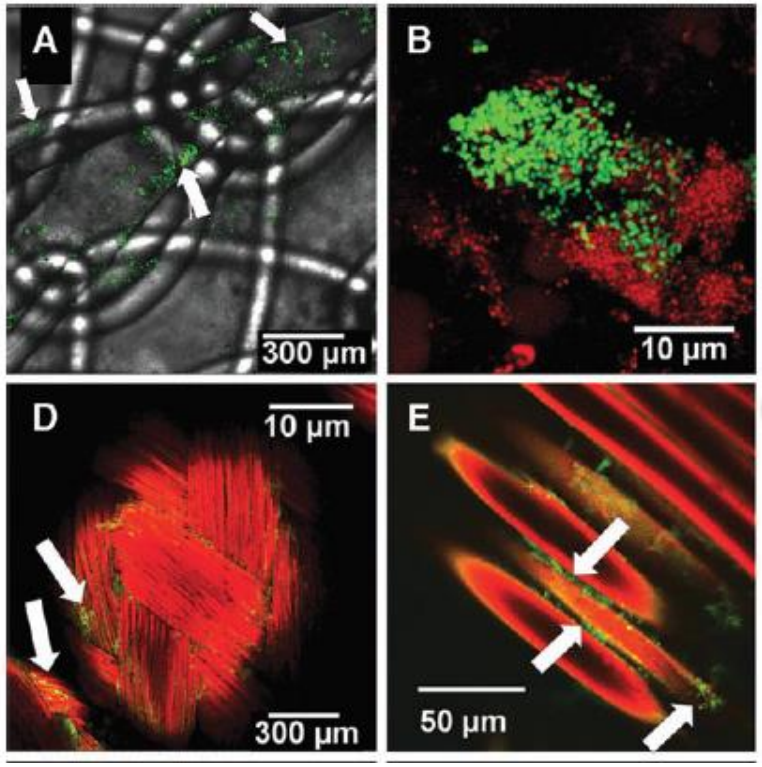
Direct biofilms identification

Confocal laser scanning microscopy (CLSM)

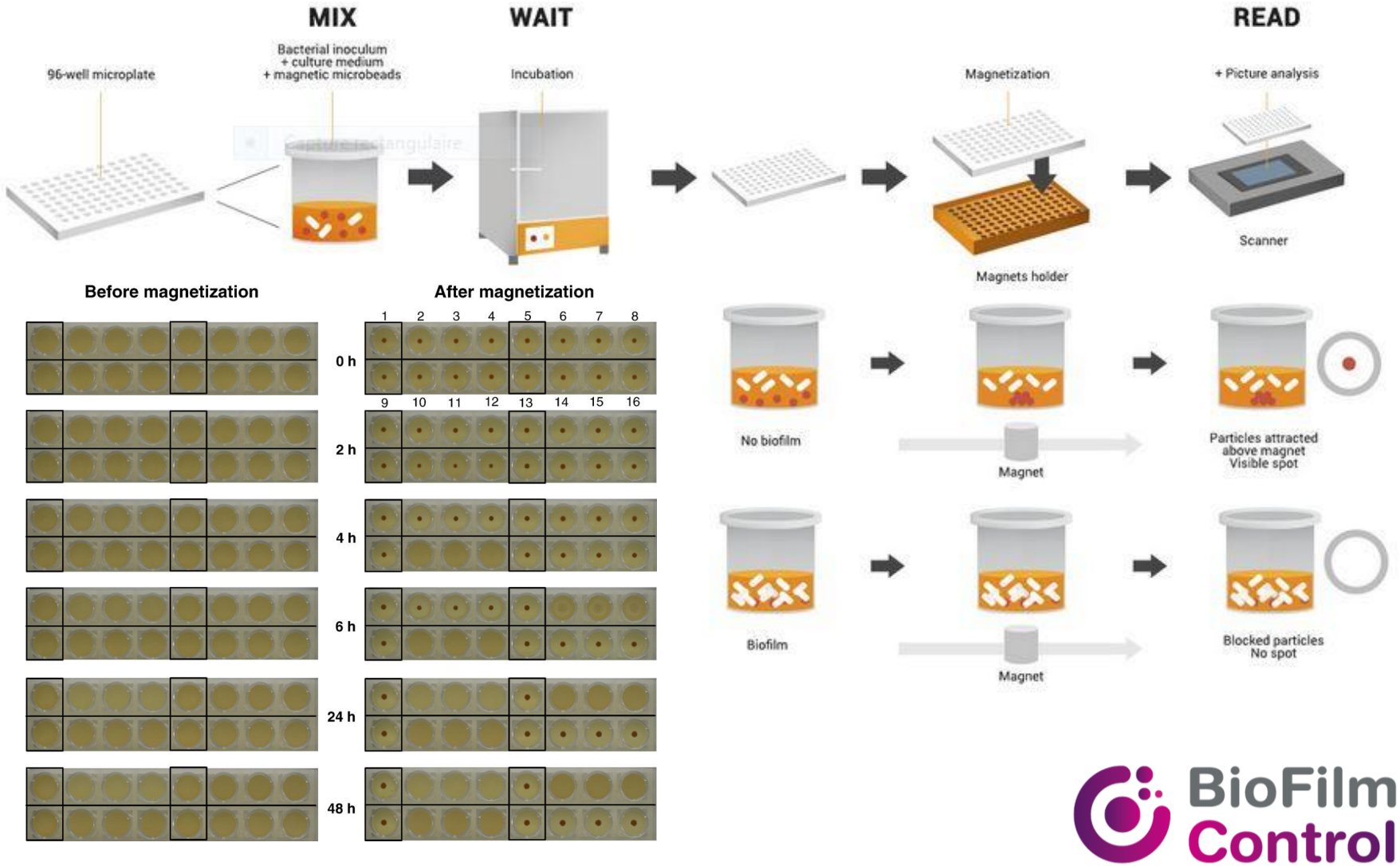
Fluorescence in situ hybridization (FISH)







BioFilm Ring Test (BRT)

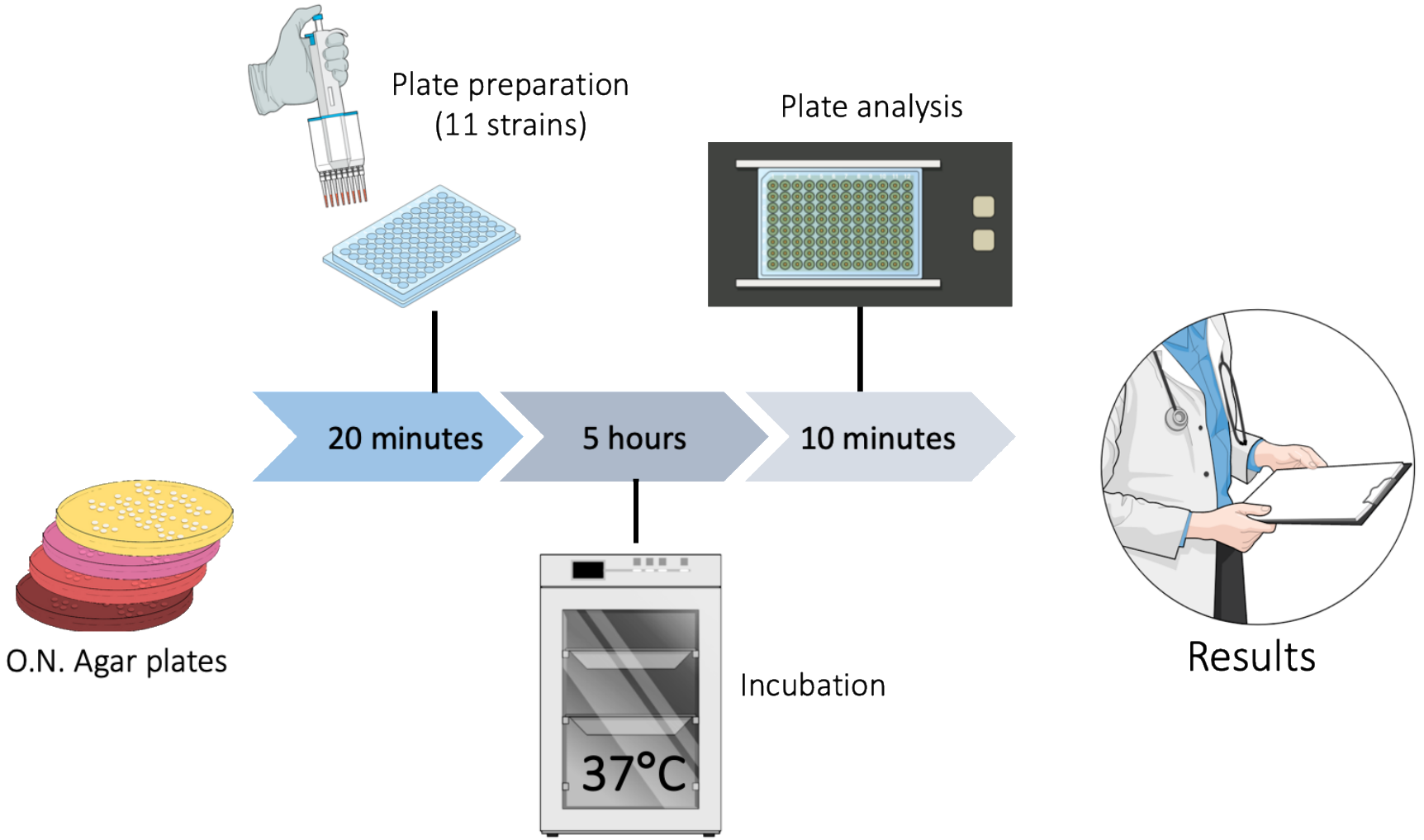


BioFilm Control

[Chavant et al 2007]

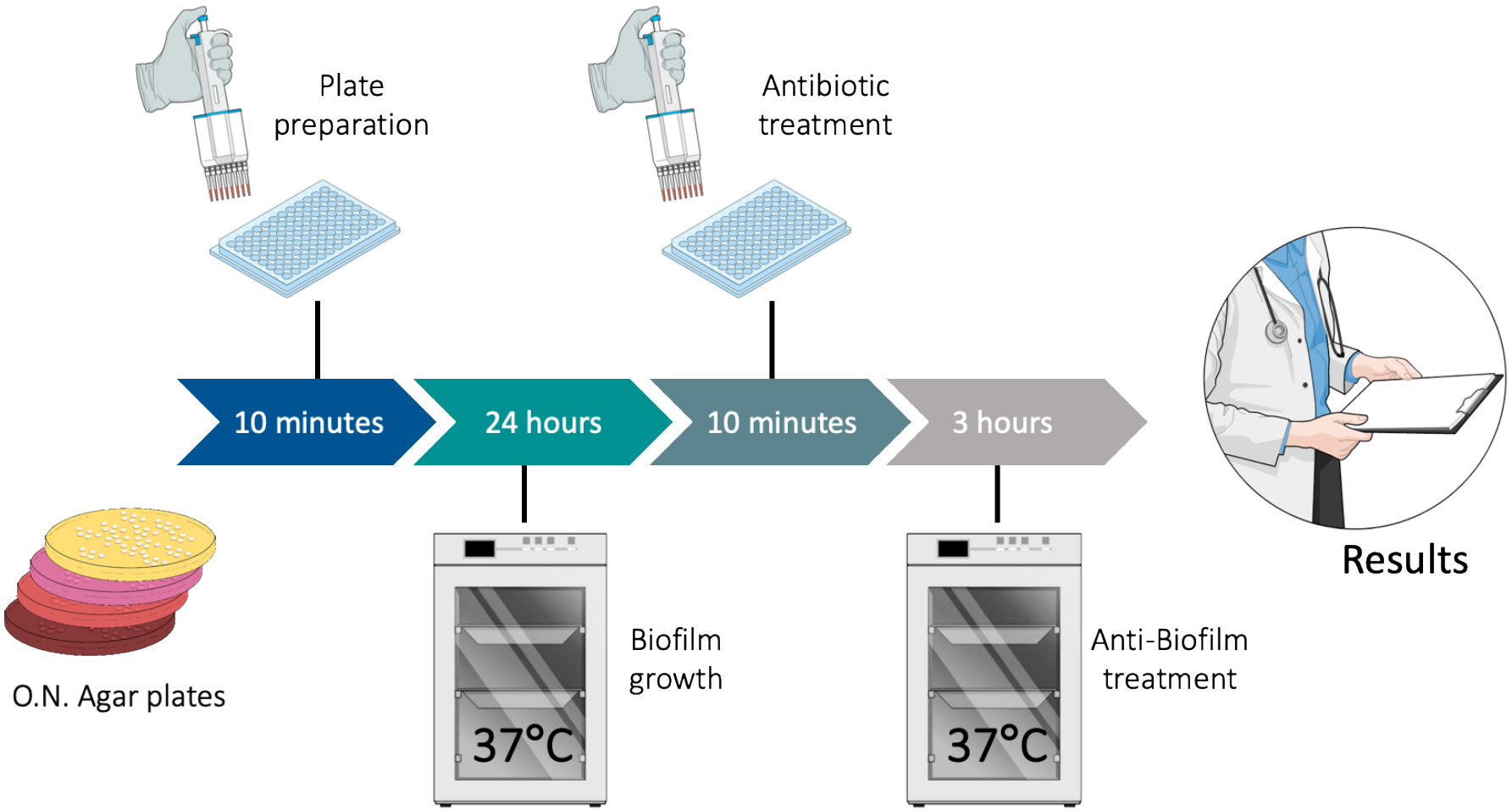
clinical BIOFILM RING TEST® (cBRT)

The working protocol



Patent number WO2017/1219461

Anti-biofilm susceptibility test (ABT)



Patent N: 102019000013983

Development of innovative strategies for biofilm-related infections

Clinical assessment

Sampling

cBRT (5 hours)

ABT (24 hours)

Report

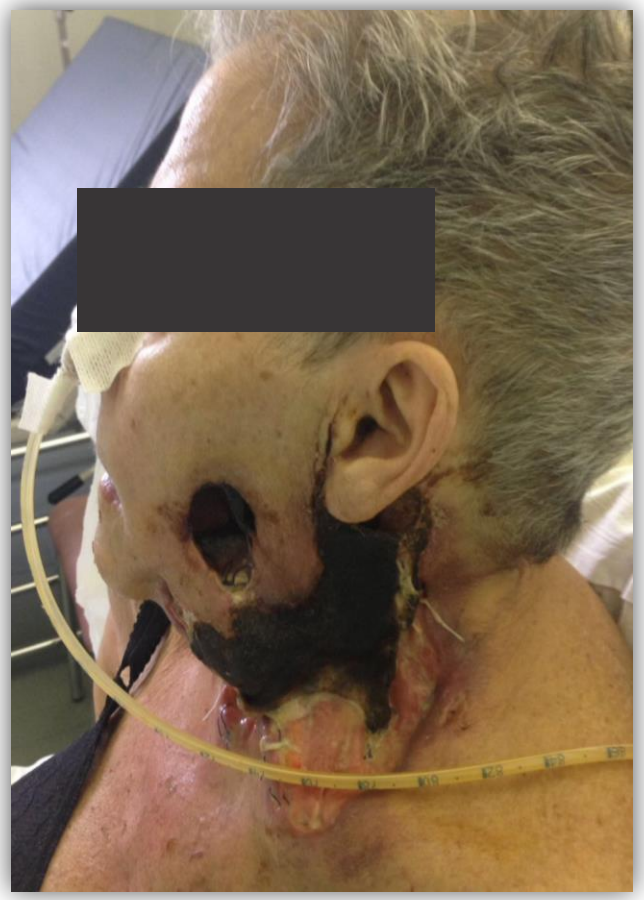
BIOFILM-ASSOCIATED SURGICAL SITE INFECTIONS

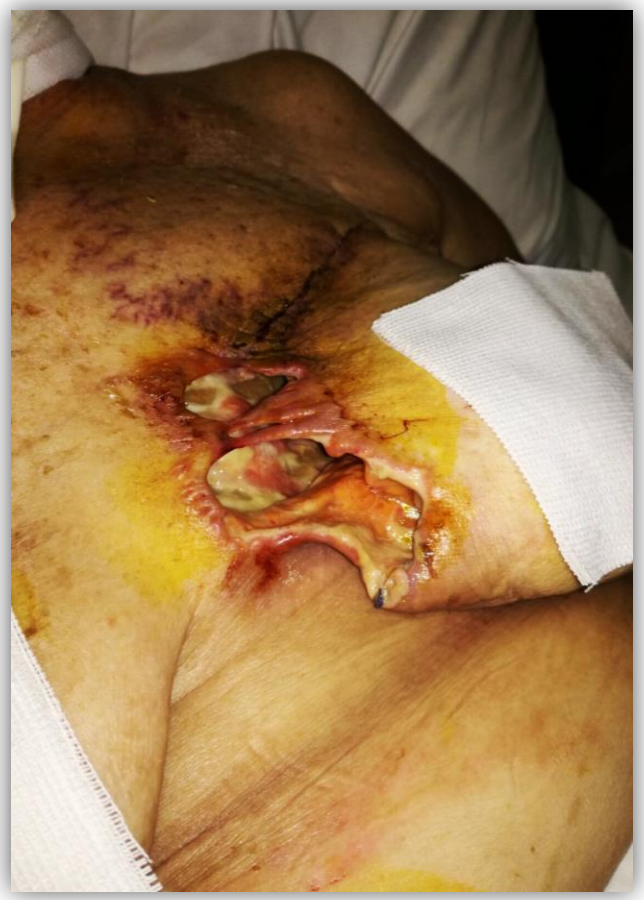
78-Year-old woman with oral cancer

Tracheostomy over the second tracheal ring

Pectoralis major flap for reconstructive head and neck surgery

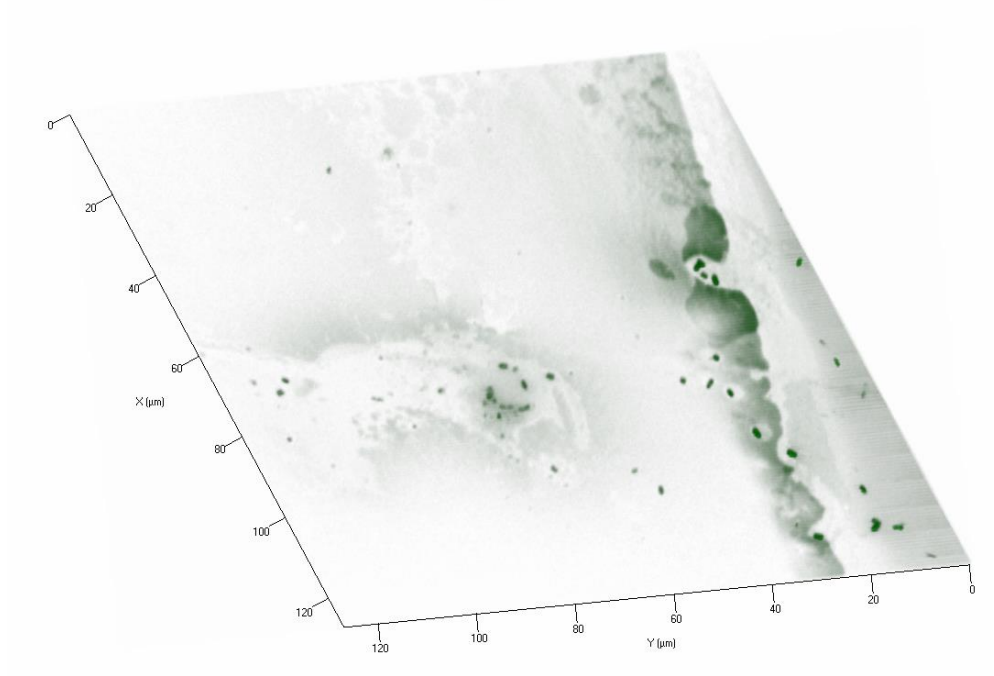
Surgical site infection by: *Staphylococcus aureus Pseudomonas aeruginosa*



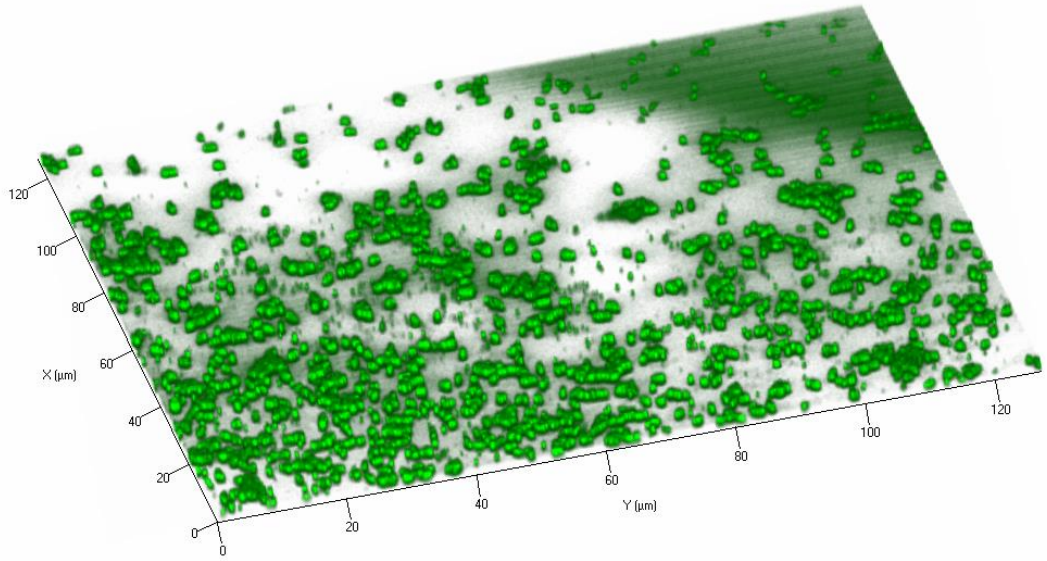


BIOFILM ON SUTURE

STAINLESS STEEL



NYLON



Co-infection by *S. aureus* and *P. aeruginosa*

I.D.: XXX Sig. **XXX** | Sesso F | MICROBIOLOGIA

Data di Nascita: XXX | Età: 78 Anni | PROVENIENZA: OTORINO | Materiale: **TAMPONE ULCERA**

	Staphylococcus aureus	*Pseudomonas aeruginosa*
STRAIN:		
RESULT:	**Moderate biofilm producer**	**High biofilm producer**

Antimicrobials	MIC (mg/L)	INT	BMIC (mg/L)	INT
Benzilpenicillin	> 0.5	R	> 8	R
Clindamycin	≤ 0. 25	S	> 2	R
Daptomycin	≤ 0. 50	S	4	R
Erythromycin	> 2	R	4	R
Fusidic Acid	≤ 0,5	S	≤ 1	S
Gentamicin	≤ 1	S	1	S
Linezolid	2	S	1	S
Oxacillin	> 2	R	> 2	R
Rifampicin	-	-	≤ 0.06	S
Teicoplanin	≤ 0,5	S	4	R
Tigecycline	0.25	S	0.25	S
TMP/SMX	≤ 10	S	≤ 10	S
Vancomycin	≤ 0,5	S	2	S

Antimicrobials	MIC (mg/L)	INT	BMIC (mg/L)	INT
Amikacin	≤ 2	S	≤ 2	S
Cefepime	≤ 1	S	> 32	R
Ceftazidime	4	S	32	R
Ciprofloxacin	≤ 0.25	S	> 2	R
Gentamicin	≤ 1	S	1	S
Imipenem	2	I	> 16	R
Meropenem	1	S	> 16	R
PIT	8	S	> 128	R

Piperacillin/Tazobactam (PIT)
TMP/SMX = Trimethoprim/Sulfamethoxazole

EUCAST Clinical breakpoints - bacteria (v 9.0)

Daptomycin
+
Meropenem

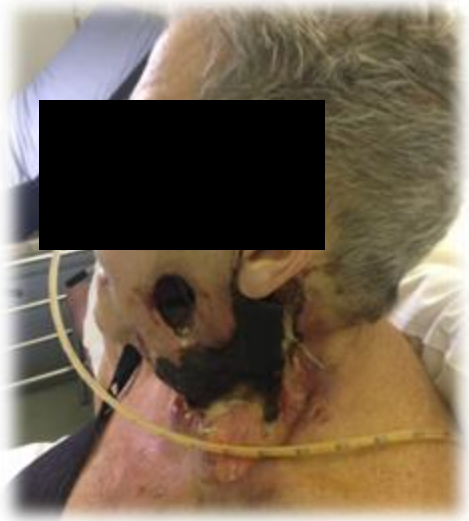
Gentamicin
+
Meropenem

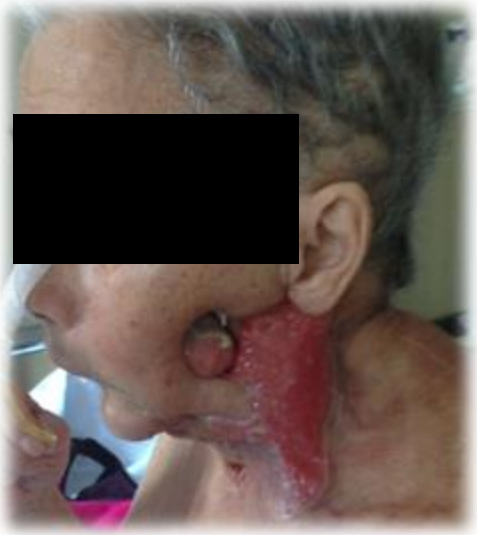
D=0

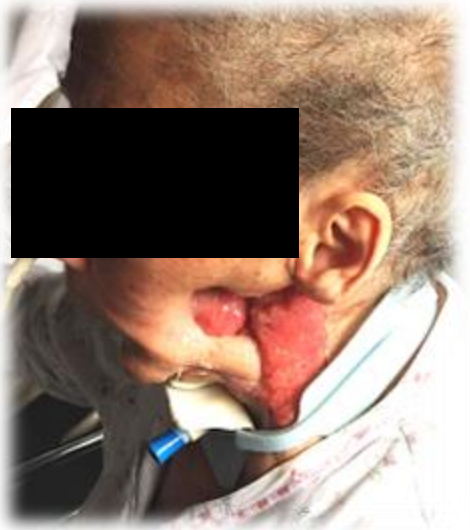
D=7

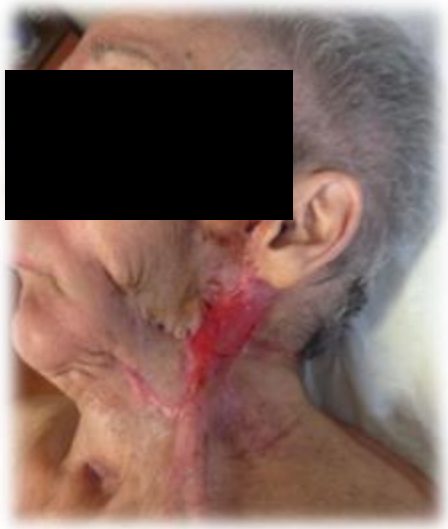
D=14

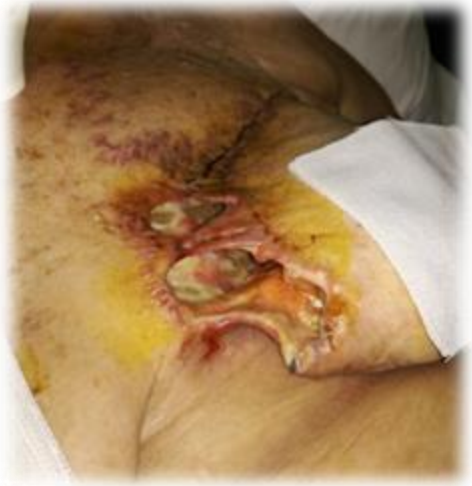
D=21

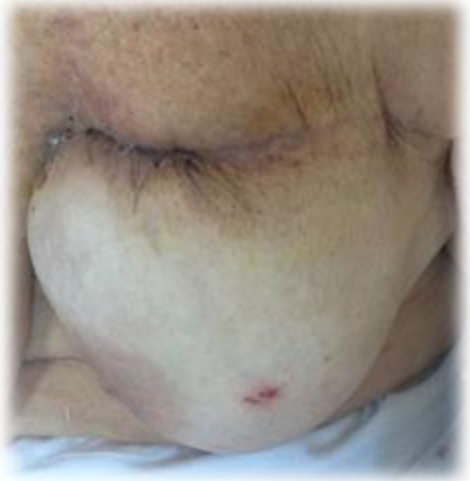


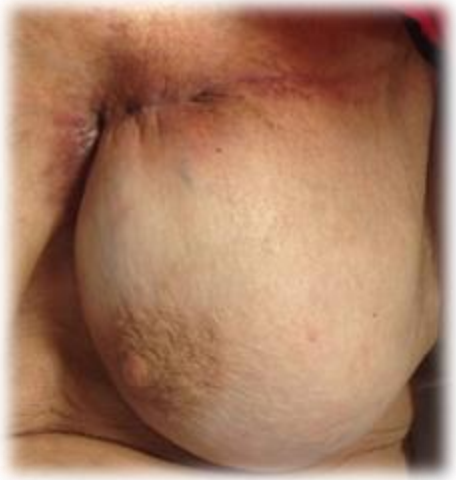












SAPIENZA
UNIVERSITÀ DI ROMA

Dipartimento di
BIOLOGIA E BIOTECNOLOGIE
CHARLES DARWIN

Bacterial biofilm poetry

Distant horizons

The distant horizons call me.
Their rolling waves seduce my heart
Oh, how I long to move into the lush stream.
Oh, how I want to glide down smooth slopes.
Alas, I can not!
Damn, this polymer matrix
Damn, this polymer matrix

(J.C. Bryers, Univ. Washington)

Dipartimento di BIOLOGIA E BIOTECNOLOGIE CHARLES DARWIN

“Nothing in Biology Makes Sense Except in the Light of Evolution”

T. Dobzhansky (1973)

THANK YOU

Questions?

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