Microbial Biofilms Formation: Stages, Matrix, Gene Expression, Quorum Sensing

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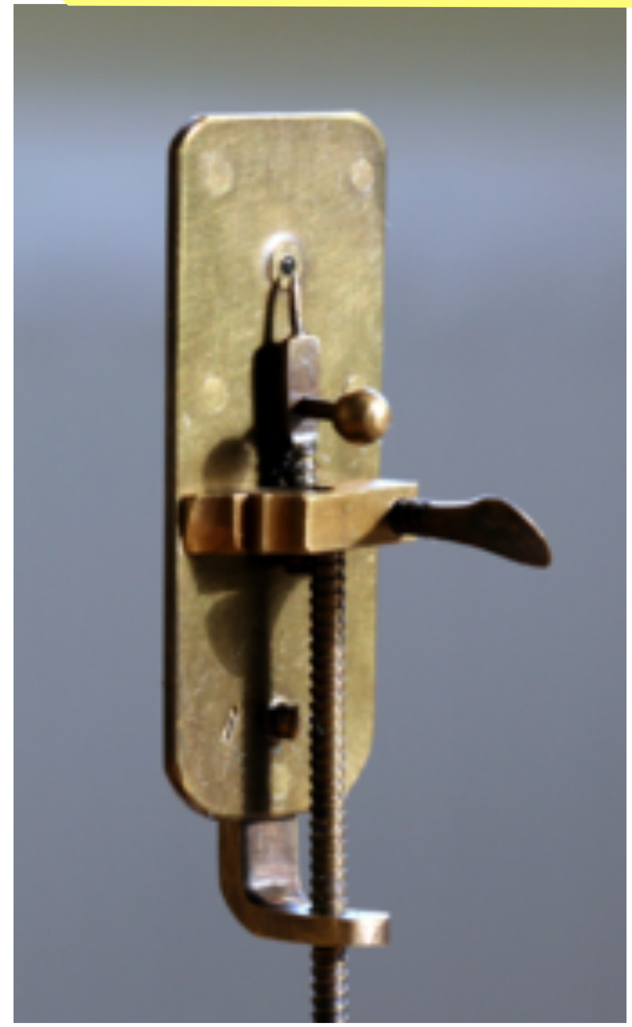
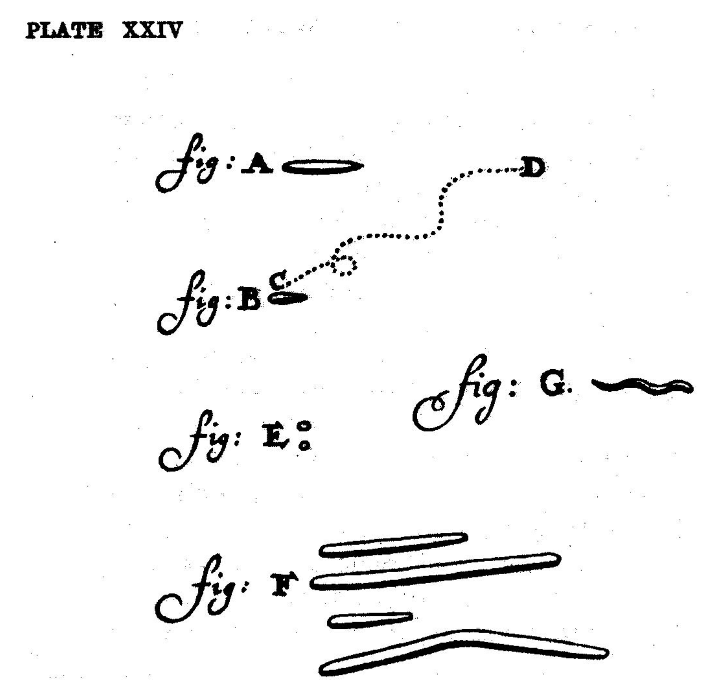
Bacteria as unicellular organisms





Antonie van Leeuwenhoek (1632-1723)
Father of Microbiology

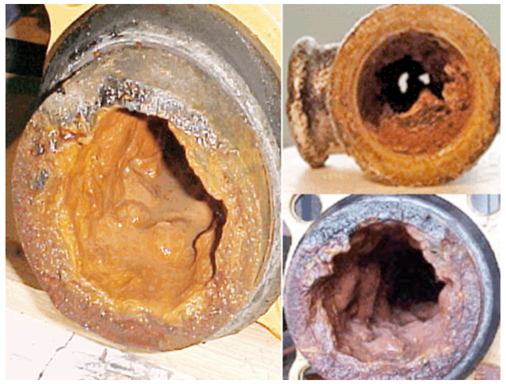
“The number of these animalcules in the scurf of a man's teeth are so many that I believe they exceed the number of men in a kingdom” (Antonie van Leewenhoek London Royal Society, 1684)

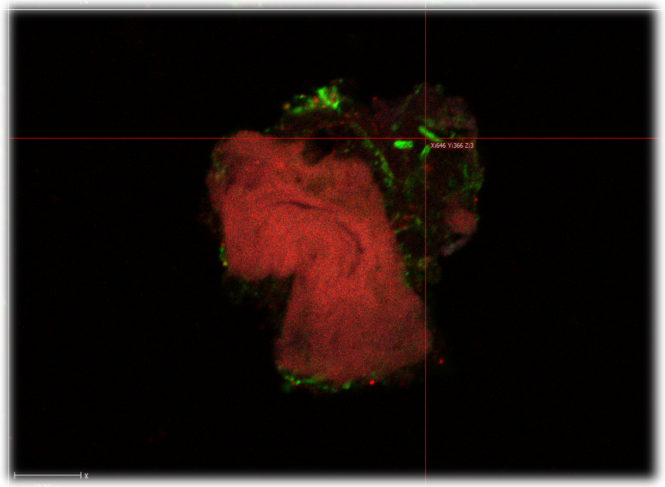
Biofilms in nature

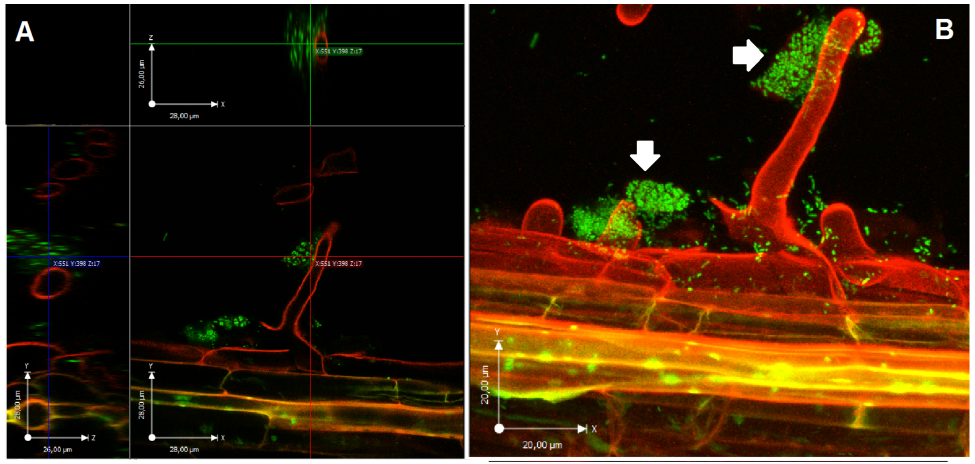
Biofilm en la tumba Abbatija tad-Dejr. Imagen: G. Zammit.

It is the predominant form of life of microorganisms in any hydrated biological system (Trautner & Darouiche, 2004).

- Cooperative community
- Universal distribution
- Can colonize any surface
    - Minerals (pipes, cement…)
    - Roots
    - Even inside cells (our cells)
- Relevant in biogeochemical process
- Present in extreme environments



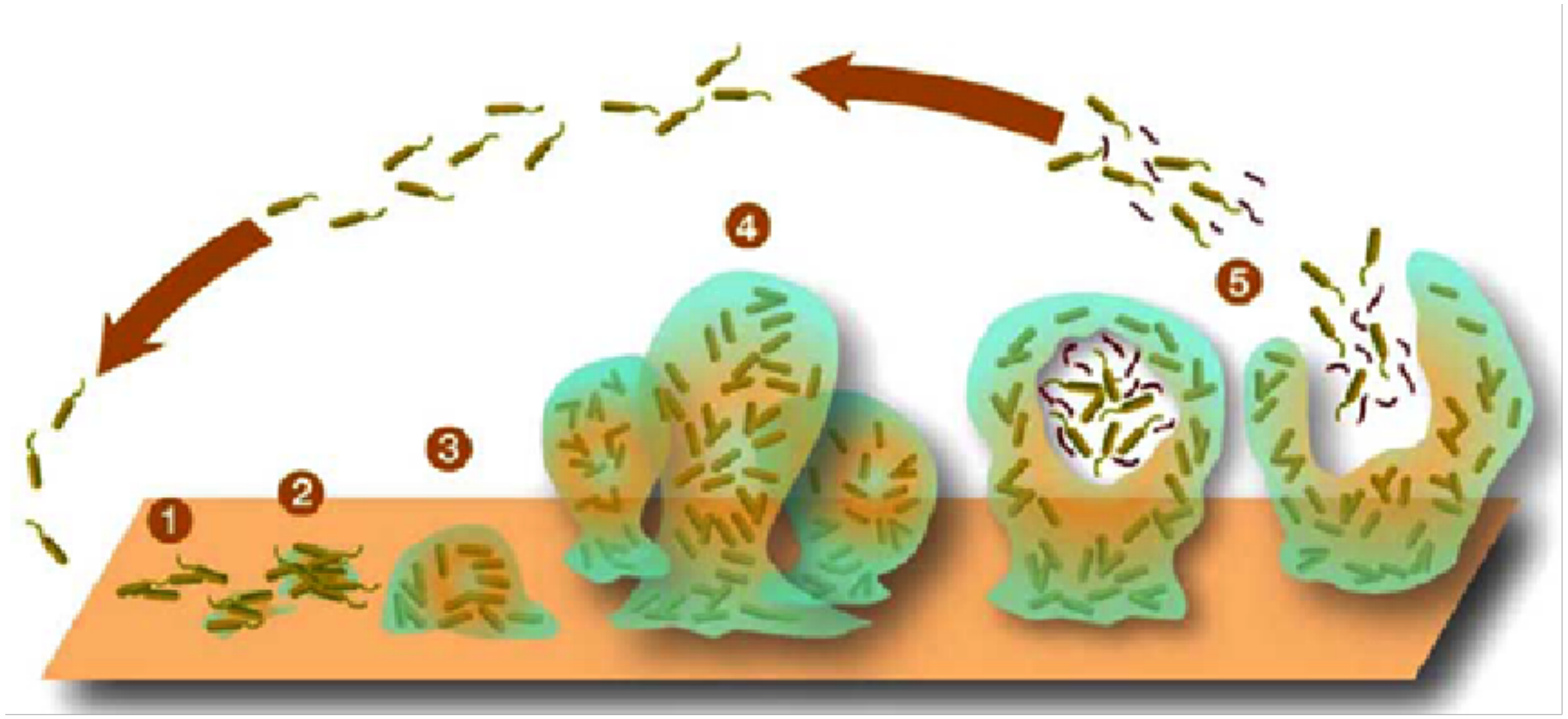




Biofilm definition

A bacterial c**ommunity** that is **irreversibly** attached to a surface, surrounded by a self-produced polysaccharide **matrix**. Microorganism in the biofilm **differ** from their planktonic counterparts in **gene expression**, **metabolic** and **physiological** status.

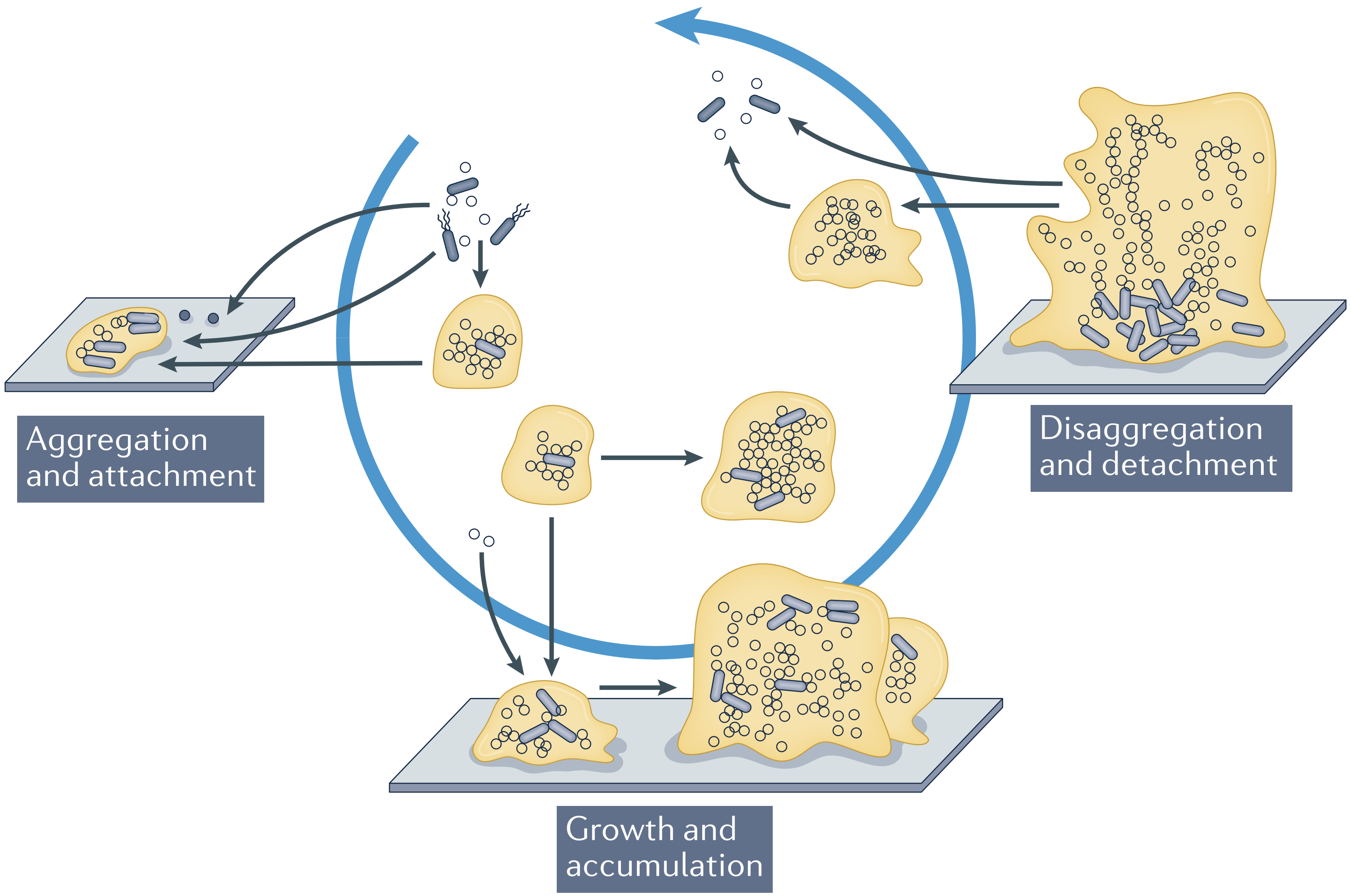
Costerton, 1999



Once mature, the biofilm generates an altered pattern of **bacterial growth**, **physiological cooperation**, and **metabolic efficiency**, which provides a community-based functional coordination that mimics **primitive eukaryotic tissue.**

Dunne, 2002

Biofilm new concepts



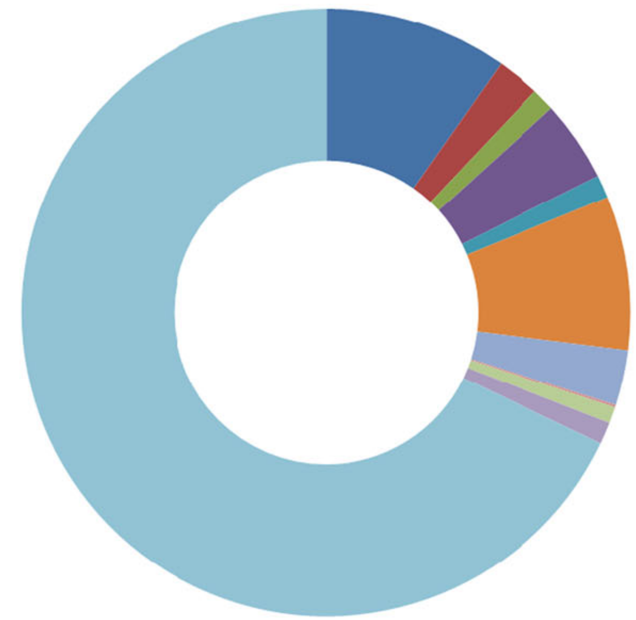
Expanded conceptual model of biofilm formation

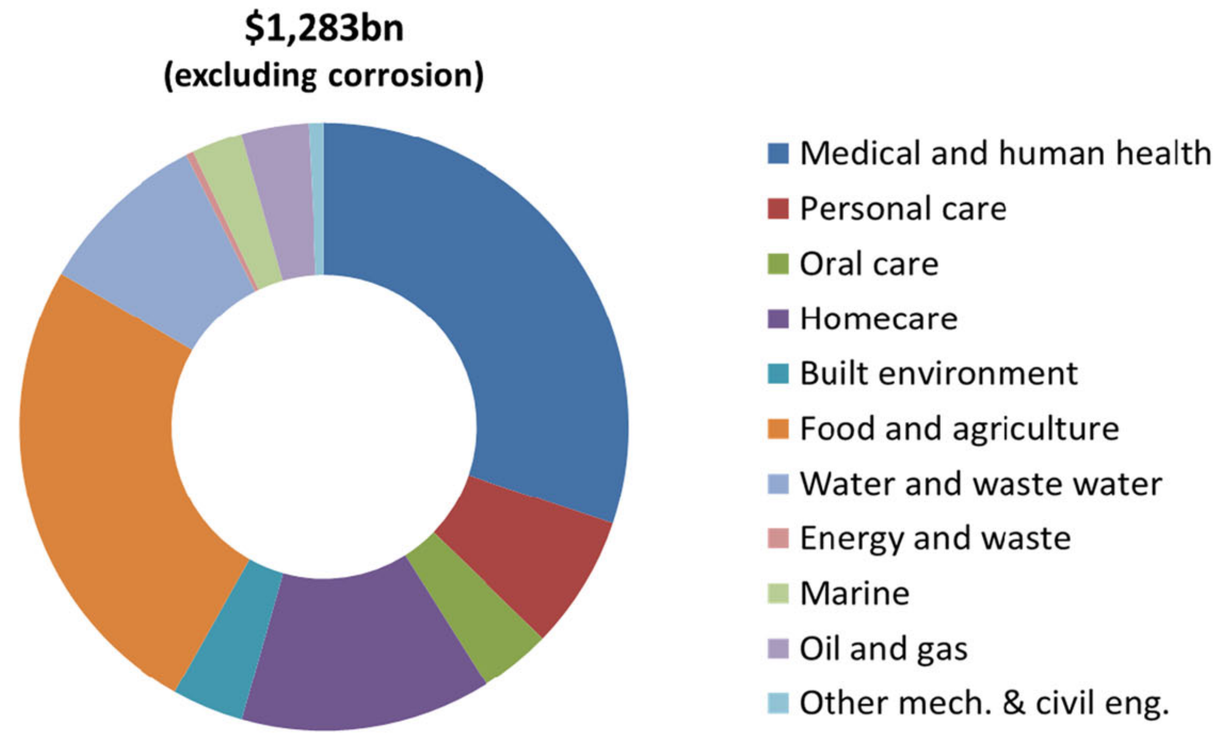
Sauer et al., 2022

Biofilm costs

Table 1. Quantification of market sectors engaging with biofilm technologies—summary of economic information[6].

Sector	Global ($bn)	Comment
Medical and human health		
Wound healing	281	Biofilms form on the surface of wounds and delay healing.
Cystic fibrosis	7.5	The mucus produced in the lungs of cystic fibrosis patients is colonised by pathogens.
Infective endocarditis	16	Biofilms in natural and artificial heart valves result in serious cardiac disease.
Chronic sinusitis	24.4	Chronic sinusitis is often associated with secondary biofilm infections which are difficult to clear.
Ophthalmology	0.759	Surfaces in the eye are prone to biofilm formation.
Human antibiotics	34.2	Bacterial infections are often linked to biofilms and are widely treated with antibiotics.
Central venous catheter bloodstream infection	11.5	Biofilms colonise catheters and can lead to infections.
Catheter-associated urinary tract infection	1	Biofilms colonise catheters and can lead to infections.
Prosthetic cardiac valves and pacemakers	0.22	The surfaces of surgically implanted devices may host biofilms that can only be treated by surgery.
Ventilator-associated pneumonia	2.3	Endotracheal tubes are prone to biofilm infections, which can result in pneumonia and protracted hospital stays.
Breast implants	0.093	The surfaces of breast implants can become infected with biofilms.
Prosthetic joints	7.8	The surfaces of surgically implanted devices may host biofilms that can only be treated by surgery.
Total medical and human health	386.8	
Personal care		
Total personal care	91	Personal care products control biofilms on skin and hair.
Oral care		
Human oral care	47	Tooth scale is a form of biofilm and central to oral health.
Animal oral care	1.85	There is increasing awareness of animal oral health.
Total oral care	48.9	
Homecare		
Homecare	161	Prevention and removal of biofilms contribute to a clean domestic environment.
Textiles	10	Fabrics hostile to biofilms contribute to hygiene.
Total homecare	171	
Built environment		
Cleaning and related hygiene products	41.5	Prevention and removal of biofilms contribute to a clean environment and are essential to some institutions and industry.
Anti-microbial surfaces	7.1	Surfaces hostile to biofilms contribute to hygiene.
Total built environment	48.6	
Food and agriculture		
Crops—microbials	5.3	Biofilm-forming bacteria are used as both biofertilisers and biopesticides.
Crops—antimicrobials	10.4	Prevention of biofilms optimises horticultural output.
Animal husbandry	4.3	Animal health and growth can be promoted by the use of antibiotics to control biofilms in the digestive system.
Food processing	accounted for in 'crops'	Food safety requires elimination of biofilms.
Preservatives	1.5	Control of biofilms is important to retaining freshness and wholesomeness.
Food packaging	303	Part of the purpose of food packaging is to prevent biofilm growth and control the environment within the packaging.
Total food and agriculture	324	
Water and wastewater		
Water	90.4	Uncontrolled biofilms in water distribution systems present health hazards.
Wastewater treatment	27	Wastewater treatment technologies use biofilms to cleanse water.
Total water and wastewater	117	
Energy and waste		
Anaerobic digestion	2	The composting of putrescible waste by biofilms produces gas and energy.
Landfill gas	3.3	Decomposition of organic matter in landfills by biofilms produces methane which can be captured and used as fuel.

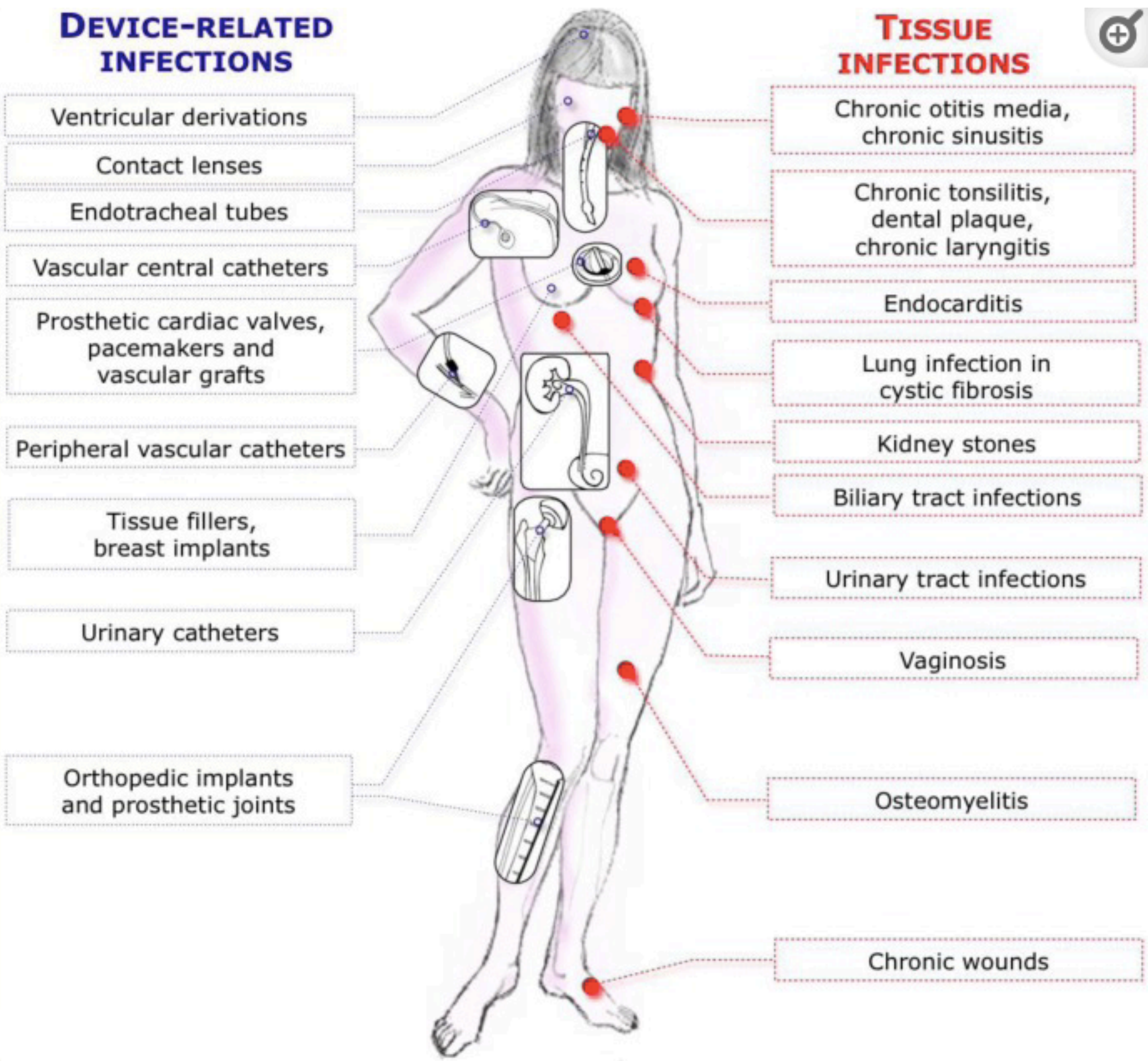




(Camara et al., 2022)

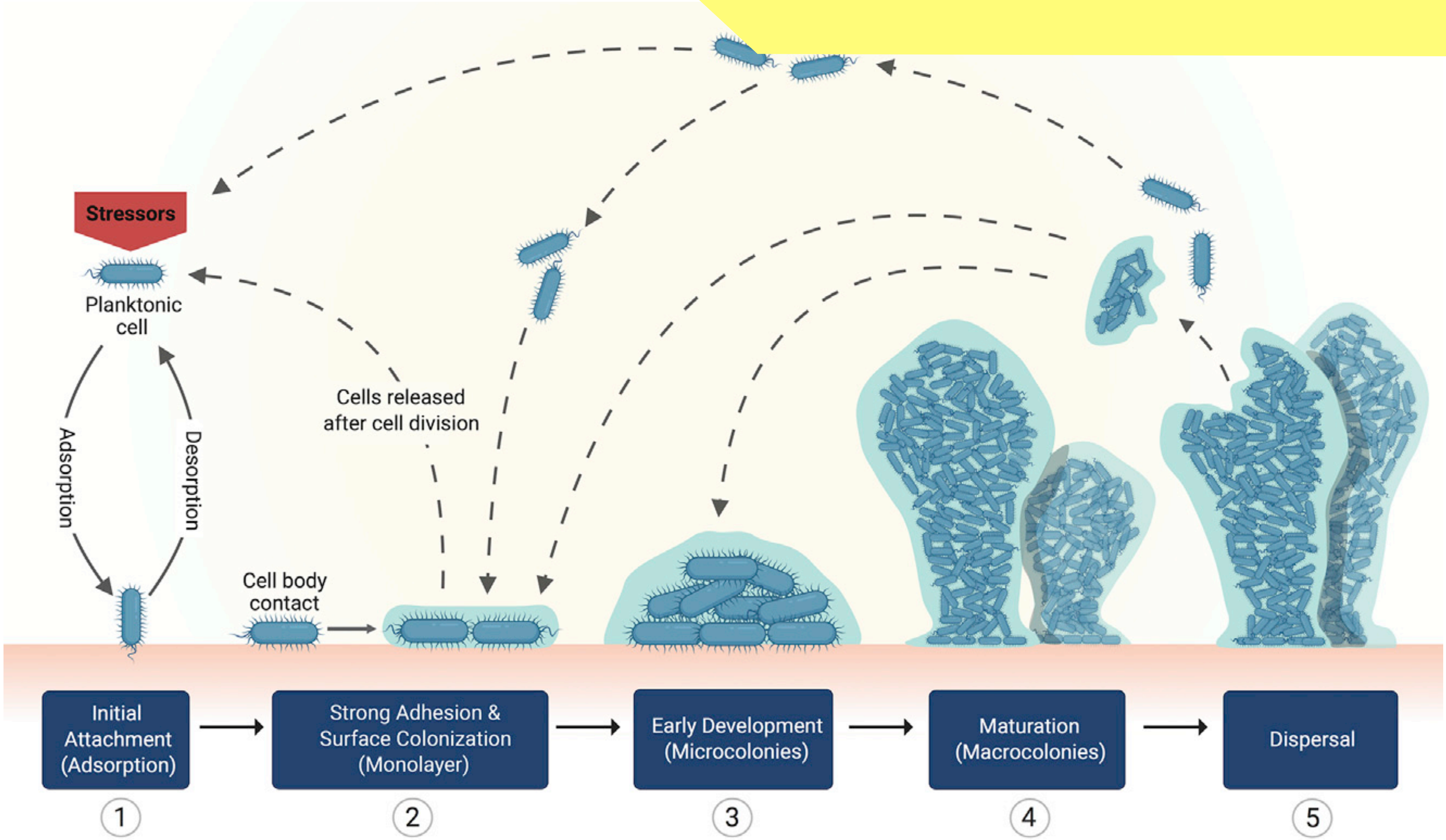
Biofilm in the medical context

More than **60%** of hospital **complications**
Around **80%** of **deaths** associated with infections are attributed to microorganisms forming **biofilms**
80% of known pathogenic bacteria are implicated in **implant-associated infections**.



Leveaux et al., 2013

Biofilm stages



Guzmán-Soto et al, 2021

1. **Reversible** adhesion
2. **Irreversible** adhesion
3. **Microcolonies** formation & **matrix** production
4. **Maturation**
5. **Dispersal**

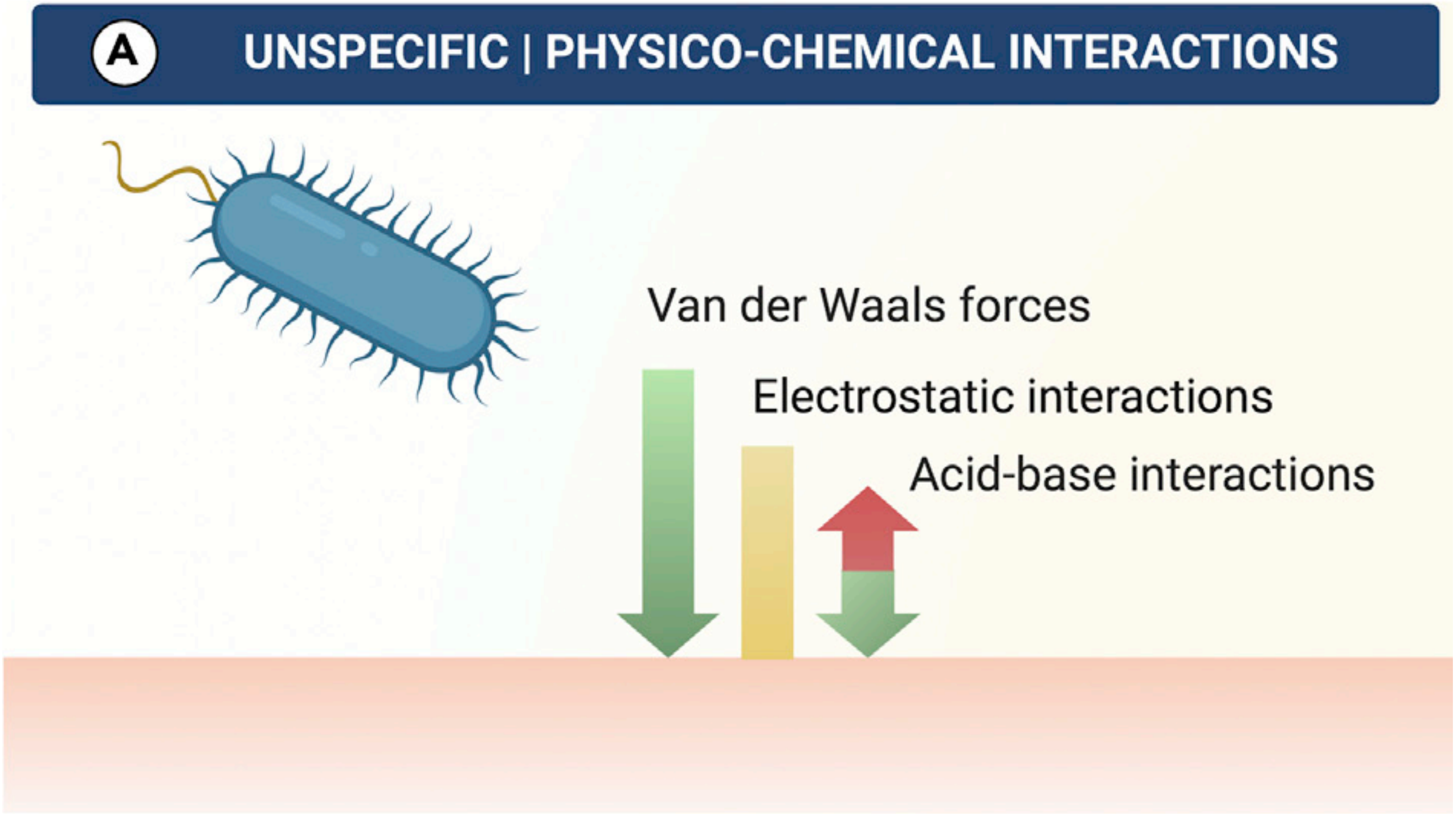
Costerton, 1999

Reversible adhesion

Adhesion and interaction with surfaces to form a biofilm are **crucial** for survival in a complex environment.

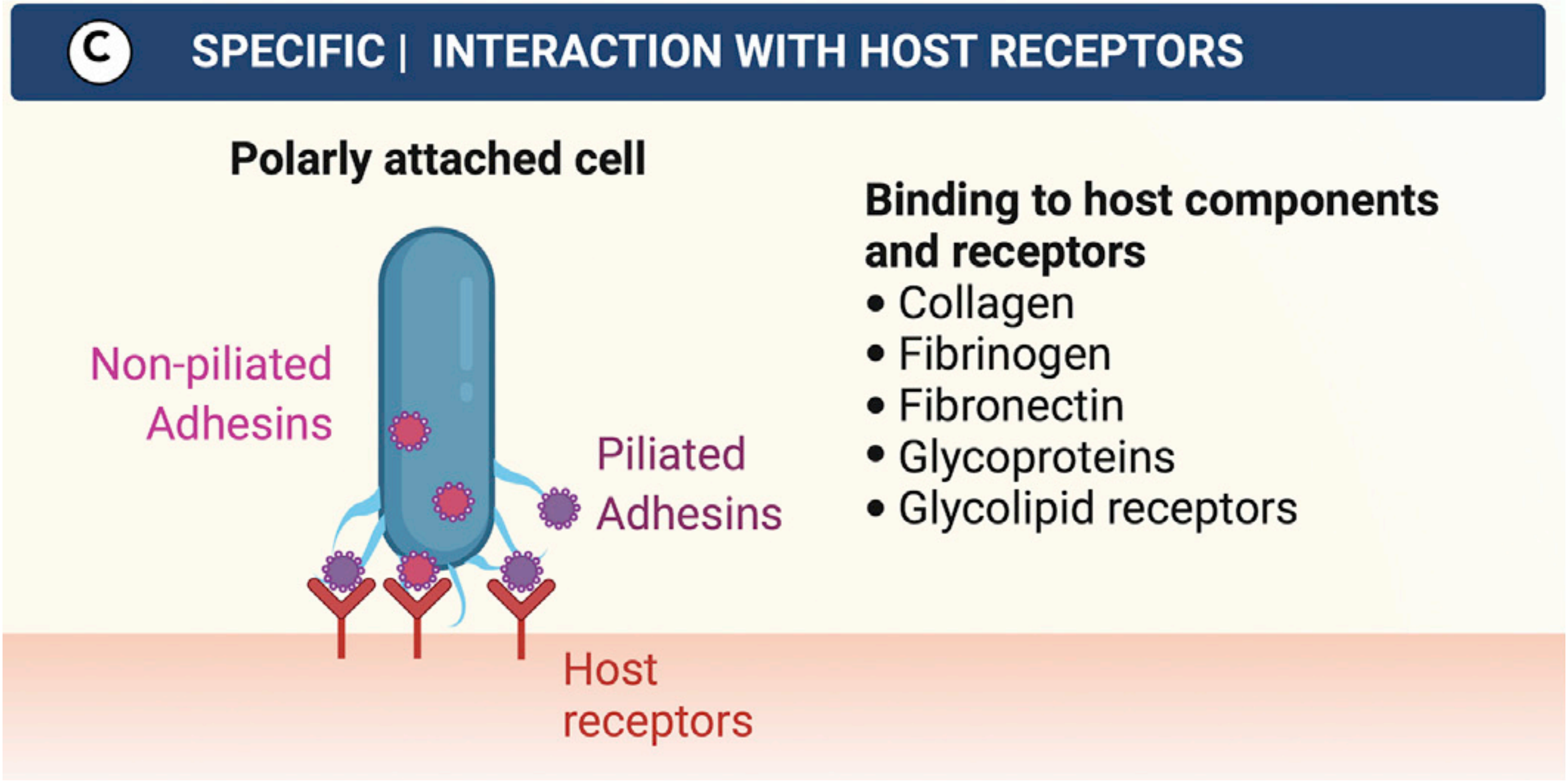
Specific and **nonspecific interactions** between bacteria and surfaces

Nonspecific interactions with **abiotic** surfaces



Reversible adhesion

In **biotic** surfaces, receptor-ligand type interactions

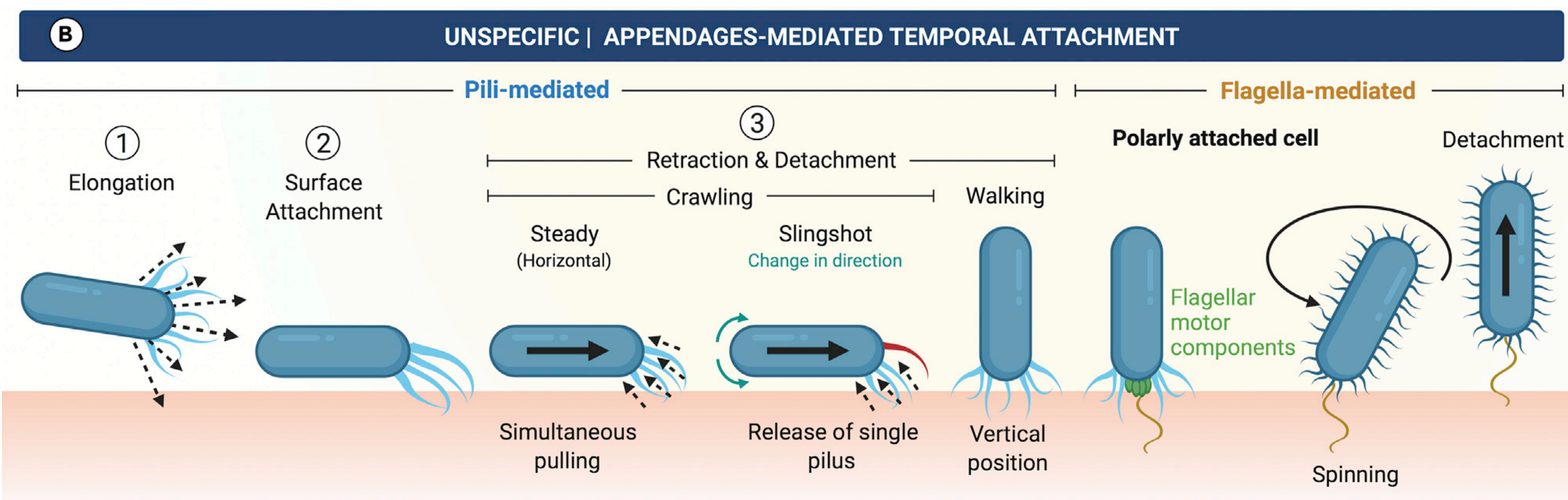


Reversible adhesion

Negative charge of the bacterial surface
Repulsive electrostatic forces
Liquid medium: Repulsive hydrodynamic forces near the surface

Fimbriae/flagella for adhesion/movement

Once on the surface, adhesion is increased by specific and nonspecific **adhesins**, leading to **irreversible adhesion**



Irreversible adhesion

It is influenced by:

environmental factors (pH, salinity, etc.)

physicochemical properties of the **surface** (roughness, hydrophobicity, charge, etc.)

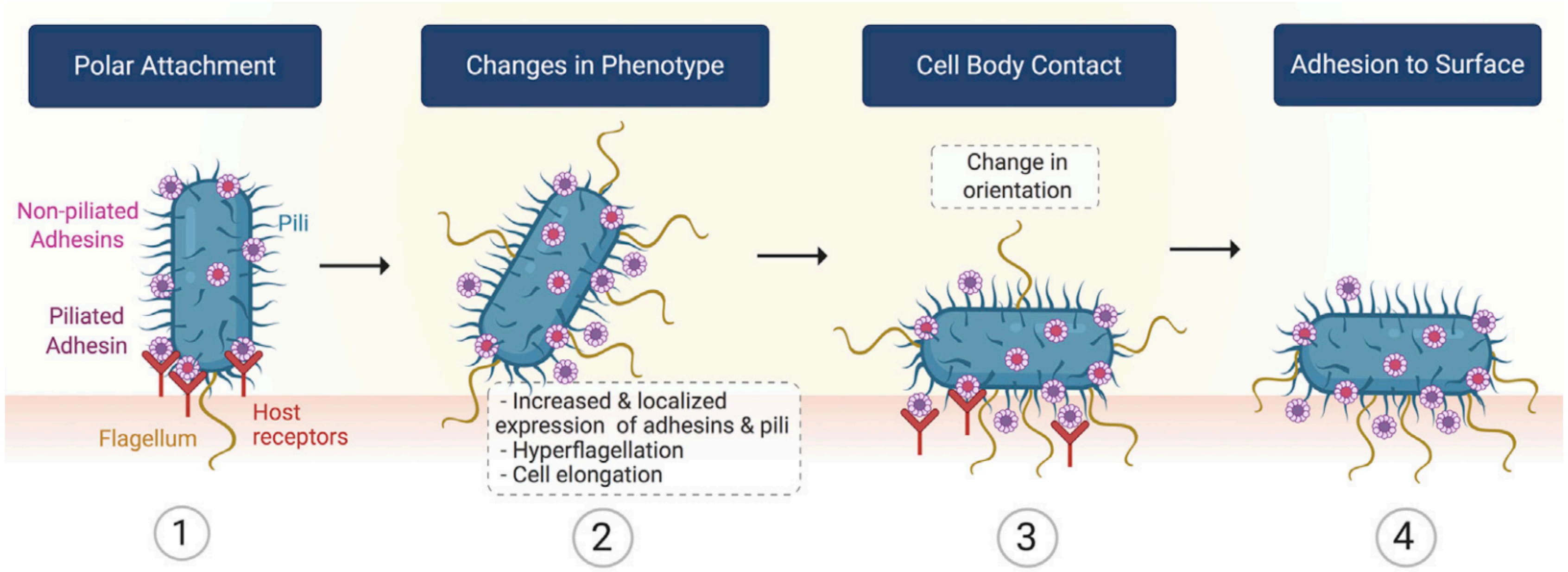
presence of a **conditioning film** (a layer of organic and inorganic compounds absorbed onto the surface)

For permanent adhesion, bacteria use nonspecific adhesin as:

fimbrial

nonfimbrial

discrete polysaccharide adhesins



Irreversible adhesion

Fimbrial/pili adhesins

A ubiquitous group of adhesins, both Gram-positive and negative, involved in adhesion to biotic/abiotic surfaces, DNA transfer, biofilm formation, relevant in early stages, mediating intercellular interaction through aggregation and microcolony formation, playing a role in biofilm secondary structure through twitching motility, and 4 subgroups defined by the type of secretion and assembly.

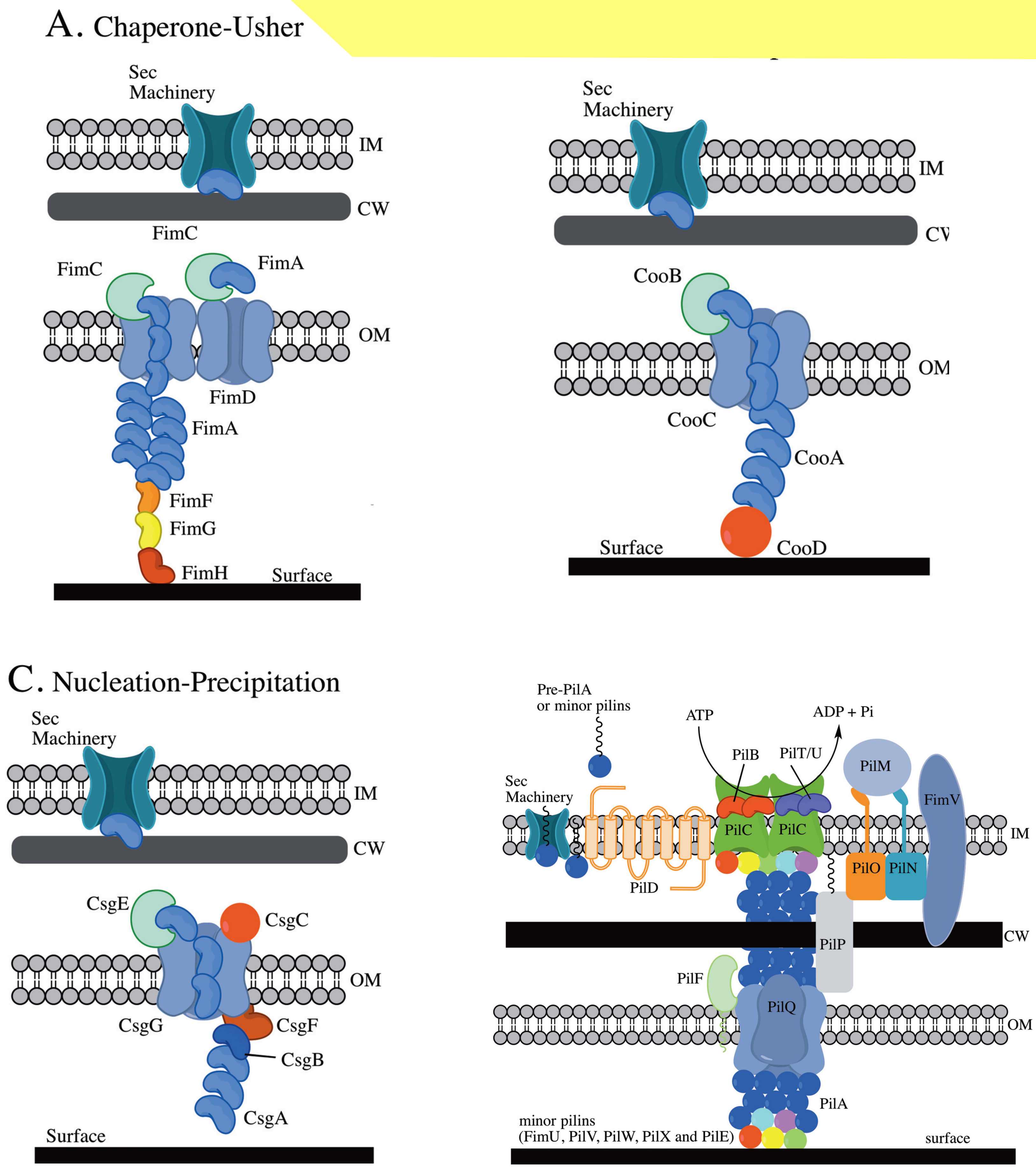
CUP (chaperon-usher pili)
Type IV fimbriae
Alternative CUP
Fimbriae assembled by extracellular nucleation-precipitation (curli)

Irreversible adhesion

TABLE 1 **Examples of fimbrial adhesins involved in biofilm form**

Pili type	Major pilus proteins	Minor proteins and assembly proteins	Bacteria	Reference
Chaperone/ usher	EcpA or MatA (ECP pili)	EcpC, EcpD, EcpE	*E. coli*	196
	FimA (type I)	FimC, FimD, FimF, FimG, FimH	*E. coli, Klebsiella pneumoniae, X. fastidiosa, Enterobacter amylovora, Serratia marcescans*	21, 197–200
	CsuA/B (type I)	CsuC, CsuD, CsuE	*Acinetobacter baummanii*	201
	MrkA (type 3)	MrkB, MrkC, MrkD	*K. pneumoniae, E. coli (UPEC), Citrobacter koseri*	202, 203
Type IV pili				
Type IVa	PilA,	PilE, PilD, PilV, PilW, PilX	*P. aeruginosa*	14, 45, 46
	PilE	PilD, PilH, PilI, PilJ, PilK, PilX , PilV, ComP, PilD	*Neiserria* spp.	204, 205
	MshA (MSHA)	MshB, C, D, E, F, G, I, J, K, L, M, N, O, P, and MshQ	*Vibrio parahemolyticus* and *Vibrio cholerae*	49, 206
	PilA (ChiRP)	PilB,PilC,PilD	*V. parahemolyticus*	49, 207
Type IVb	BfpA (bundle forming)	BfpP, BfpI, BfpJ, BfpK,	*E. coli* (EPEC)	208
	TcpA (Tcp)	TcpB, C, D, E, F, TcpJ	*V. cholerae*	50
Tad	Flp	TadA, TadB/C, TadD, TadE, TadF, TadG, TadV, RcpA, RcpB, TadZ,	*Aggregatibacter actinomycetemcomitans*	58, 64
	PilA	CpaA, CpaB, CpaC, CpaD, CpaE, CpaF	*C. crescentus*	11, 66
	Flp	TadA, TadB, TadC, TadD, TadF, TadG, FppA, RcpA, RcpC, TadZ	*P. aeruginosa*	56, 209
	CtpA (common pili)	CtpA, CtpB, CtpC, CtpD, CtpE, CtpF, CtpG, CtpH, CtpI	*Agrobacterium tumefaciens*	252
Alternative CU	CooA (CS1)	CooB, CooC, CooD	*E. coli* (ETEC)	26, 28
	CblA (cable pilus)	CblB, CblC, CblD	*Burkholderia cepacia* complex	36
Nucleation/ precipitation	CsgA(Curli)	CsgB, CsgG, CsgE, CsgF, CsgD	*E. coli, Enterobacter cloacae, Citrobacter* spp.	72, 77
	AgfA (Tafi)	AgfB, AgfC, AgfD, AgfE, AgfF	*Salmonella enteritidis*	210, 211
	FapC	FapA, FapB, FapD, FapE, FapF	*Pseudomonas* spp.	212

Irreversible adhesion



Irreversible adhesion

Non fimbrial adhesins

T1SS

T5SS

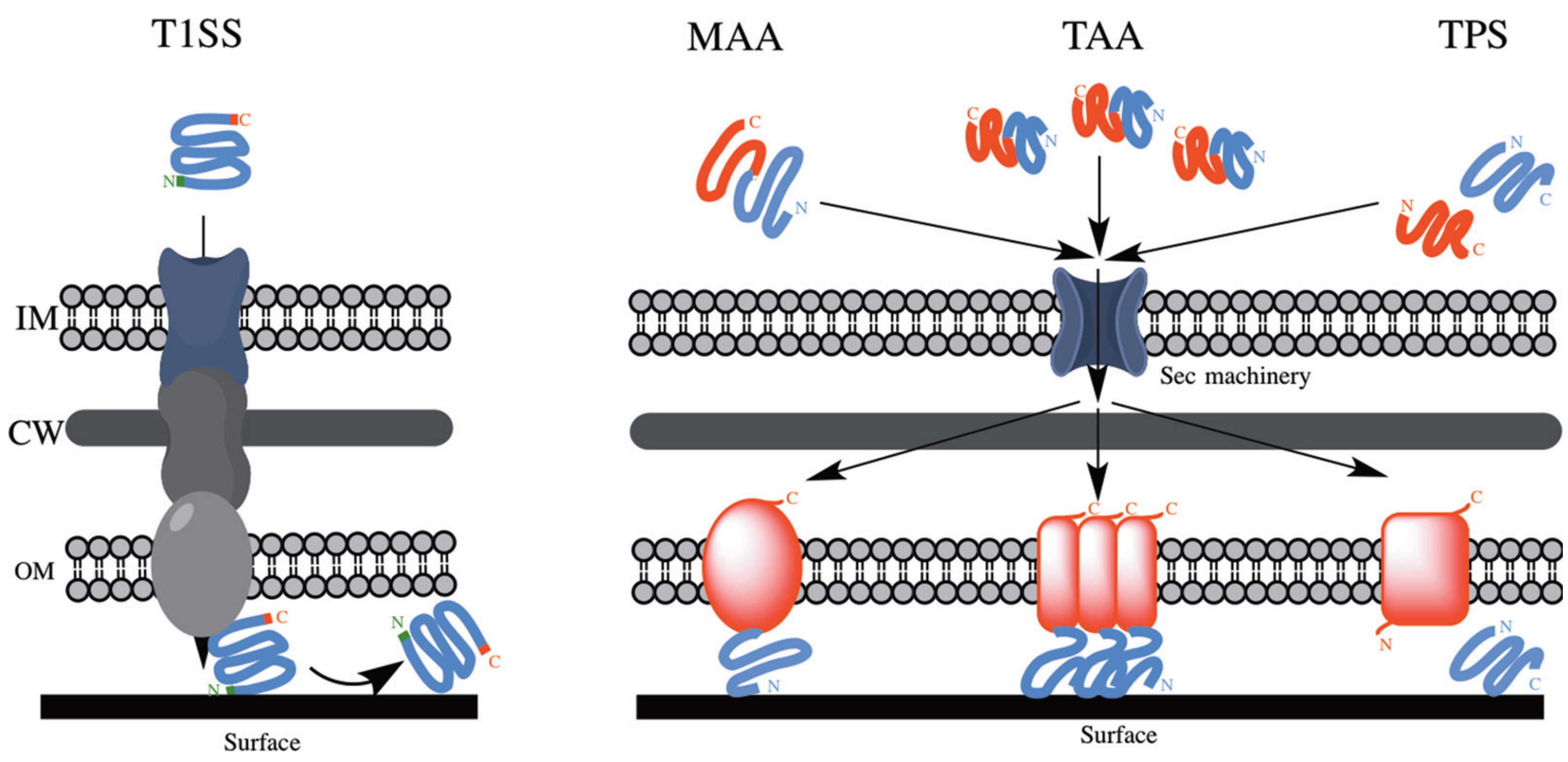


FIGURE 3 Schematic overview of the various secretion systems of nonfimbrial adhesins. The type 1 secretion system (T1SS) and three classes of type 5 secretion system (T5SS) (monomeric autotransporter adhesins [MAA], trimeric autotransporter adhesins [TAA], and two-partner secretion [TPS] systems) are represented. In T1SS, the adhesin is exported directly from the cytoplasm to the extracellular milieu via a pore comprised of three proteins. In T5SS, the adhesin is translocated from the cytoplasm to the periplasm by the Sec machinery and auto-assembled in the outer membrane. See text for more details. Abbreviations: IM, inner membrane; CW, cell wall; OM, outer membrane. doi:10.1128/microbiolspec.MB-0018-2015.f3

TABLE 3 Selected examples of nonfimbrial adhesins experimentally shown to be involved in biofilm formation by Gram-negative bacteria

Protein	Organism	Size (aa)	Reference
Biofilm associated proteins (Bap) – T1SS			
LapA	*Pseudomonas putida*	8,682	84
BapA / AdhA	*B. cenocepacia*	2,924	213
LapA	*Pseudomonas fluorescens*	4,920	85
BapA	*S. enterica*	3,825	214
YeeJ	*E. coli*	2,358	102
Bap	*Acinetobacter baumannii*	8,621	215
LapF	*P. putida*	6,310	89
BfpA	*Shewanella oneidensis*	2,768	216
MRP	*Pectobacterium atrosepticum*	4,558	217
BfpA	*Shewanella putrefaciens*	4,220	218
Cat-1	*Psychrobacter articus*	6,715	219
Monomeric autotransporter adhesins – T5SS			
Ag43	*E. coli*	1,039	104
Cah	*E. coli*	2,850	220
AIDA	*E. coli*	1,237	107
TibA	*E. coli*	989	221
YfaL/EhaC	*E. coli*	1,250	102
YpjA/EhaD	*E. coli*	1,526	102
YcgV	*E. coli*	955	102
Hap	*H. influenzae*	1,392	127
EhaA	*E. coli*	1,328	222
EhaB	*E. coli*	980	223
UpaH	*E. coli*	2,845	224
UpaC	*E. coli*	996	225
UpaI	*E. coli*	1,254	226
MisL	*S. enterica*	955	227
Trimeric autotransporter adhesins – T5SS			
YadA	*Yersinia pseudotuberculosis*	434	119
UspA1	*Moraxella catarrhalis*	955	228
Hap/MID	*M. catarrhalis*	2,090	228
UpaG	*E. coli*	1,779	120
SadA	*S. enterica*	1,461	121
AtaA	*Acinetobacter* sp. Tol5	3,630	229
EhaG	*E. coli*	1,589	230
BbfA	*Burkolderia pseudomallei*	1,527	122
Hemagglutin-like adhesins – T5SS			
HxfB	*X. fastidiosa*	3,376	231
HxfA	*X. fastidiosa*	3,458	231
HMW1	*H. influenzae*	1,536	127
HMW2	*H. influenzae*	1,477	127
XadA	*X. fastidiosa*	763	125
YapH	*Xanthomonas fuscans*	3,397	124
FhaB	*X. fuscans*	4,490	124
XacFhaB	*Xanthomonas axonopodis*	4,753	232
CdrA	*P. aeruginosa*	2,154	129
FHA	*B. pertussis*	3,590	128
BcpA	*Burkholderia thailandensis*	3,147	233

Polysaccharide adhesins

strongly associated with the bacterial surface, forming the **capsule** (capsular polysaccharides)

loosely associated or secreted (extracellular polysaccharides, EPS)

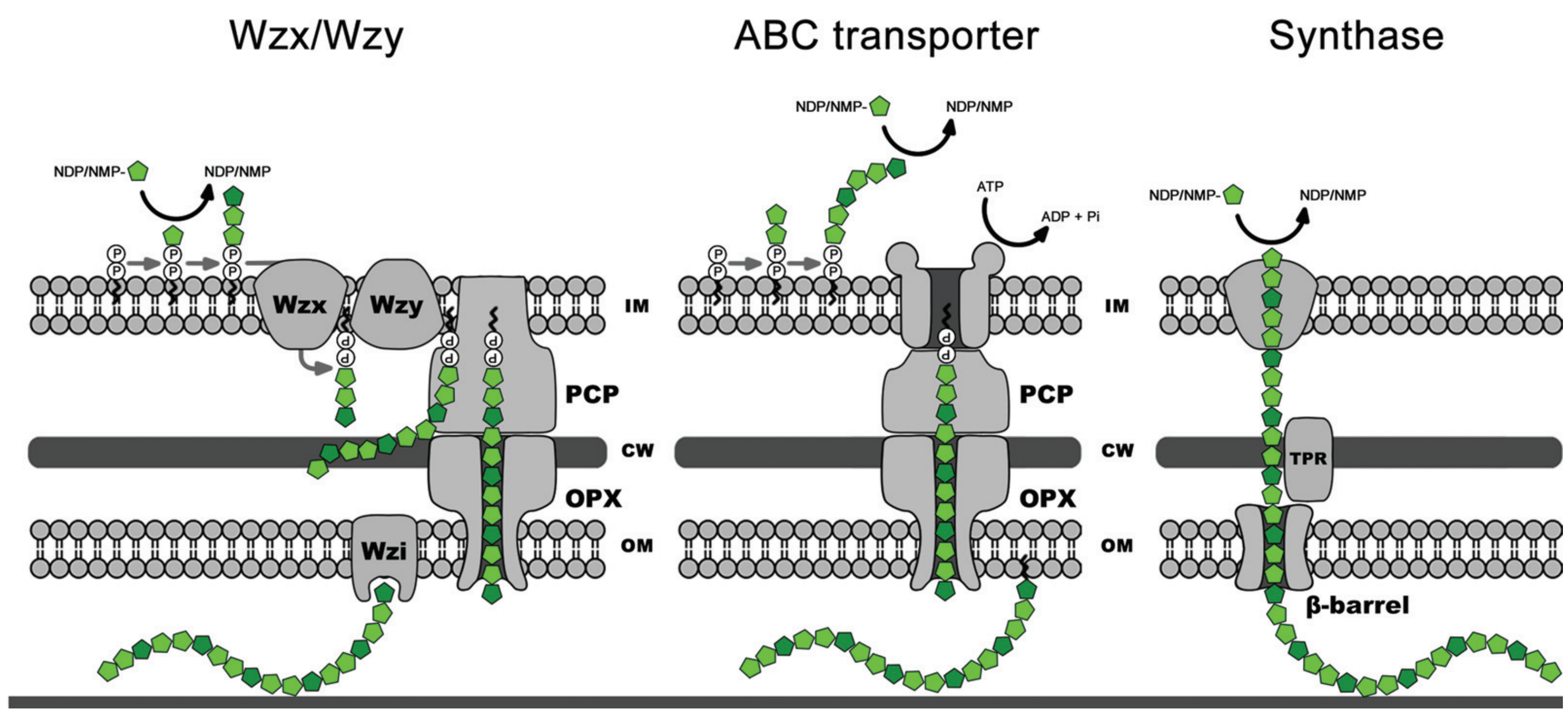
differences are experimentally defined and have limited physiological relevance

from an adhesive perspective:

protective polysaccharides form a protective barrier
aggregative polysaccharides (EPS) adhesive/cohesive properties

Irreversible adhesion

Aggregative polysaccharides



Aggregative polysaccharides

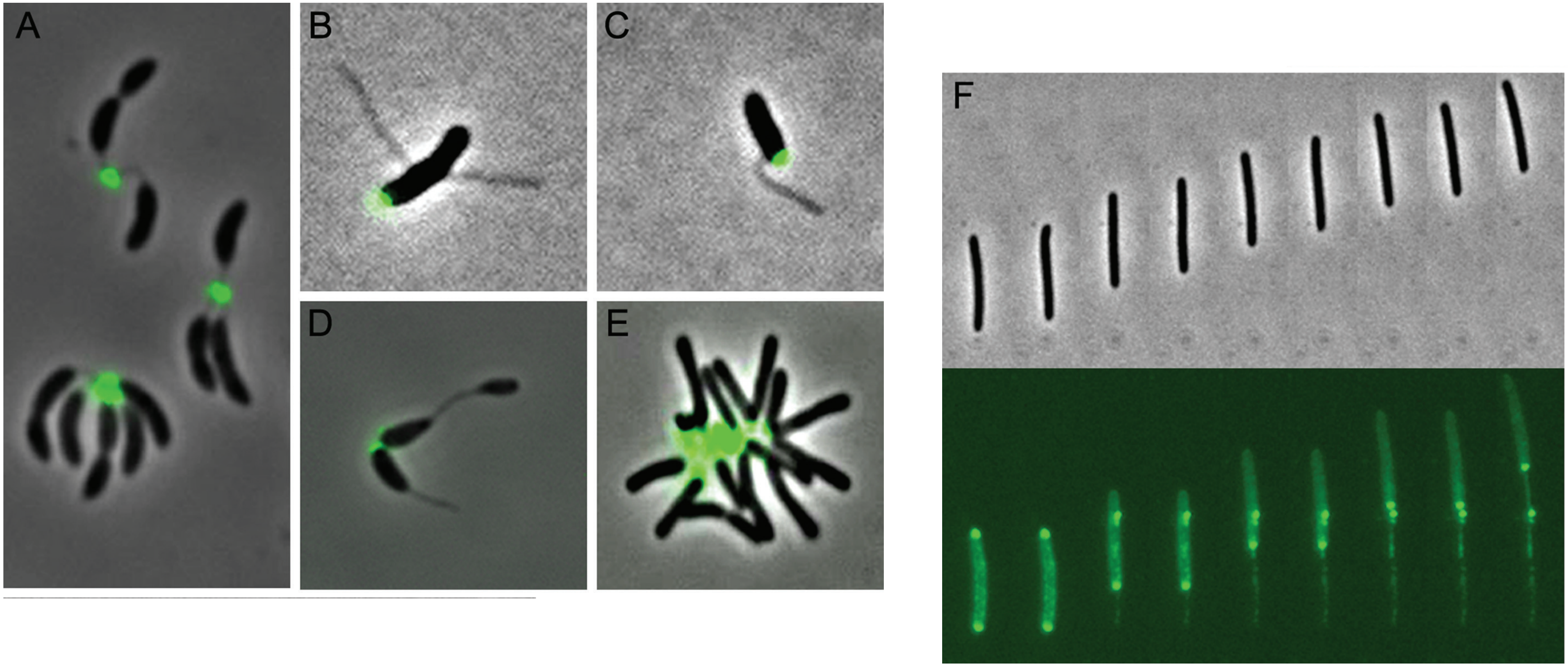


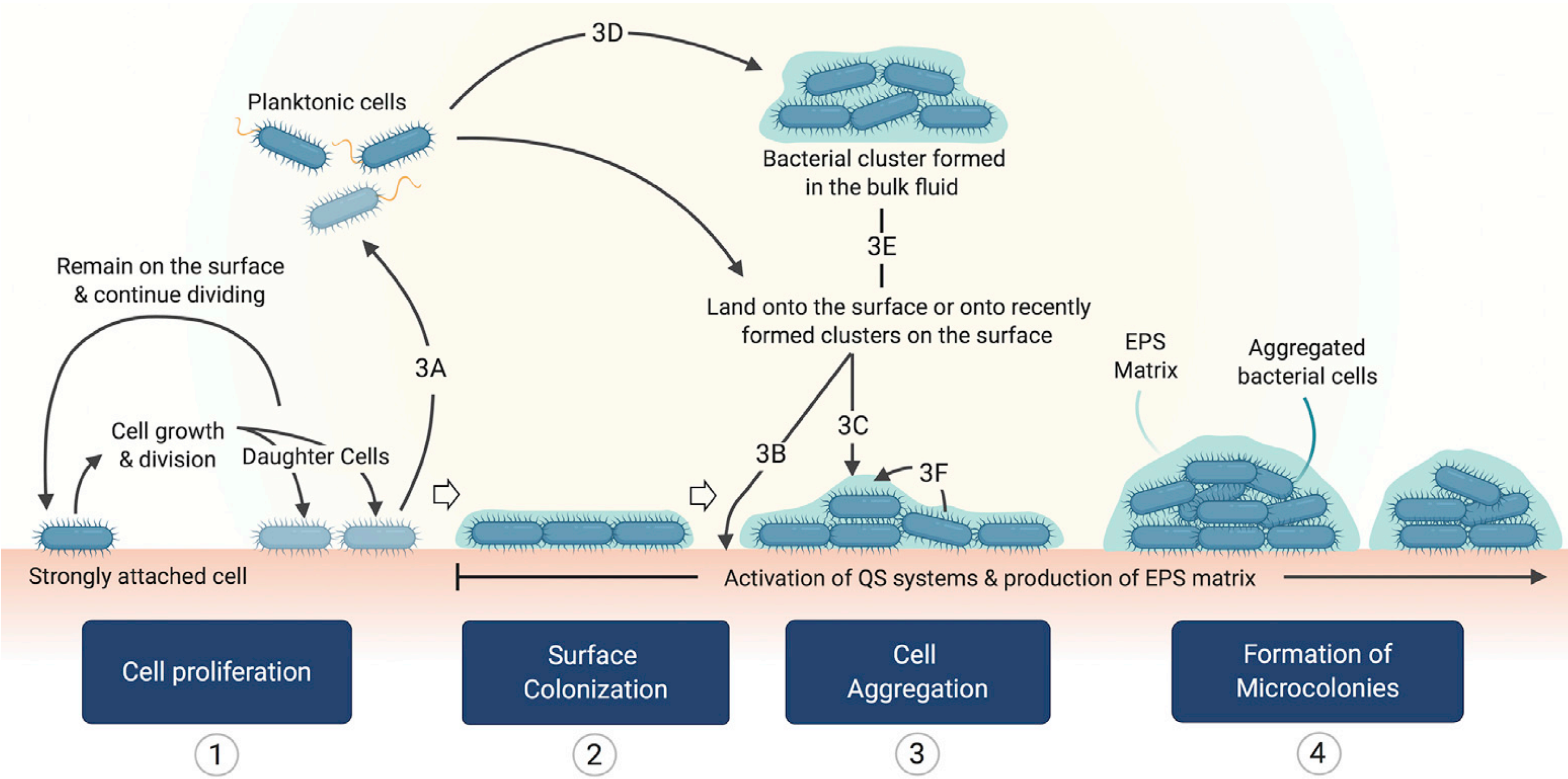
FIGURE 6 **Selected examples of discrete polysaccharides. AF488-conjugated wheat germ agglutinin lectin labelling of the holdfast in (A) *C. crescentus*, (B) *A. biprosthecum* (courtesy of Chao Jiang), (C) *Asticcacaulis excentricus* (courtesy of Chao Jiang), and (D) *Hyphomicrobium vulgare* (courtesy of Ellen Quardokus). (E) AF488-conjugated wheat germ agglutinin lectin labelling of the UPP in *A. tumefaciens*. (F) FITC-conjugated ConA lectin labelling of the slime in *M. xanthus*. doi:10.1128/microbiolspec.MB-0018-2015.f6**

Aggregative polysaccharides

TABLE 4 **Selected examples of aggregative polysaccharides experimentally shown to be involved in biofilm formation by Gram-negative bacteria**

Polysaccharide	Organism	Composition/structure	Reference
Alginate	*P. aeruginosa*	β-1,4-linked mannuronic acids and guluronic acids	234
Cellulose	*Gluconacetobacter xylinus, A. tumefaciens, Rhizobium leguminosarum* bv. Trifolii, *Sarcina ventriculli, Salmonella* spp., *E. coli, K. pneumoniae*	β-1,4-linked D-glucose	235–239
Holdfast	*Caulobacter* spp., *Asticcacaulis biprosthecum, Hyphomonas adherens, Hyphomonas rosenbergii, Hyphomicrobium zavarzinii, Maricaulis maris, Oceanicaulis alexandrii*	Suspected to contain β-1,4-linked N-acetyl-D-glucosamine, but the exact composition and structure remain unknown	160, 163, 166, 240–243
PGA	*E. coli, Yersinia pestis, Bordetella* spp., *Actinobacillus* spp., *P. fluorescens*	β-1,6-linked N-acetyl-D-glucosamine	244, 245
Psl	*P. aeruginosa*	Repeating pentasaccharide of 3 mannose, 1 rhamnose, and 1 glucose	246–248
Pel	*P. aeruginosa, P. fluorescens*	Unknown, but reported to be a glucose-rich polysaccharide polymer	246, 249, 250
Slime	*M. xanthus*	Suspected to contain α-D-mannose or α-D-glucose residues, but the exact composition and structure remain unknown	192
UPP	*A. tumefaciens*	Suspected to contain N-acetyl-D-glucosamine residues, but the exact composition and structure remain unknown	68, 179

Microcolonies



Microcolonies

The basic structure of the biofilm
varies depending on the **bacterial species**
under identical conditions: Pseudomonas putida and P. knackmussii

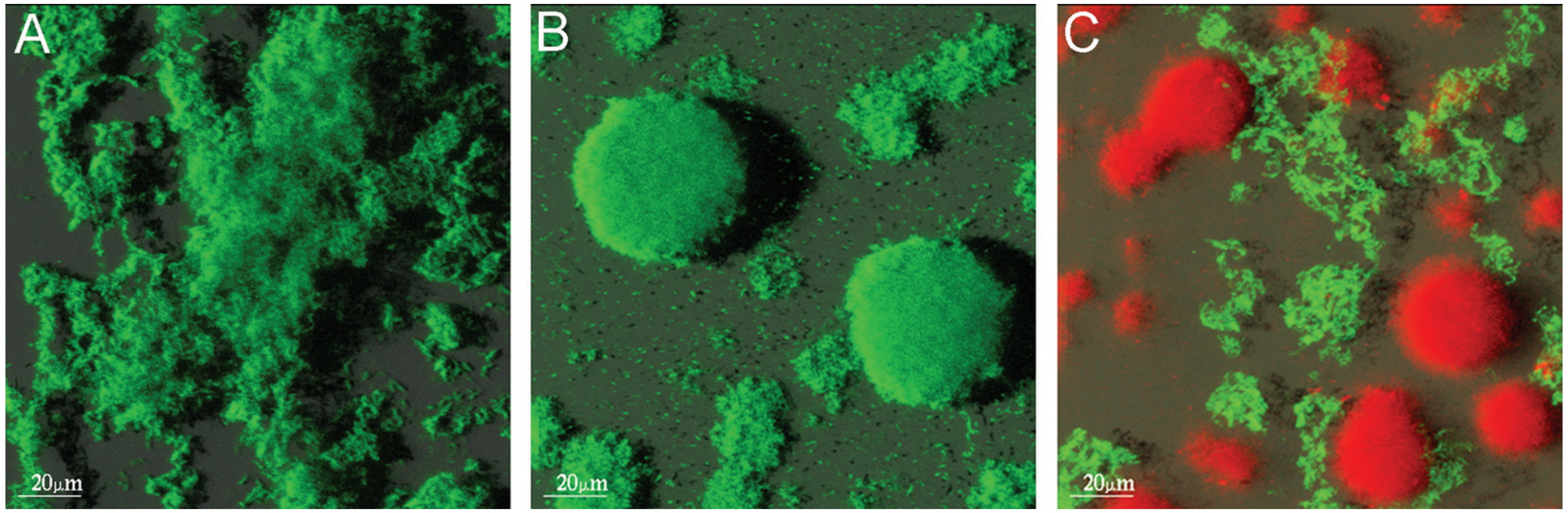


FIGURE 1 Confocal laser scanning microscopy (CLSM) images showing spatial structures in flow-chamber-grown 5-day-old biofilms formed by (A) Gfp-tagged (green fluorescent) *P. putida*, (B) Gfp-tagged *P. knackmussii*, and (C) a mixture of Gfp-tagged *P. putida* and DsRed-tagged (red fluorescent) *P. knackmussii*. Bars, 20 µm. Adapted from reference 43 with permission from the American Society for Microbiology. doi:10.1128/microbiolspec.MB-0001-2014.f1

Differences in the components of the matrix could give rise to differences in the structures of microcolonies

Microcolonies

Varies depending on **environmental conditions**

Mushroom-shaped P. aeruginosa microcolonies under flow conditions with glucose-containing medium

Flat biofilms in P. aeruginosa under flow conditions with citrate-containing medium

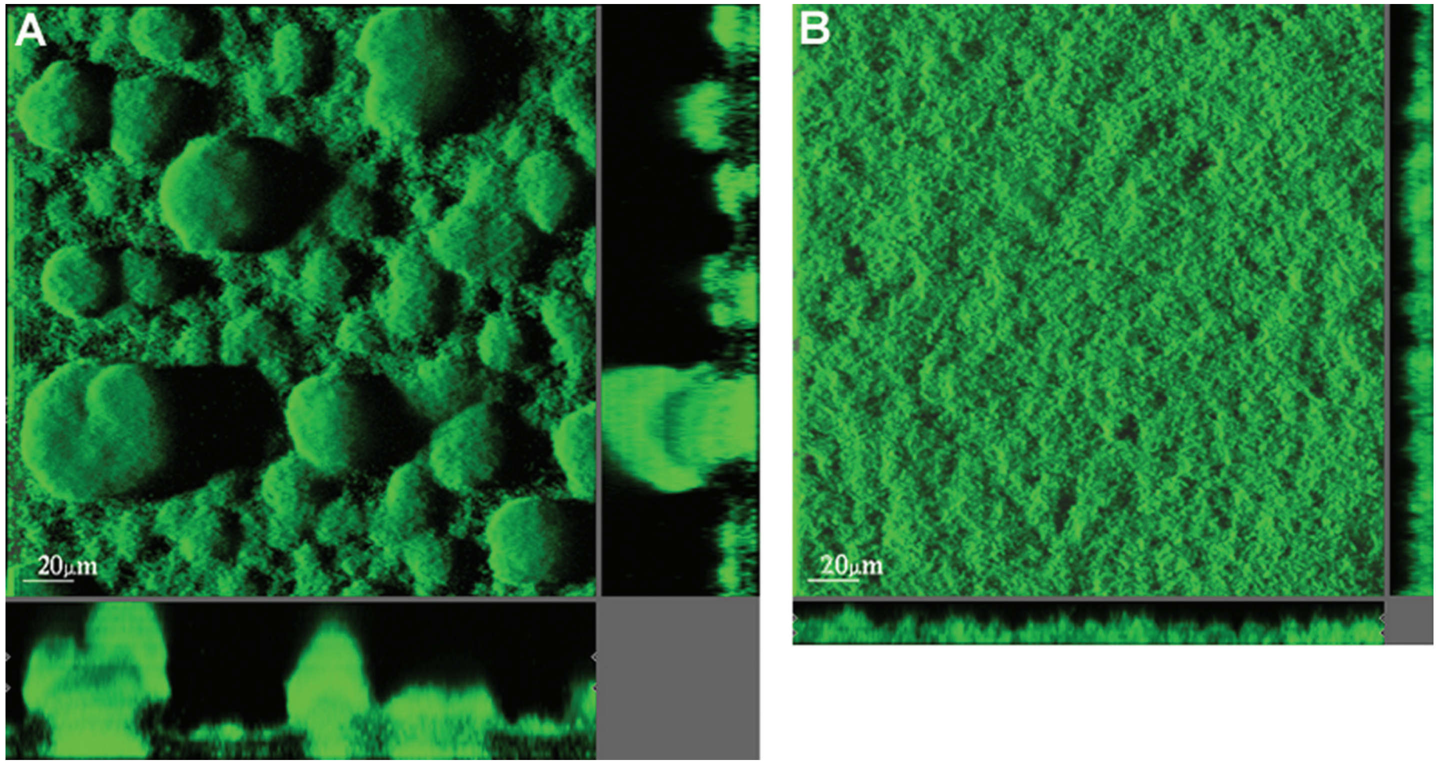


FIGURE 2 CLSM micrographs acquired in 5-day-old *P. aeruginosa* PAO1 biofilms grown on (A) glucose minimal medium and (B) citrate minimal medium. The central pictures show-top down fluorescence projections, and the flanking pictures show vertical sections. Bars, 20 μm. Adapted from reference 47 with permission from Wiley-Blackwell publishing. doi:10.1128/microbiolspec.MB-0001-2014.f2

Matrix

contributes to **structure** and **stability**

Exact components vary between microorganisms and culture conditions

50-90% **exopolysaccharides**
Proteins (adhesins, components of fimbriae and flagella, secreted extracellular proteins, and outer membrane vesicle proteins (OMVs)
Nucleic acids (**eDNA**)
97% **water**

Matrix proteome
Large amount of periplasmic, cytoplasmic, outer, and inner membrane proteins

Matrix

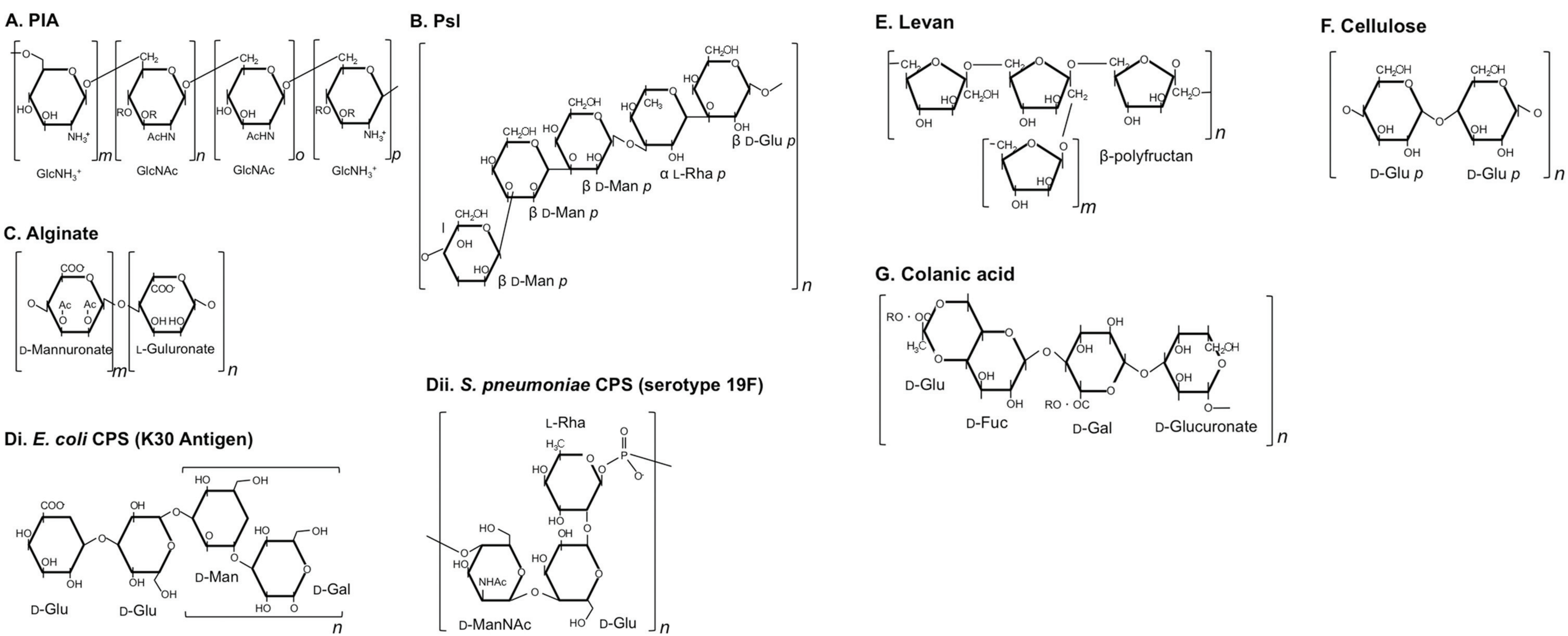
exopolysaccharides
vary in composition and therefore in physicochemical properties
size
in association with lectins, proteins, lipids, and DNA

TABLE 1 Summary of the cellular location, chemical composition, and functions of bacterial polysaccharides important for biofilm formation

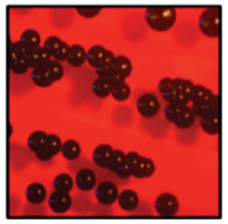
			Functions		
	Localization	Charge	Aggregative	Protective	Architectural
Pel	Secreted	NA	X	X	X
Psl	Secreted/cell associated	Neutral	X	X	X
PIA	Secreted	Polycationic	X		X
Cellulose	Secreted	Neutral	X	X	
Alginate	Cell associated	Polyanionic		X	X
CPS	Covalently attached	Polyanionic		X	
Levan	Cell associated	Neutral	X	X	
Colanic acid	Cell associated	Polyanionic			X
VPS	Secreted	NA	X	X	X
***Bacillus* EPS**	Secreted	Neutral			X

Matrix

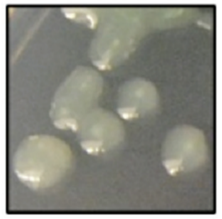
exopolysaccharides



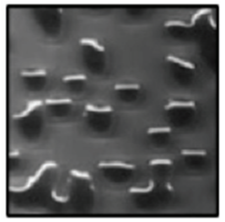
A. *S. aureus* PIA



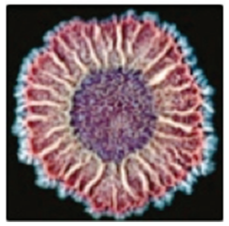
D. *P. aeruginosa* Alginate



E. *E. coli* Colanic acid



G. *B. subtilis* EPS

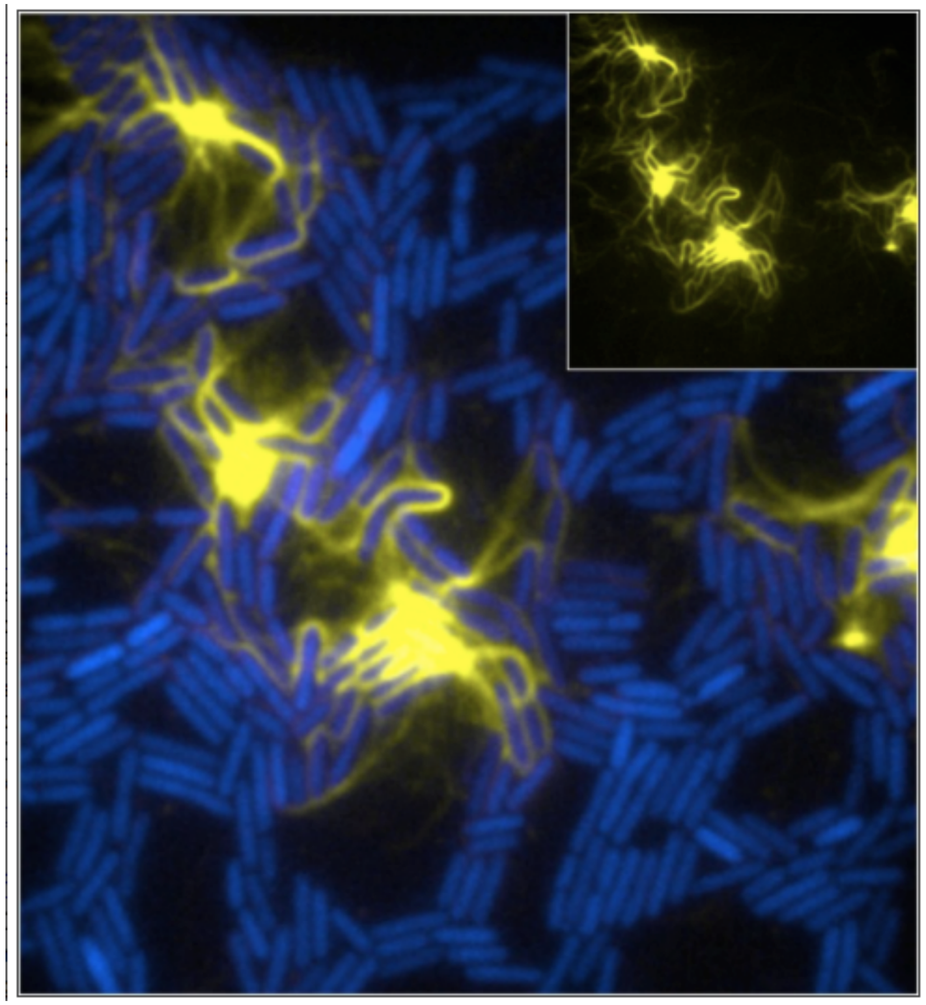


H. *E. coli* Cellulose

Matrix

Extracellular DNA

DNAse treatment prevents microcolony formation
In some cases, it results from lysis of certain bacteria
It is regulated in time and space
Interacts with EPS to produce defined agglomerates



Extracellular DNA is visualized within an P. aeruginosa biofilm. A biofilm of P. aeruginosa expressing cyan fluorescent protein (blue) that has been cultured at the interface of a glass coverslip and solidified nutrient medium supplemented with a fluorescent dye that stains extracellular DNA (yellow). Turnbull et al. report that extracellular DNA facilitates large-scale self-organization of cells in actively expanding biofilms of P. aeruginosa. The findings provide insights into how the intricate patterns of trails emerge during this complex multicellular behavior. Image courtesy of Erin S. Gloag, Lynne Turnbull, and Cynthia Whitchurch.
Karine A. Gibbs, et al. J Bacteriol. 2015 Jul;197(13):2084-2091.

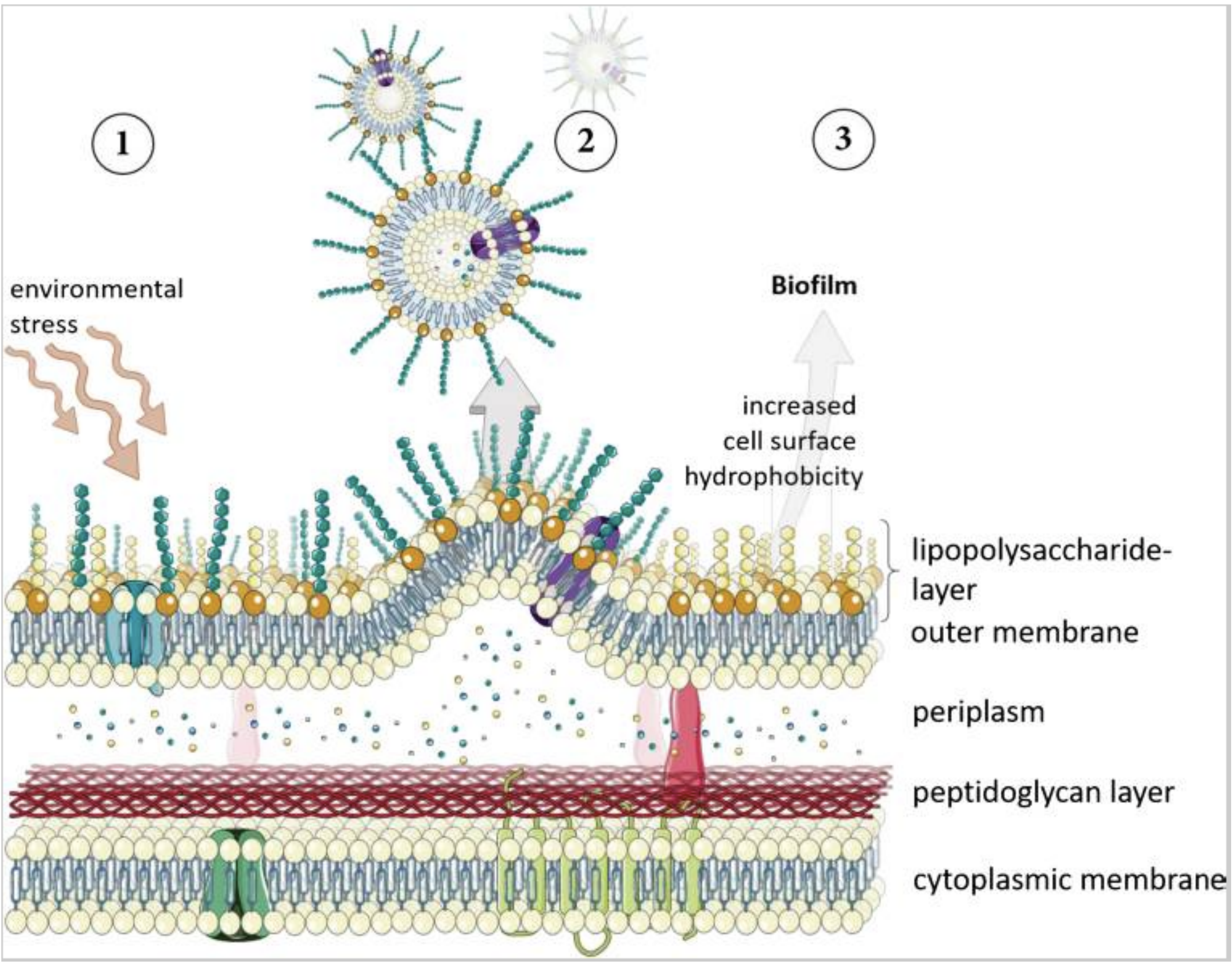
Outer Membrane Vesicles (OMV)

Small spherical structures produced by Gram-positive bacteria (10-300 nm) containing cytoplasmic and periplasmic contents (proteases, phospholipases, toxins, OMPS, LPS, etc.)
Involved in biofilm formation, pathogenesis, quorum sensing, nutrient acquisition, horizontal gene transfer, and antibiotic resistance
Relationship with biofilms… contribute to communication, nucleation, nutrient acquisition, and defense, but this remains unclear.

Table 1. The relationship between biofilm and vesicle in some bacteria.

Species	Strain	Factor	Effect	Reference
Helicobacter pylori	TK1402	22-kDa protein	Plays an important role in biofilm formation.	Yonezawa *et al.* (2011)
Francisella	-	OMV	Involved in biofilm formation and forming part of biofilm matrix.	van Hoek (2013)
Pseudomonas aeruginosa	PAO1	CPA	Its absence causes structural defects which limit the development of mature biofilms.	Murphy *et al.* (2014)
Vibrio cholerae	El Tor strain C6706	OMV-associated protein DegP	Required for the secretion of biofilm matrix components and the activity strongly influences biofilm formation.	Altindis, Fu and Mekalanos (2014)
Pseudomonas putida	DOT-T1E	OMV	Lead to an increased hydrophobicity of cells surface which enhanced their ability to form biofilms	Baumgarten *et al.* (2012)

Outer Membrane Vesicles (OMV)



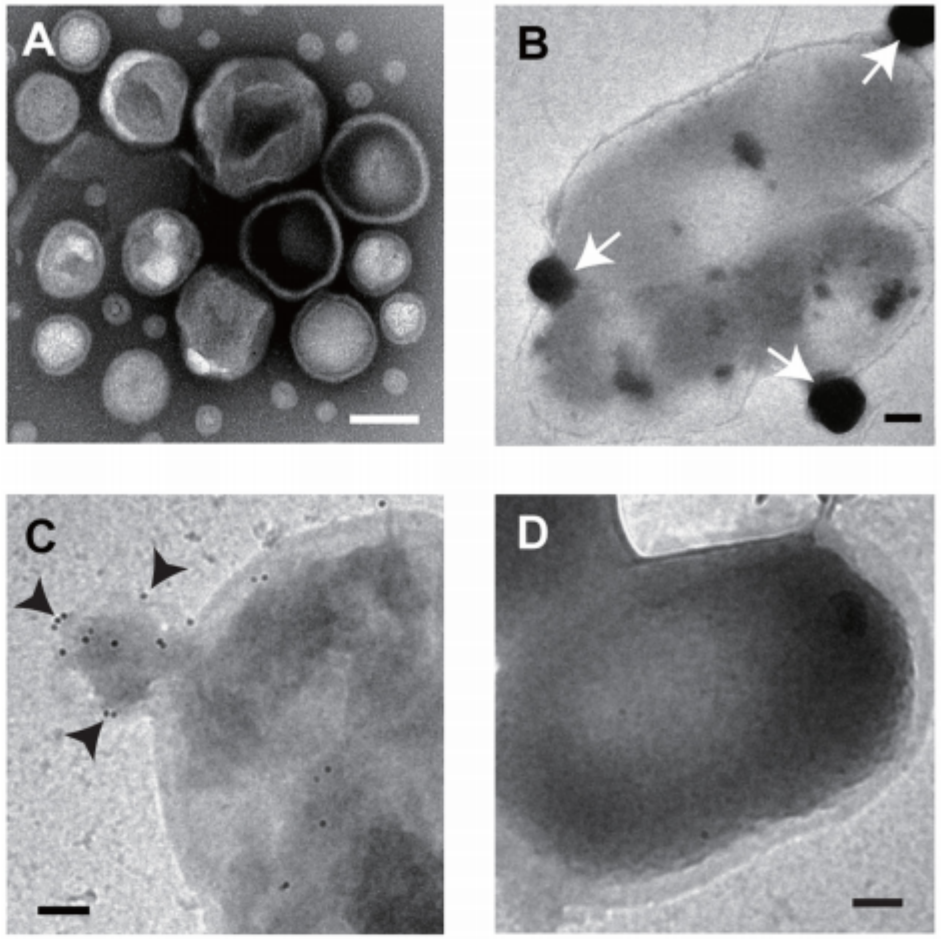
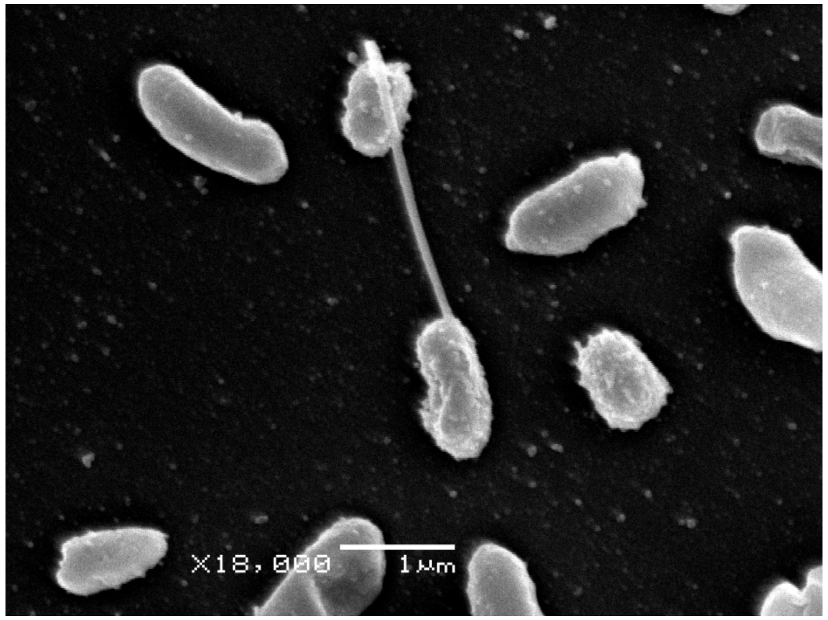
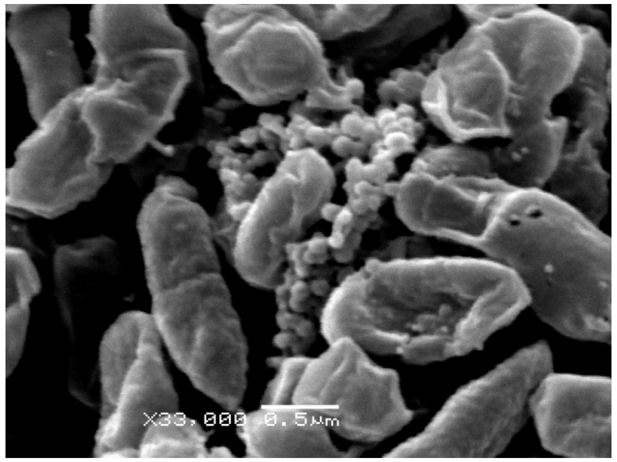


FIGURE 3 | TEM imaging. (A) Image of purified MVs derived from *B. agrestis* CUETM77-167. **(B)** Association of FM4-64-labeled MVs with cells. The white arrows indicate MVs, which have a high density due to FM4-64 labeling. **(C)** Association of FITC-labeled MVs with cells. Cell-associated MVs were detected by small gold particles (black arrows) through the FITC antibody. **(D)** Cells with no addition of MVs were reacted with FITC antibody. All bars indicate 100 nm.





Outer Membrane Vesicles (OMV)

There are differences in the composition of planktonic OMVs vs. biofilm OMVs.
Role in protecting DNA from degradation.
Maintaining matrix integrity.
How DNA enters OMVs and its role is a major debate.

Maturation

Microcolonies and Macrocolonies

Biofilm coated with matrix (proteins, polysaccharides, nucleic acids, and others)
Protective barrier against bacteriophages, amoebas, immune response, and antibiotics

c-di-GMP and bacterial communication (QS)

Transition from microcolonies to macrocolonies is poorly understood

Mechanism
Simply continuous growth over time
Induction of macrocolony formation at a given time
Combination of genetic determinants + physiological factors

They differ in the distribution of some key molecules

Formation and maintenance of channels between macrocolonies, necessary for the transport of nutrients, metabolites, and waste

Quorum sensing (QS): The process by which bacteria synthesize, recognize, and respond to extracellular signaling molecules known as **autoinducers** (AIs) that mediate intercellular communication.

They use AI concentrations in the medium to monitor changes in bacterial numbers and coordinate the expression of specific QS genes.

Genes involved in bacterial behavior, ATB production, biofilm production, bioluminescence, genetic competition, spin, and virulence.

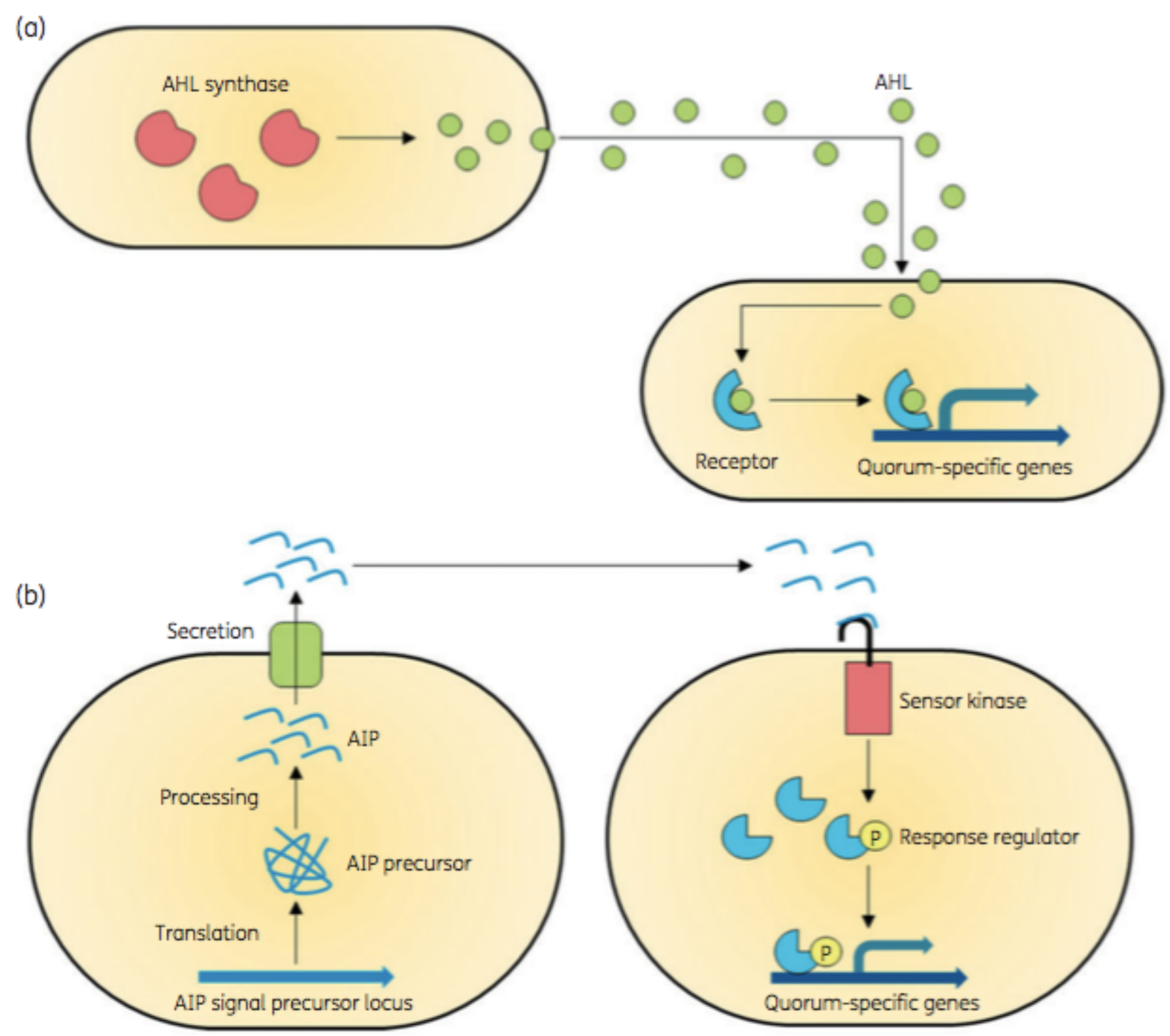
QS Principles:
AI Synthesis
AI Detection by Receptors
Activation of Specific QS Genes

Some bacteria do not produce AIs but have receptors

Quorum Sensing

Gram -
Acyl-homoserine lactones (AHLs) as AI
N-acetylated homoserine lactones that vary in size and modifications synthesized by AHL **synthases** (**LuxI**), diffuse, or are transported
Receptors (LuxRs) in the absence of AHLs are degraded.
AHL-LuxR dimerizes and binds to DNA and transcribes QS-specific genes

Gram +
Oligopeptides secreted as AIPs
Acyclic oligopeptides (cyclic lactones) **precursors** within the cell mature into AIPs, which are then secreted.
Two-component histidine kinase **receptors**, kinase activity results in **autophosphorylation**.
The kinase transfers phosphate to an intracellular response regulator, which, once activated, binds to DNA to initiate transcription of QS genes.

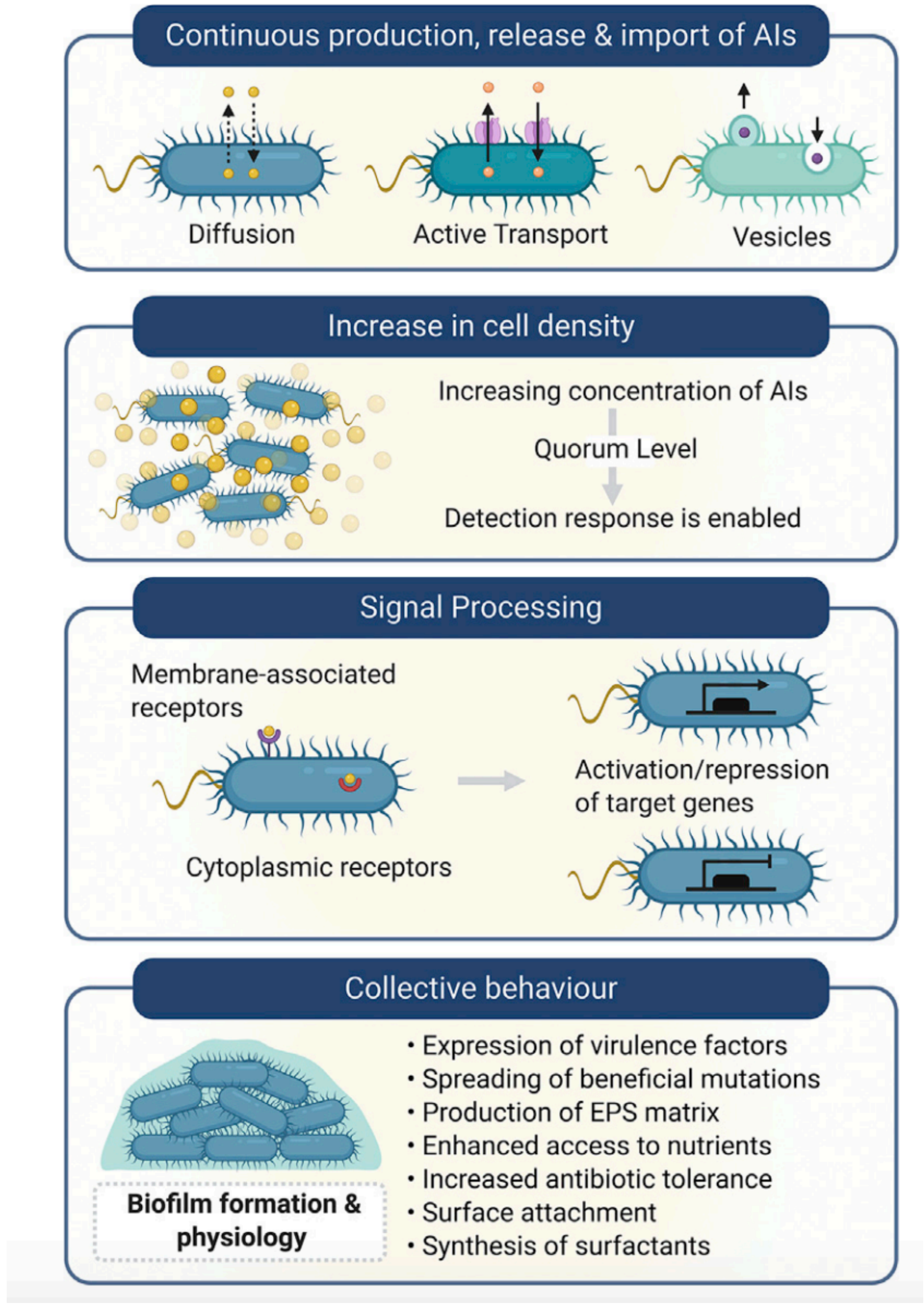


Existen AI comunes a G- y G+ como **AI-2** que median QA interespecies.

Maturation
Quorum Sensing

QS and biofilms

QS mutants of P. aeruginosa form flat, undifferentiated biofilms.
QS inhibition affects the biofilm.
Associated with DNA and matrix generation.
Controls the production of rhamnolipids, lectins, and siderophores.
Associated with dispersion.

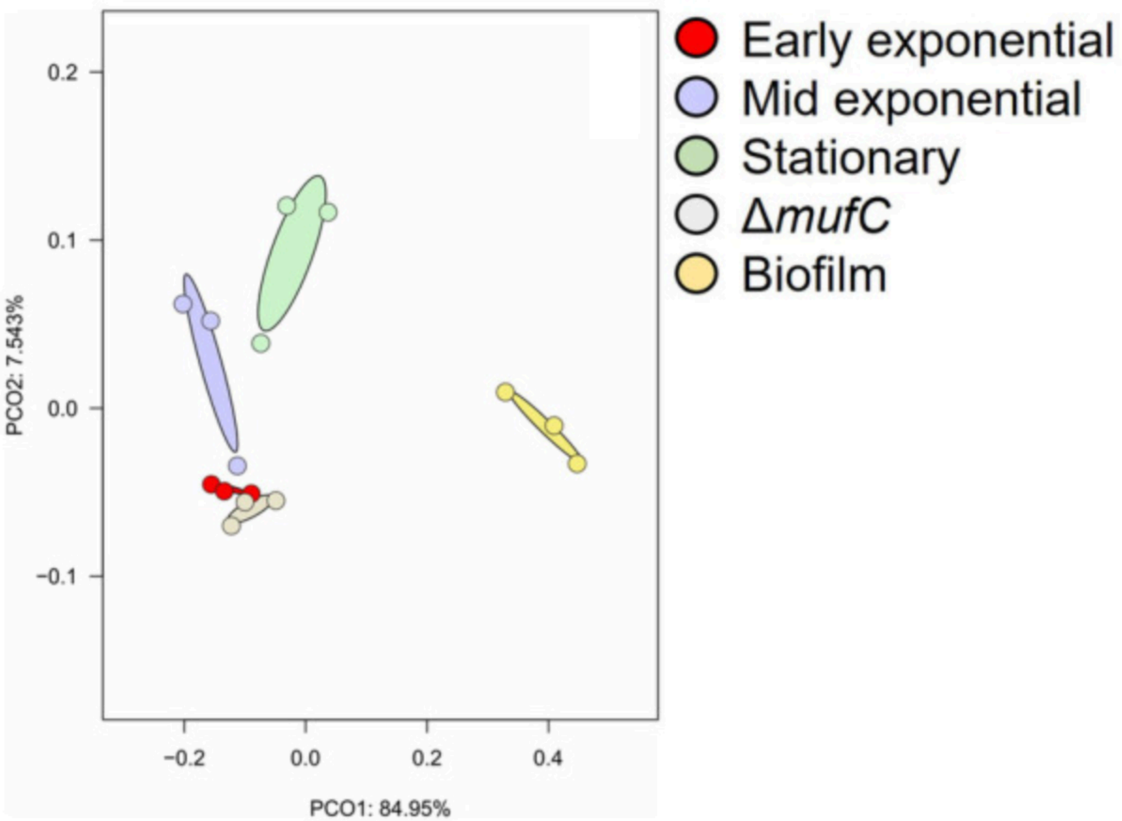


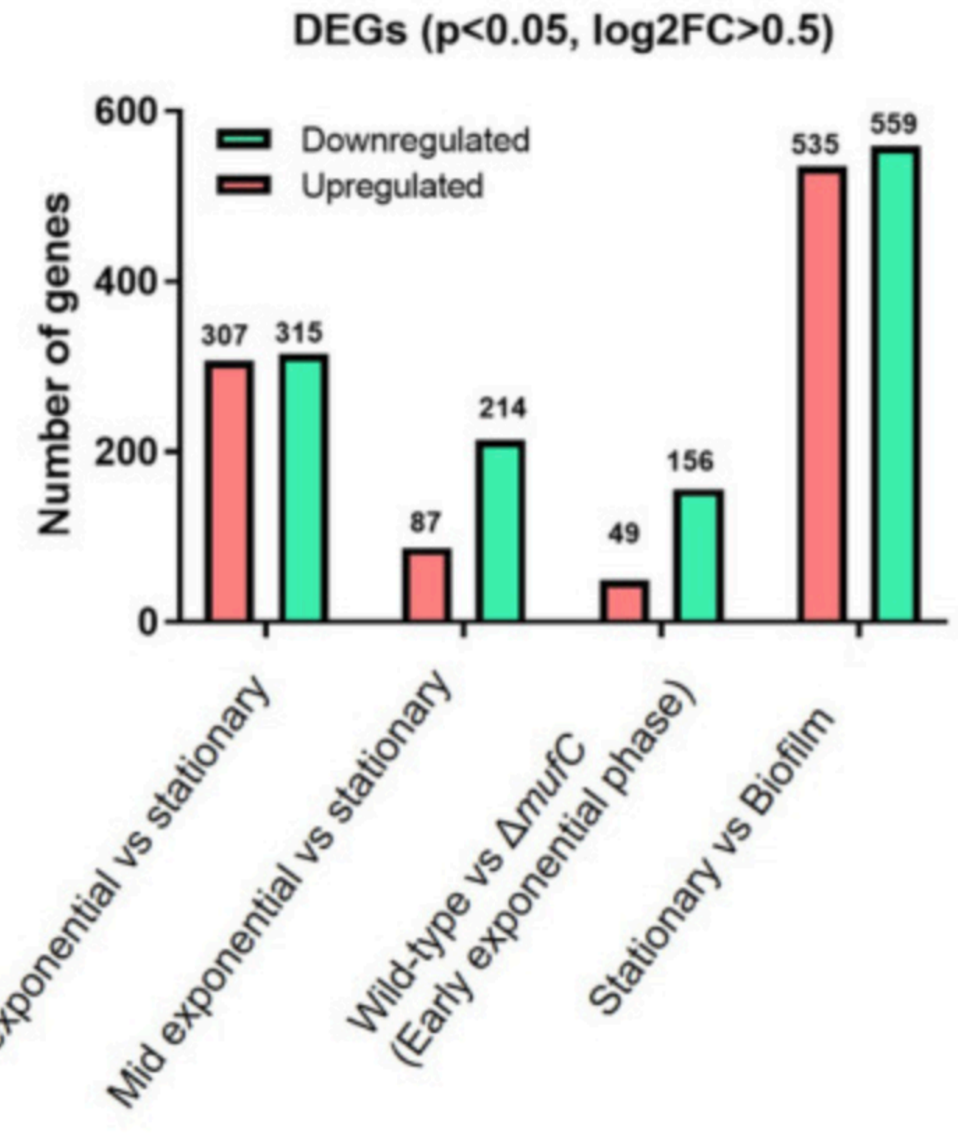
A highly programmed process

would be expected to have a set of expressed "**biofilm genes**."

Transcriptomic analyses have **failed** to identify biofilm regulons.
It would then be governed by adaptive responses dependent on nutritional conditions that change in response to environmental conditions.
If it requires the expression of genes associated with matrix products,
omics research will contribute to the understanding of biofilms.

Gene Expression

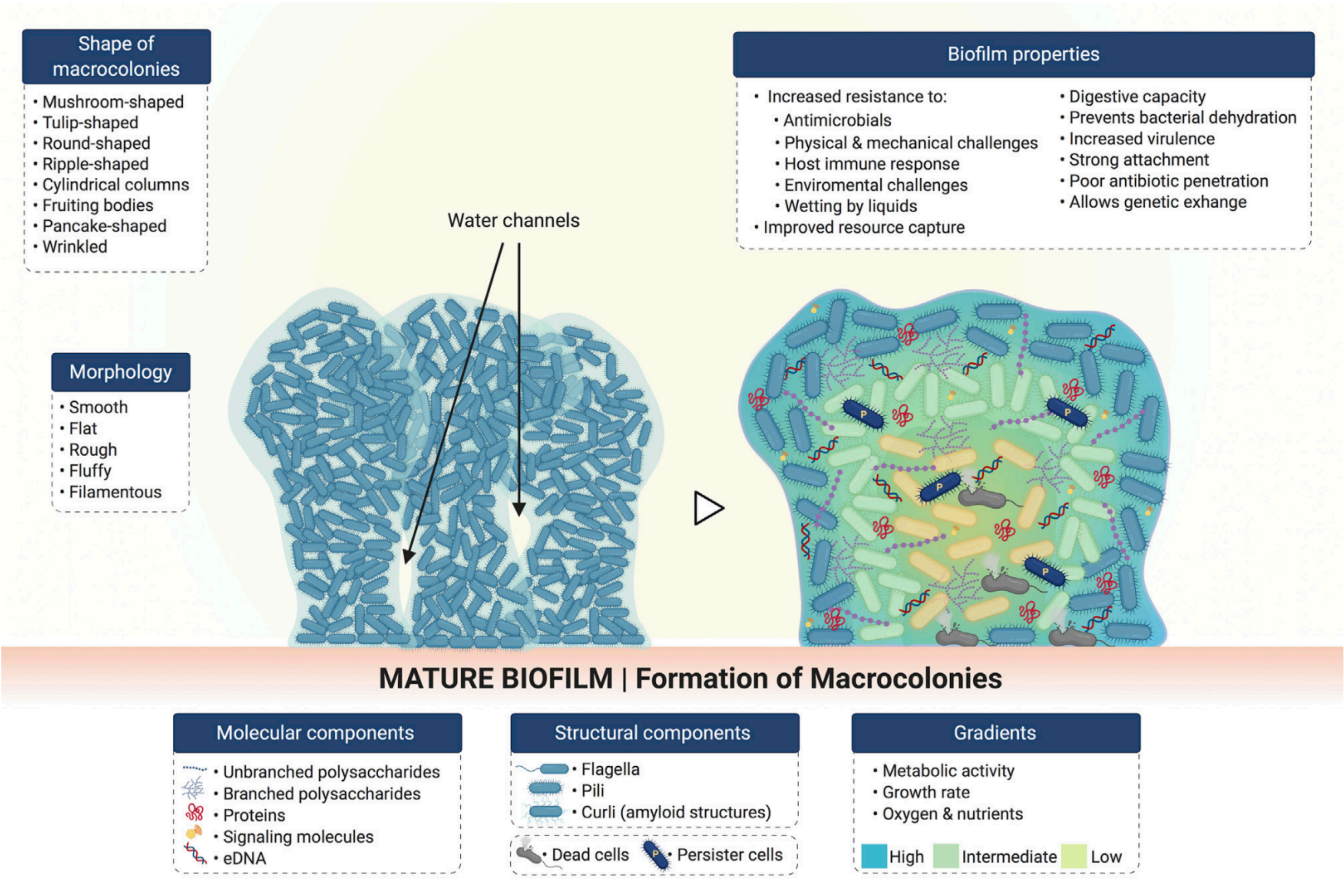




Comparison of transcriptomic profiling of *S. mutans* NMT4863 WT from different growth phases. **(A)** Principal component analysis of the samples. **(C)** Number of differentially expressed genes in the various growth phases and in biofilm.

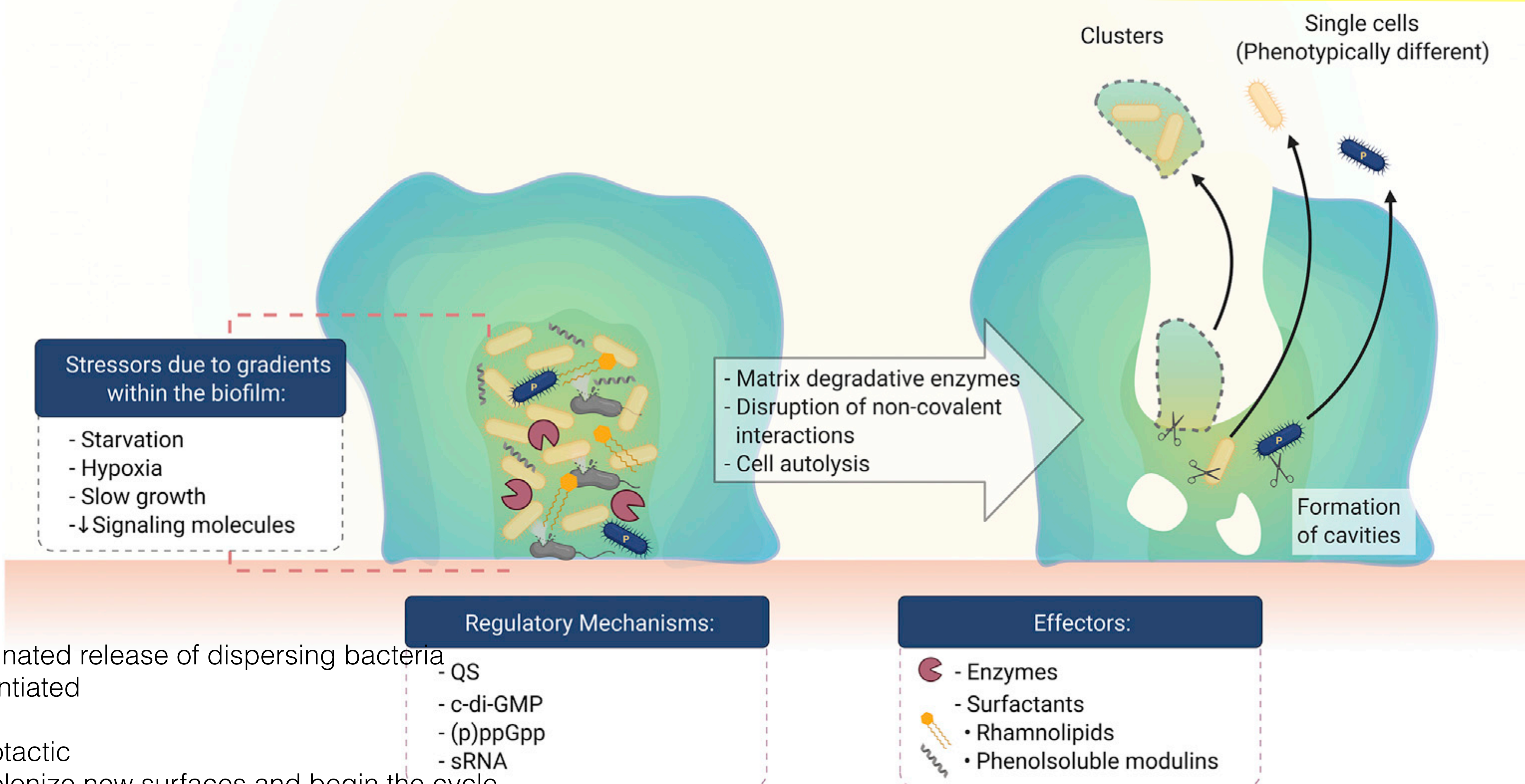
doi: 10.3389/froh.2025.1535034

Maturation



Shape of macrocolonies
• Mushroom-shaped
• Tulip-shaped
• Round-shaped
• Ripple-shaped
• Cylindrical columns
• Fruiting bodies
• Pancake-shaped
• Wrinkled
Morphology
• Smooth
• Flat
• Rough
• Fluffy
• Filamentous
Water channels
Biofilm properties
• Increased resistance to:
• Antimicrobials
• Physical & mechanical challenges
• Host immune response
• Enviromental challenges
• Wetting by liquids
• Improved resource capture
• Digestive capacity
• Prevents bacterial dehydration
• Increased virulence
• Strong attachment
• Poor antibiotic penetration
• Allows genetic exhange
MATURE BIOFILM | Formation of Macrocolonies
Molecular components
• Unbranched polysaccharides
• Branched polysaccharides
• Proteins
• Signaling molecules
• eDNA
Structural components
• Flagella
• Pili
• Curli (amyloid structures)
• Dead cells
• Persister cells
Gradients
• Metabolic activity
• Growth rate
• Oxygen & nutrients
High
Intermediate
Low

Dispersion



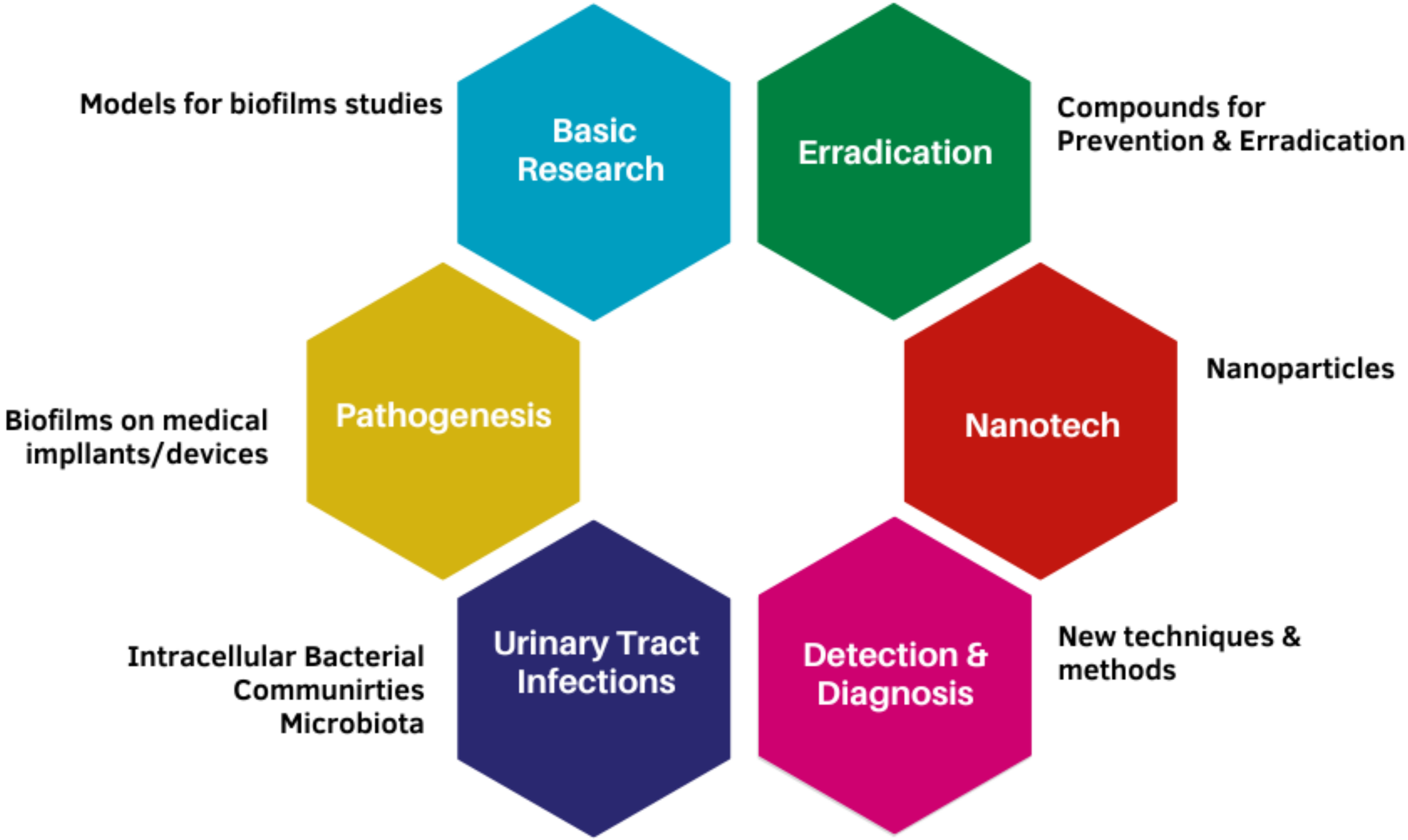
Coordinated release of dispersing bacteria
differentiated
motile
chemotactic
that colonize new surfaces and begin the cycle

may correlate with the death program of bacterial subpopulations

associated with specific nutrient and O_2 gradients

down-regulation of biofilm phenotype genes such as exopolysaccharides and fimbriae and up-regulation of flagella and chemotaxis factors

Laboratorio de Biofilms Microbianos

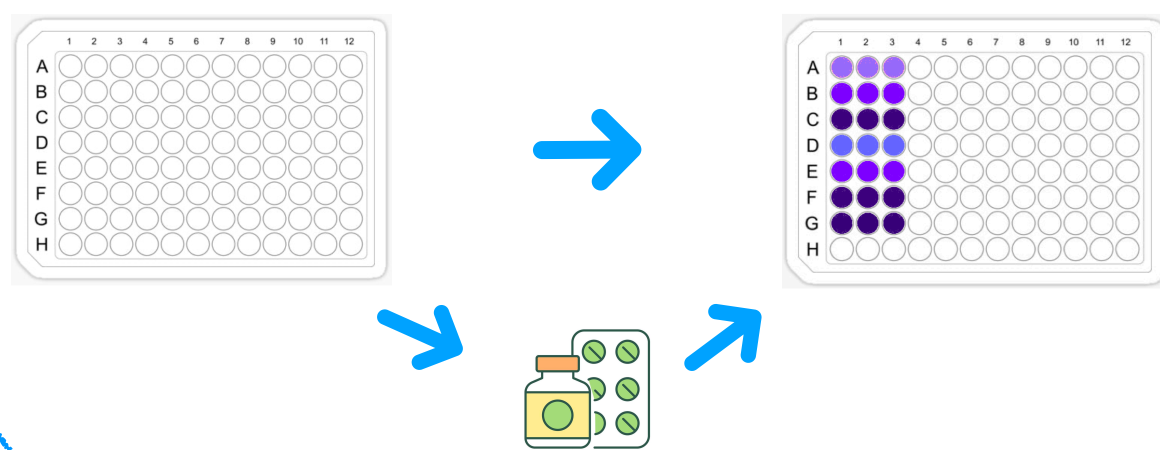


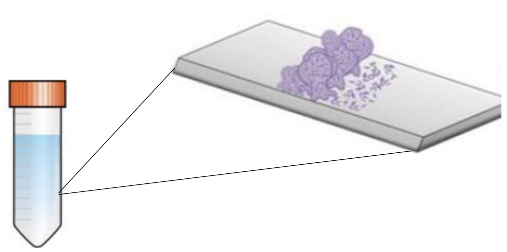
Biofilm formation

1. Development of models

ESTÁTICOS

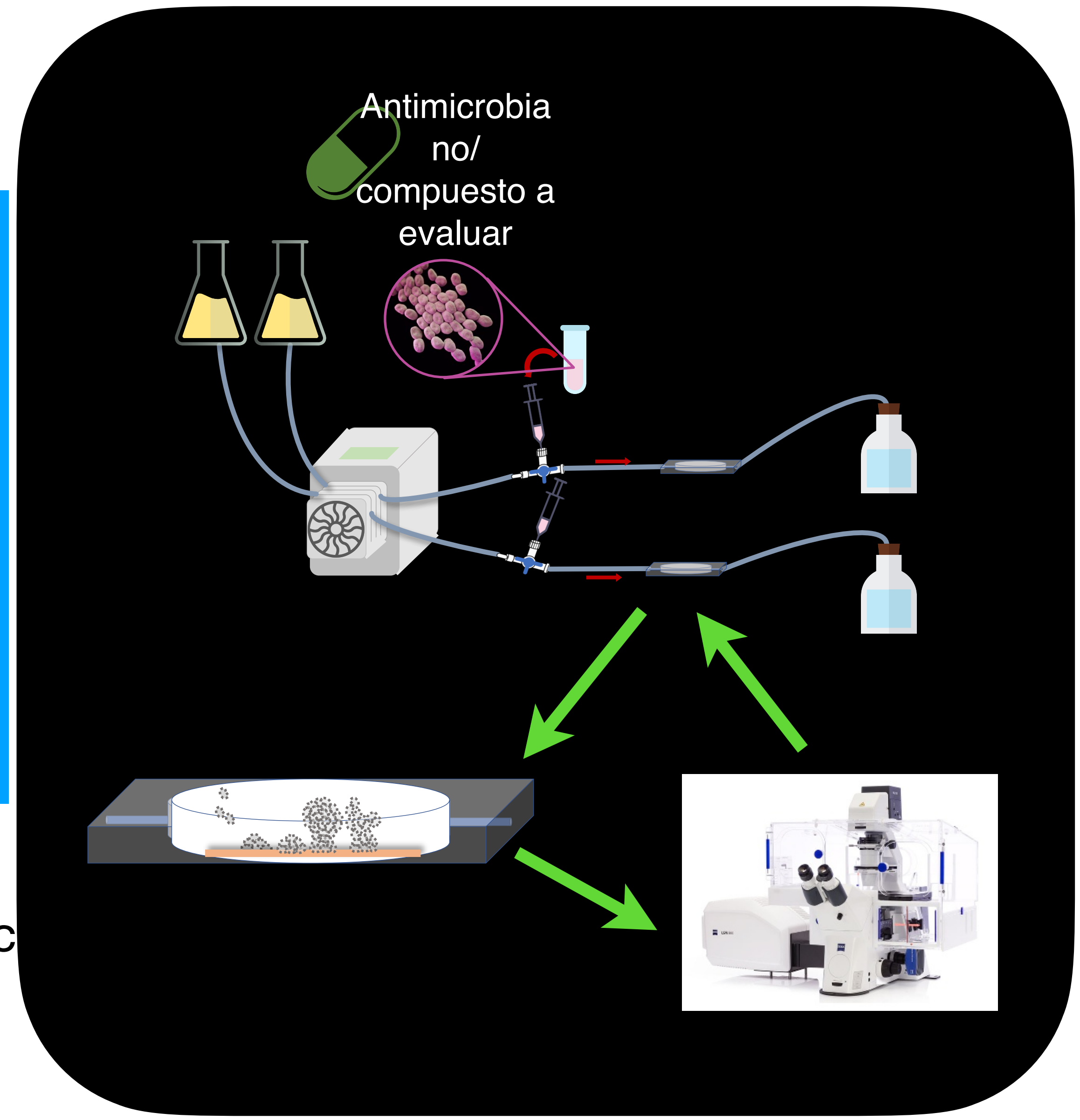
Screening





- Tiempo
- Condiciones ambientales
- Cuantificación morfotopológic

DINÁMICOS

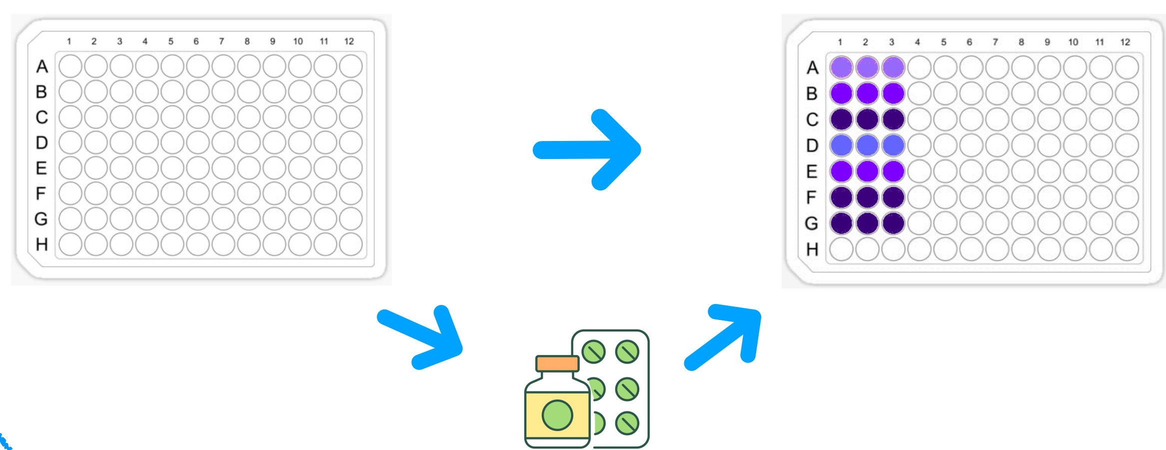


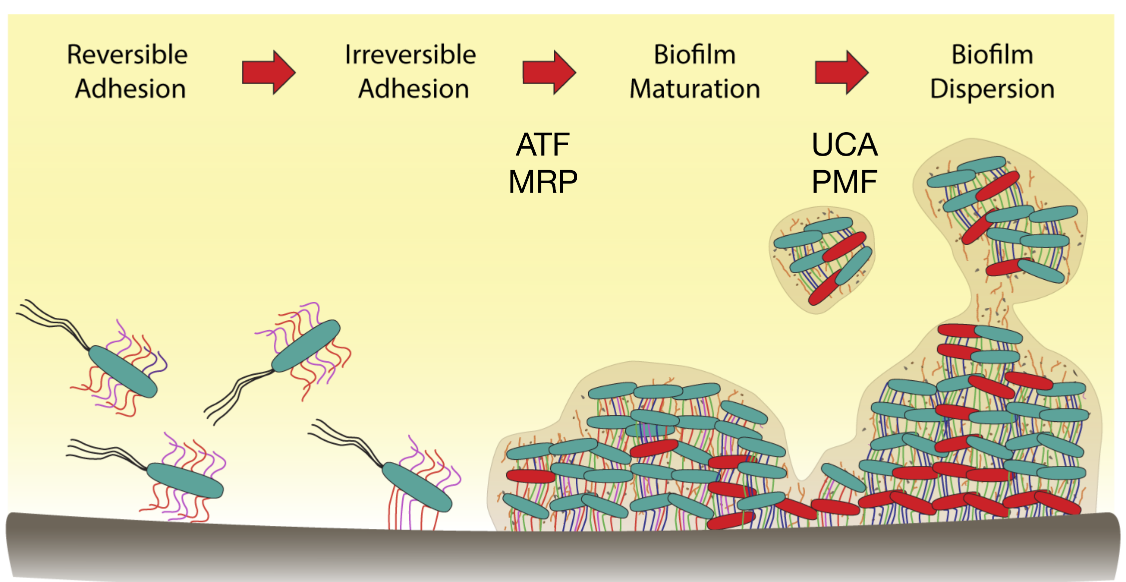
Biofilm formation

ESTÁTICOS

1. Development of models

Screening





OXFORD FEMS JOURNALS investing in science
Pathogens and Disease, 74, 2016, ftw033
doi: 10.1093/femspd/ftw033
Advance Access Publication Date: 17 April 2016
Research Article

RESEARCH ARTICLE

Fimbriae have distinguishable roles in *Proteus mirabilis* biofilm formation

Paola Scavone[1], Victoria Iribarnegaray[1], Ana Laura Caetano[1], Geraldine Schlapp[1], Steffen Härtel[2] and Pablo Zunino[1,*]

Current Research in Microbial Sciences 2 (2021) 100060
Contents lists available at ScienceDirect
Current Research in Microbial Sciences
journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences
ELSEVIER

Relevance of iron metabolic genes in biofilm and infection in uropathogenic *Proteus mirabilis*

V Iribarnegaray[a,d], MJ González[b], AL Caetano[a], R Platero[c], P Zunino[a], P Scavone[b,*]

Revista Argentina de Microbiología 55 (2023) 226–234
asociación argentina de microbiología
REVISTA ARGENTINA DE MICROBIOLOGÍA
www.elsevier.es/ram

ORIGINAL ARTICLE

Role of *Proteus mirabilis* flagella in biofilm formation

Paola Scavone[a], Victoria Iribarnegaray[a,c], María José González[a], Nicolás Navarro[a], Nicole Caneles-Huerta[b], Jorge Jara-Wilde[b], Steffen Härtel[b], Pablo Zunino[a,*]

[a] Department of Microbiology, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
[b] Laboratory for Scientific Image Processing (SCIAN-Lab), Biomedical Neuroscience Institute (BNI), Institute of Biomedical Sciences (ICBM), Faculty of Medicine, University of Chile, Santiago, Chile
[c] Department of Pathobiology, Facultad de Veterinaria, Universidad de la República, Montevideo, Uruguay

Received 5 September 2022; accepted 25 January 2023
Available online 17 April 2023

Biofilm formation

ESTÁTICOS

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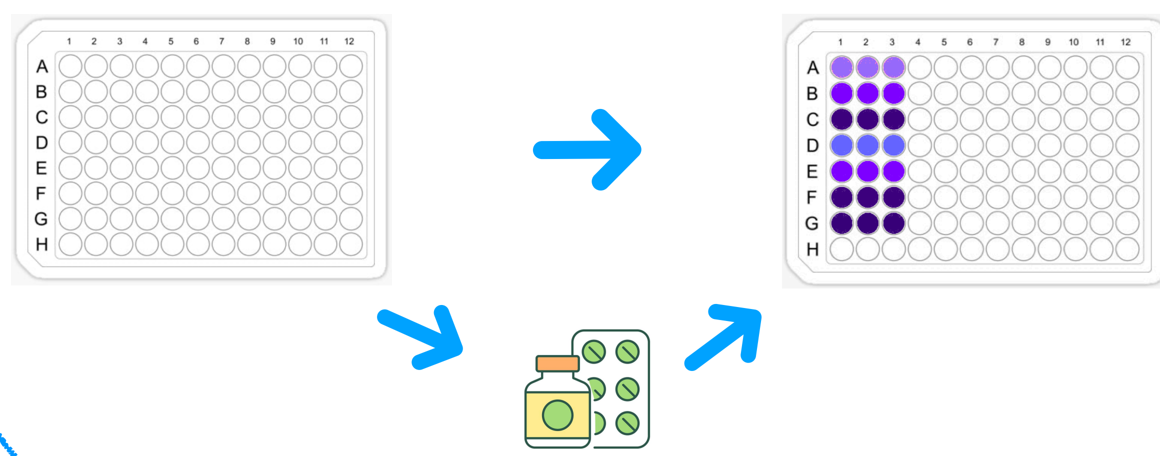


Pathogens and Disease, 75, 2017, ftx053

doi: 10.1093/femspd/ftx053
Advance Access Publication Date: 12 May 2017
Research Article

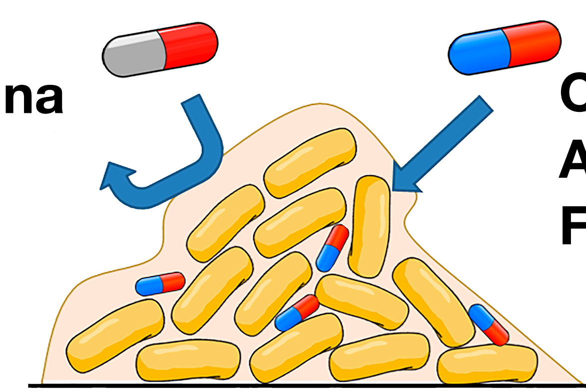
RESEARCH ARTICLE

Effect of different antibiotics on biofilm produced by uropathogenic *Escherichia coli* isolated from children with urinary tract infection

María José González[1,†], Luciana Robino[2,†], Victoria Iribarnegaray[1], Pablo Zunino[1] and Paola Scavone[1,*]

116 aislamientos
41% forma BF
Asociación pili P y BF

Ampicilina



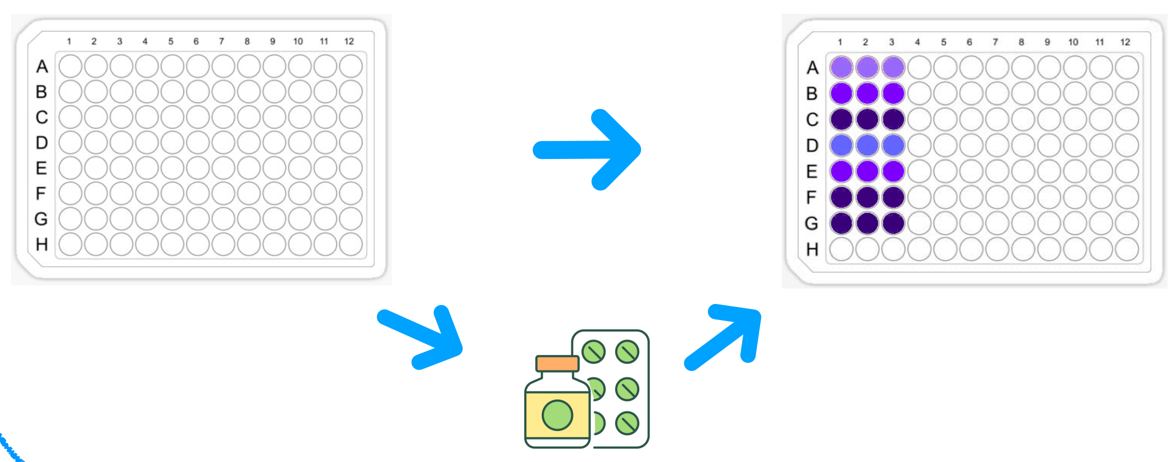
Cefalosporinas
Aminoglicósidos
Fluoroquinolonas

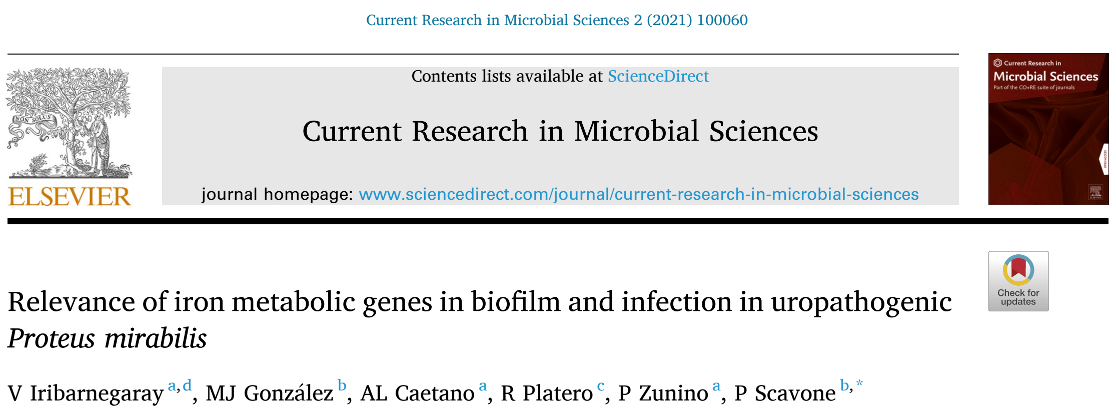
Biofilm formation

ESTÁTICOS

1. Development of models

Screening




Current Research in Microbial Sciences 2 (2021) 100060

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Relevance of iron metabolic genes in biofilm and infection in uropathogenic *Proteus mirabilis*

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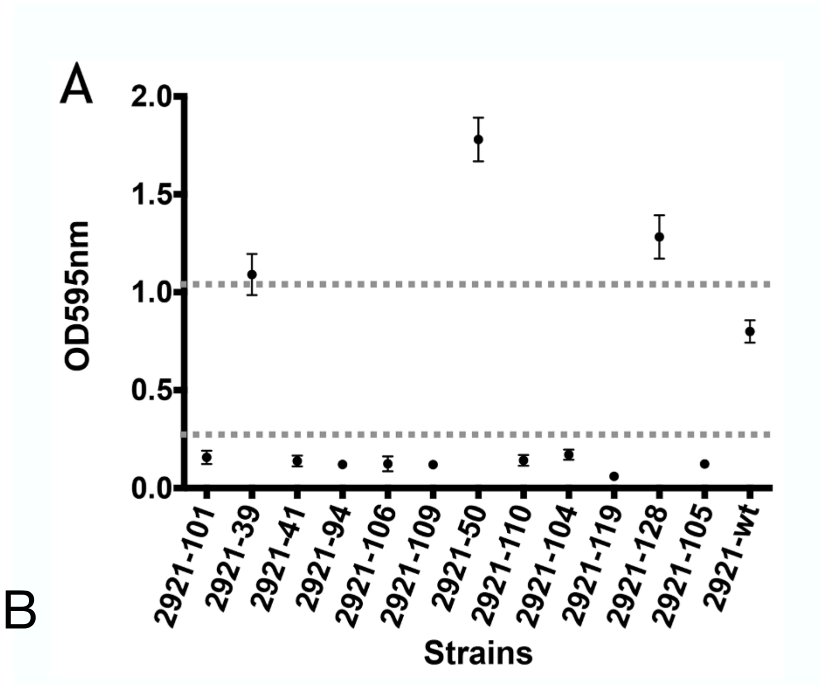


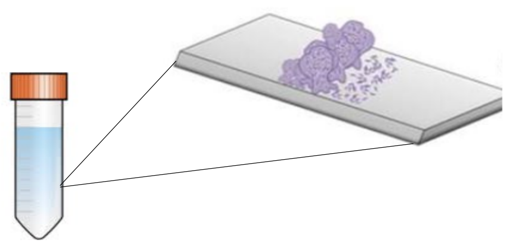
2921-50. Ferritina

2921-110 Receptor tipo TonB

Biofilm formation

ESTÁTICOS

1. Development of models



- Tiempo
- Condiciones ambientales
- Cuantificación morfotopológ

Journal of Microbiological Methods 87 (2011) 234–240

Contents lists available at SciVerse ScienceDirect

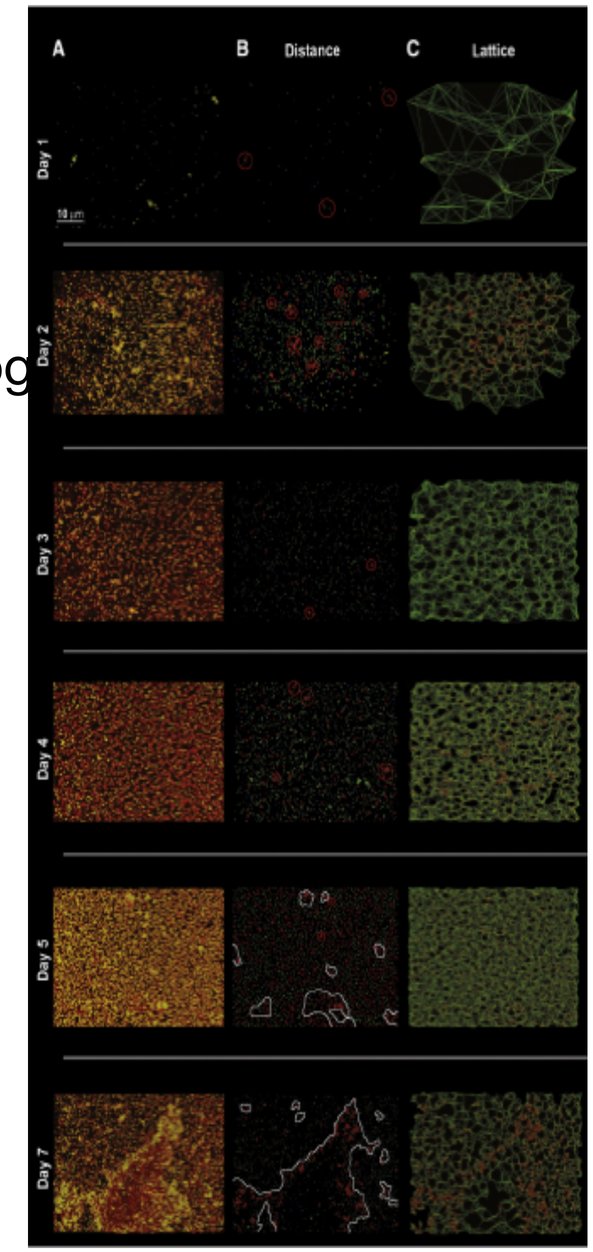
Journal of Microbiological Methods

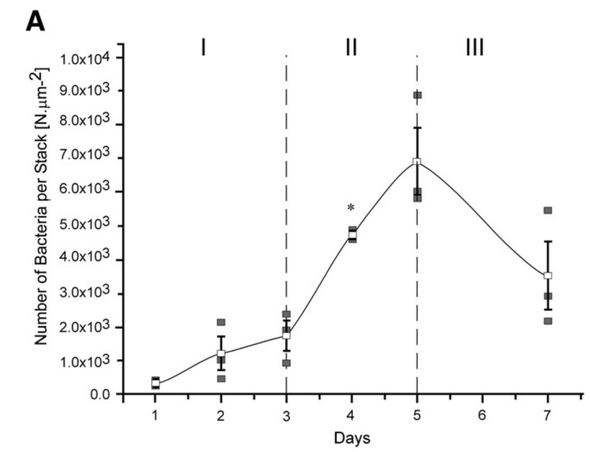
journal homepage: www.elsevier.com/locate/jmicmeth

ELSEVIER

Development of 3D architecture of uropathogenic *Proteus mirabilis* batch culture biofilms—A quantitative confocal microscopy approach

G. Schlapp [a,1], P. Scavone [a,b,1], P. Zunino [a], S. Härtel [b,*]





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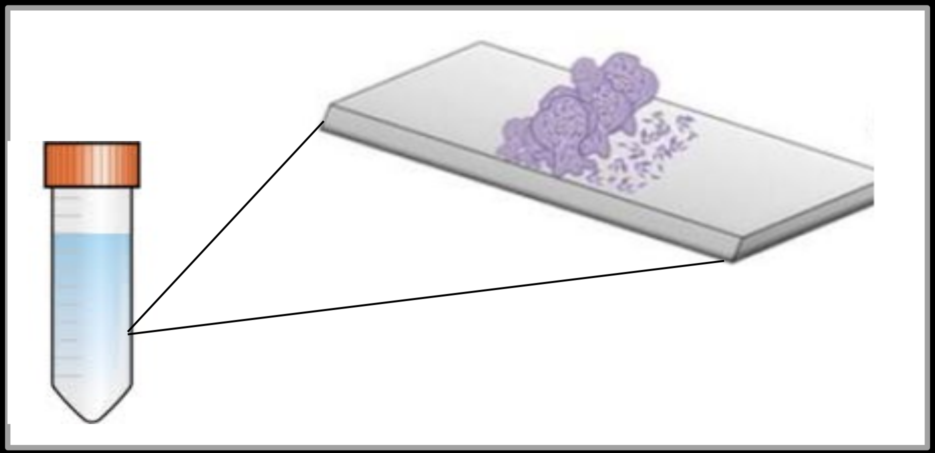
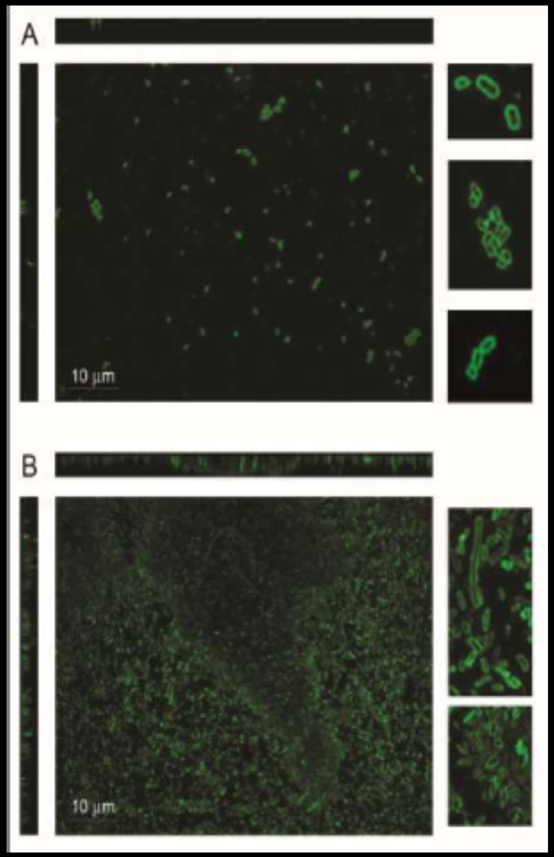
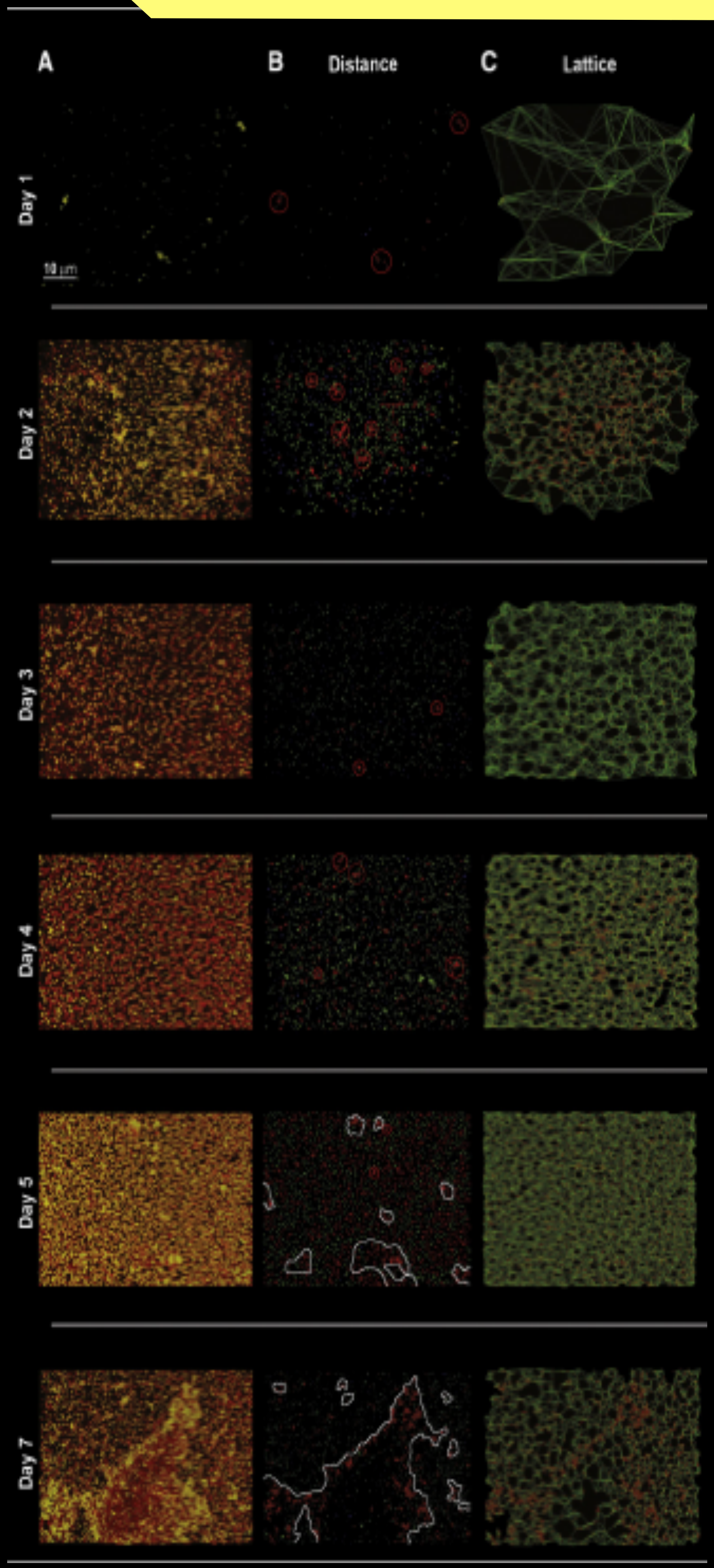
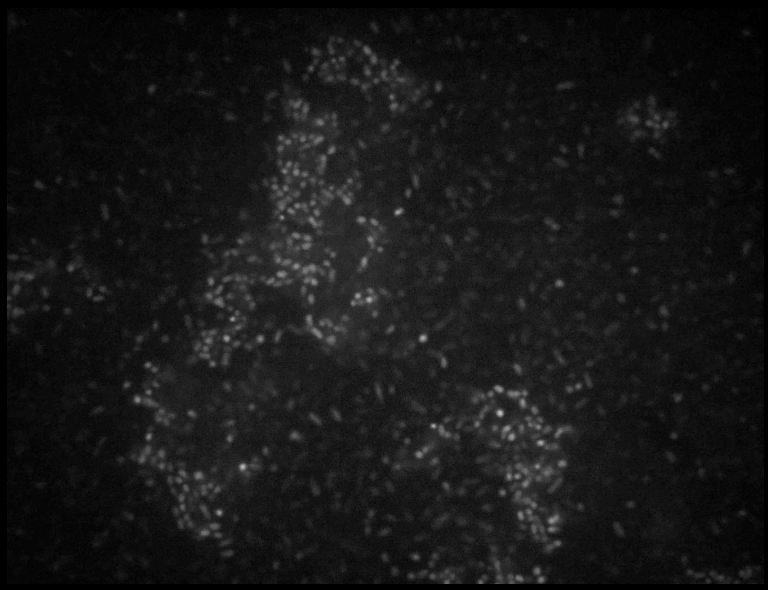
Biofilm formation









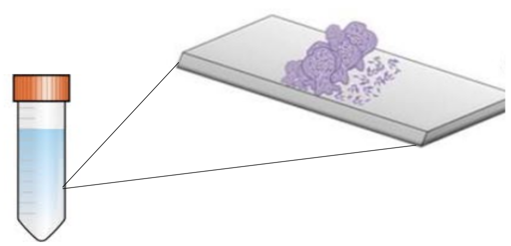
Schlapp et al., 2011

Laboratory for SCIAN Scientific Image Analysis

Biofilm formation

ESTÁTICOS

1. Development of models



- Tiempo
- Condiciones ambientales
- Cuantificación morfotopológ

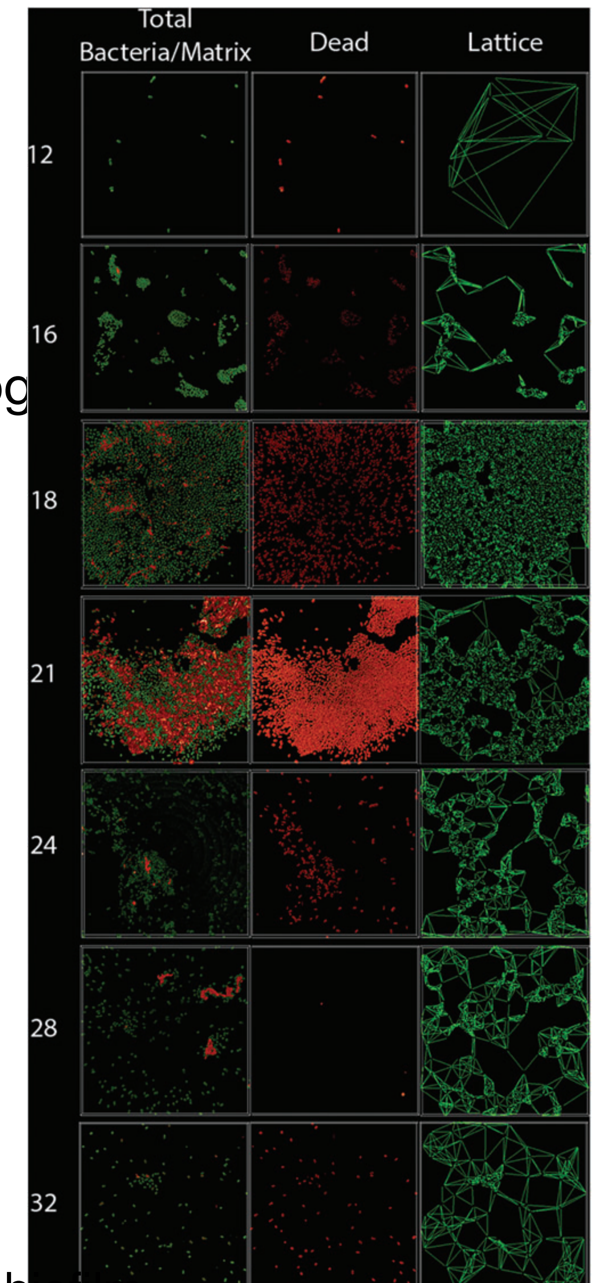
MICROBIAL DRUG RESISTANCE
Volume 00, Number 00, 2019

DOI: 10.1089/mdr.2019.0145

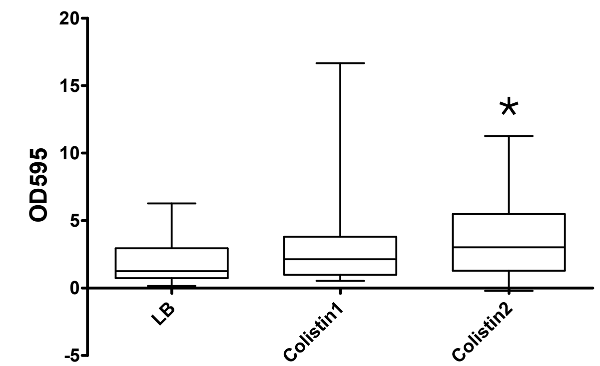
Characterization of the Different Stages of Biofilm Formation and Antibiotic Susceptibility in a Clinical *Acinetobacter baumannii* Strain

Paula Da Cunda,[1] Victoria Iribarnegaray,[1] Romina Papa-Ezdra,[2] Inés Bado,[2]
María José González,[1] Pablo Zunino,[1] Rafael Vignoli,[2] and Paola Scavone[1]

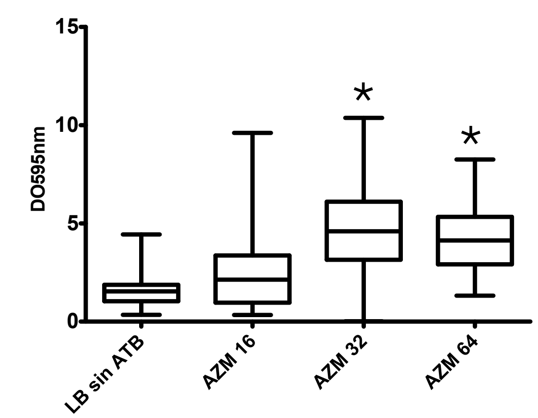
Microorganismos planctónicos susceptibles
Resistentes en el biofilm
Promoción de la formación de biofilm en modelo estático de biofilm



A. baumannii
susceptible a Colistin
Todas las cepas forman biof



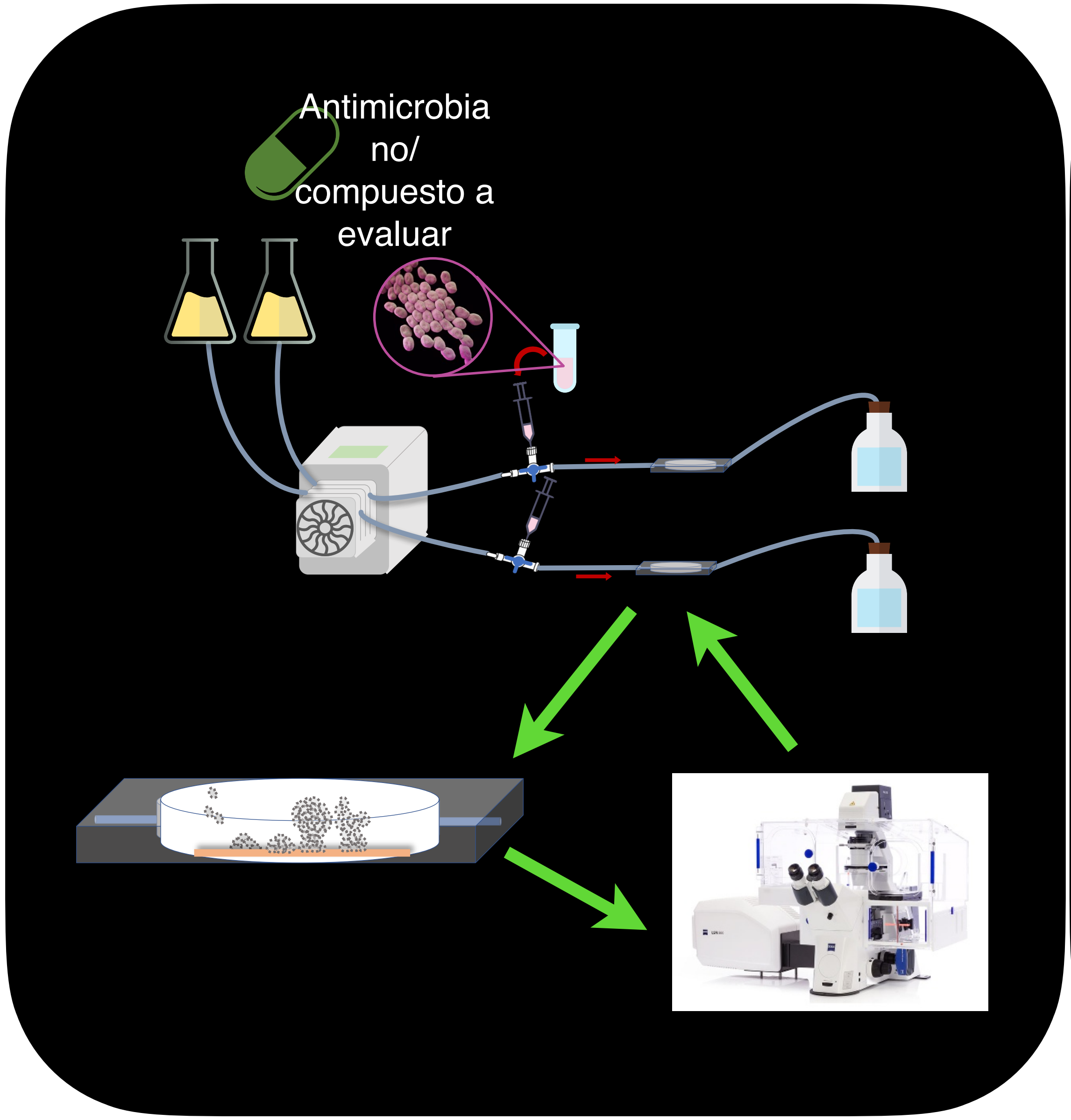
A. baumannii
resistente Azitromicina

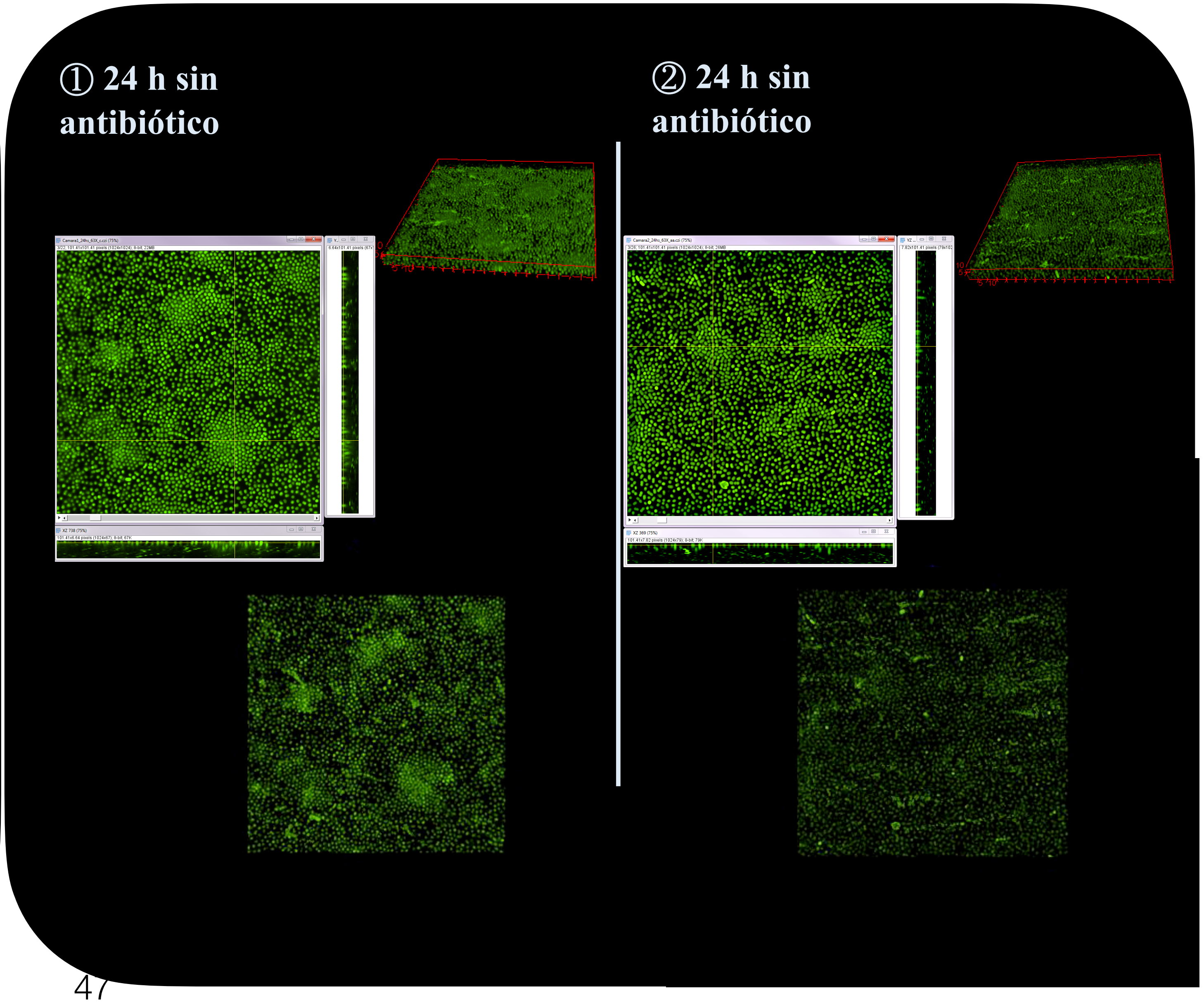


Biofilm formation

1. Development of models

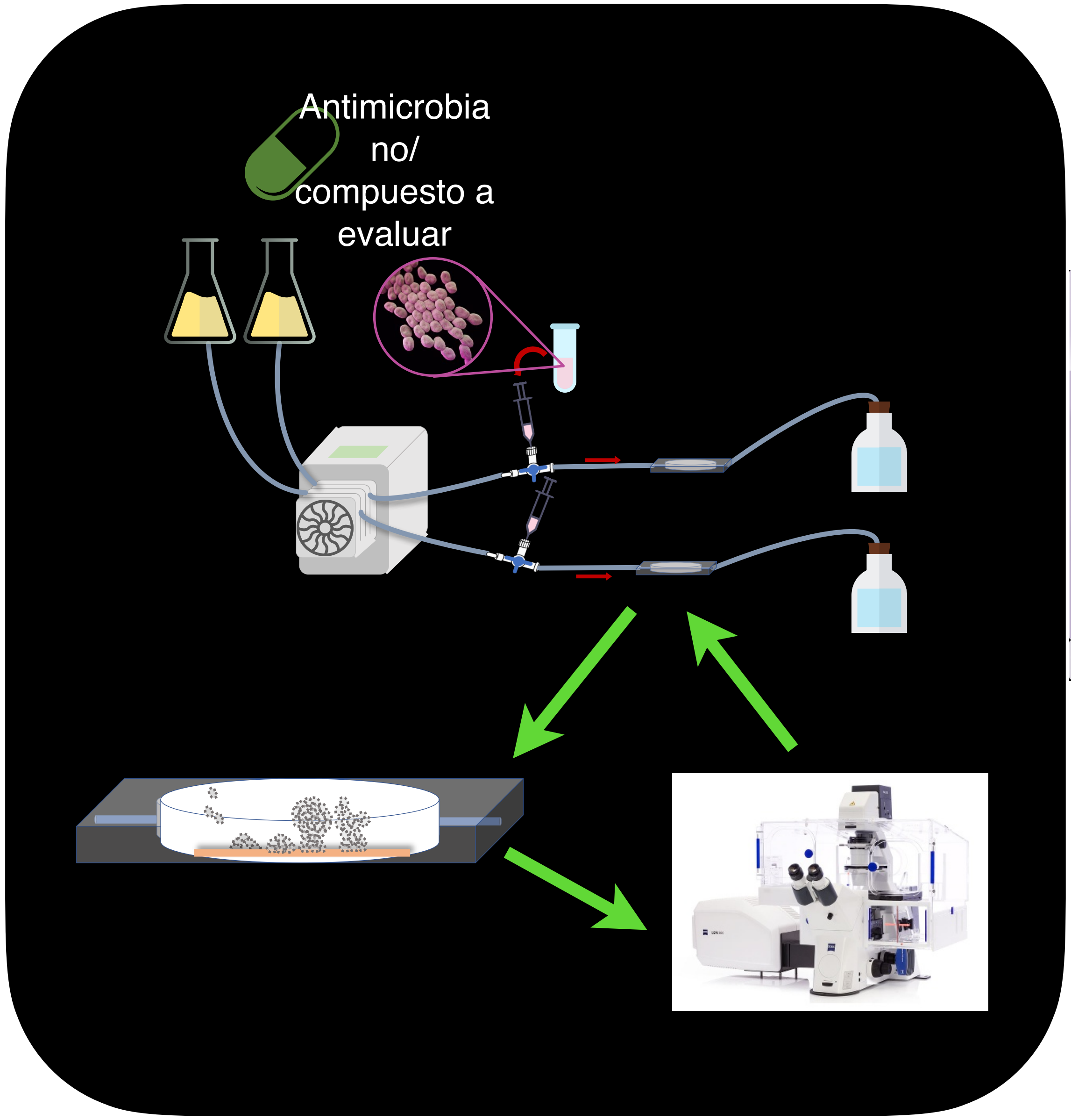
DINÁMICOS

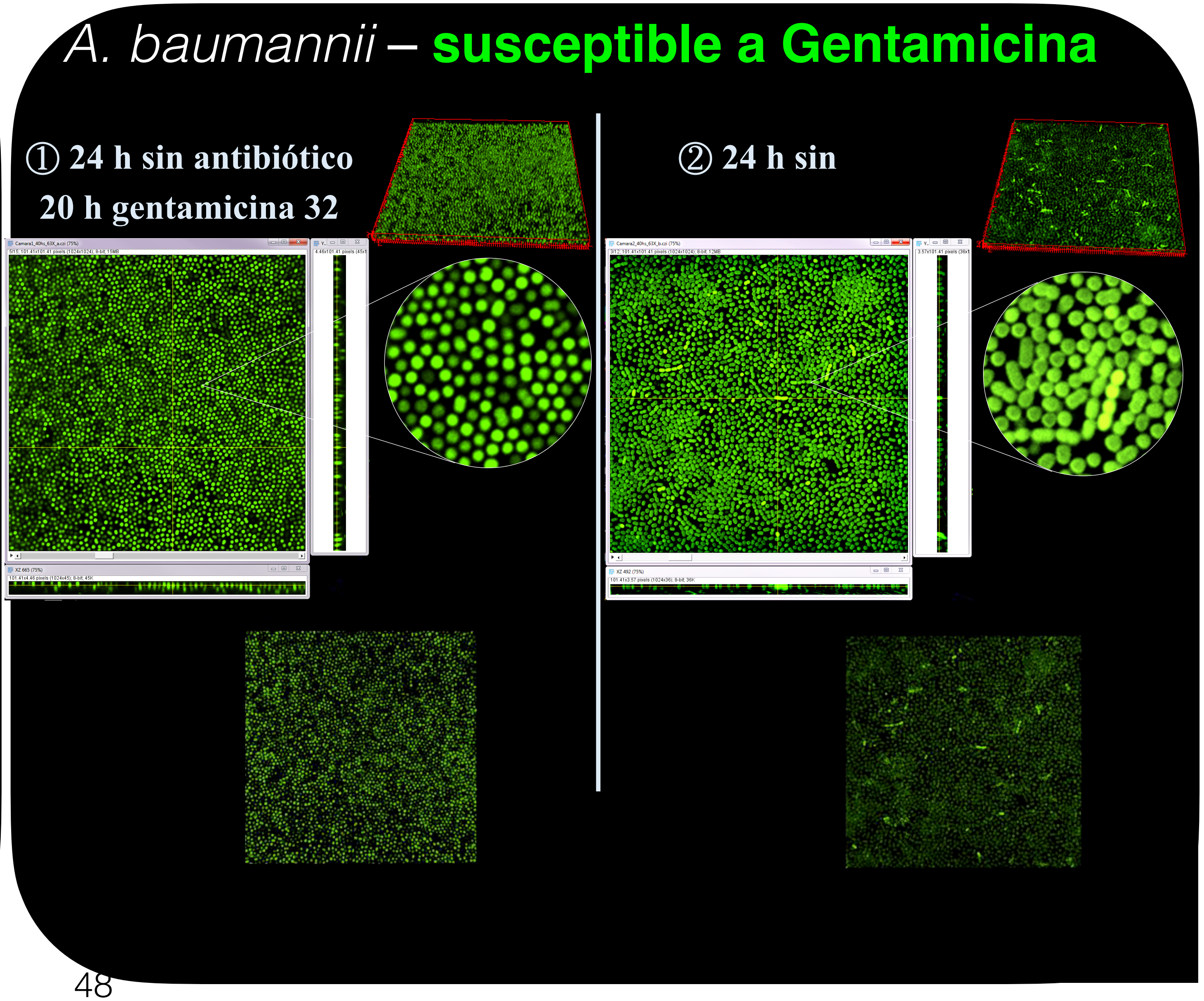




DINÁMICOS

1. Desarrollo de modelos de estudio de biofilms

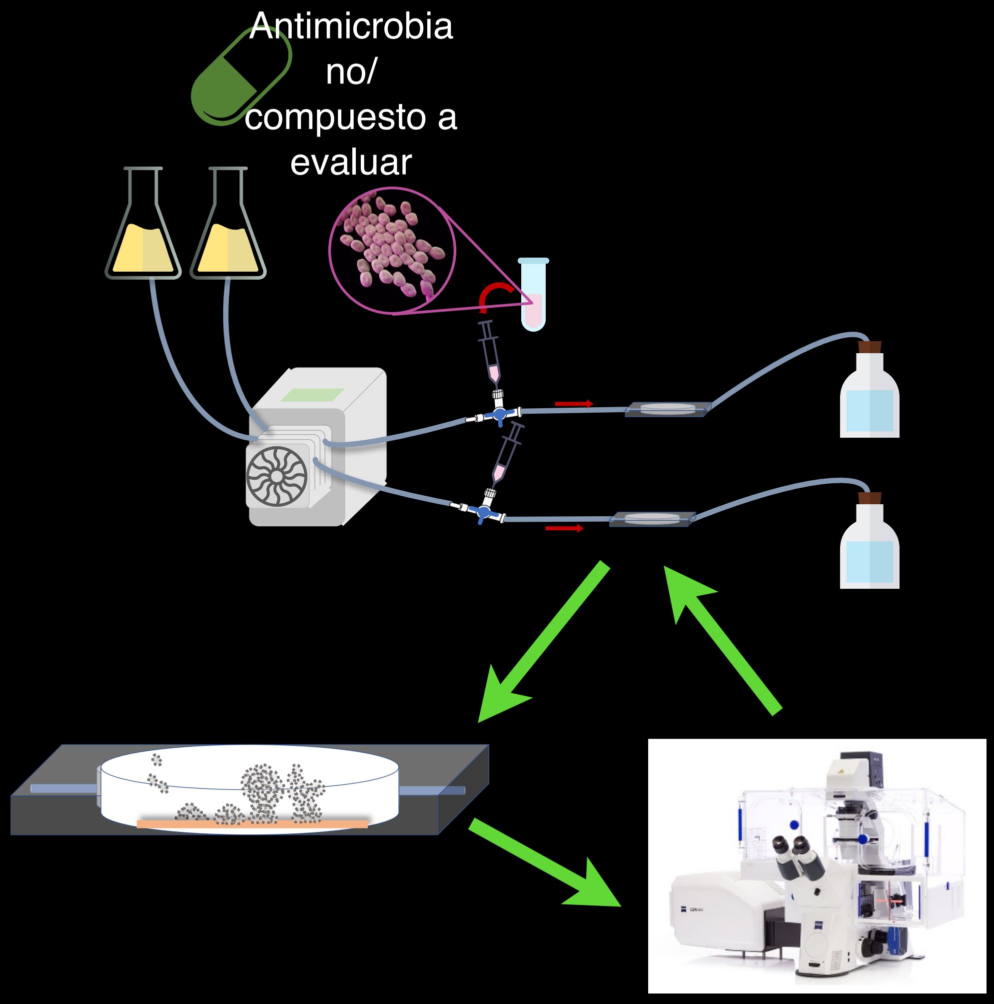




Biofilm formation

DINÁMICOS

1. Development of models



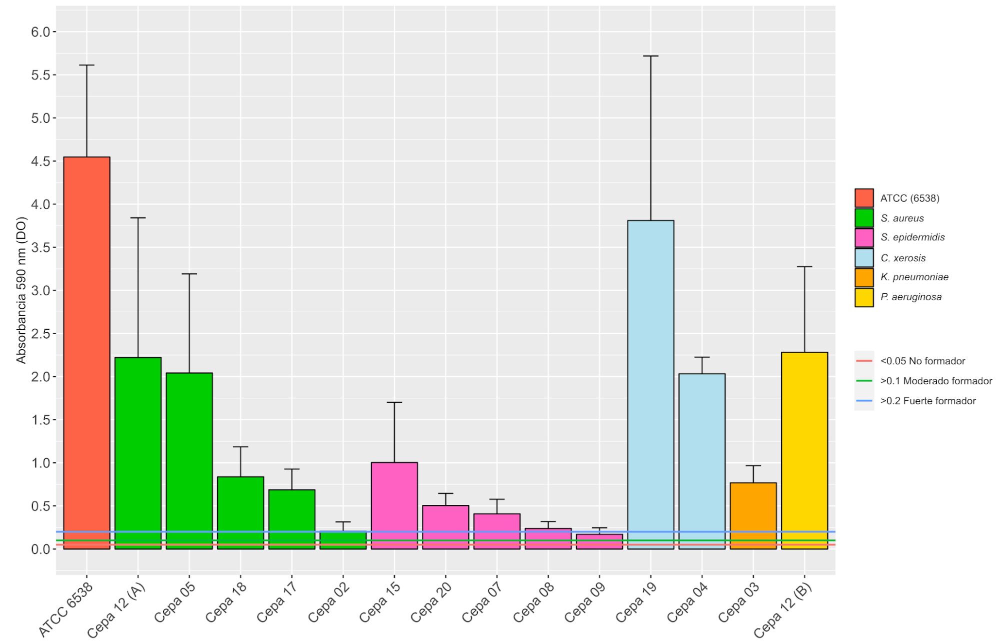
A. baumannii – **susceptible a Gentamicina**

	Con antibiótico	Sin antibiótico
Biofilm	Presente	Presente
Aspecto de bacterias	Redondeadas, más pequeñas	Cocobacilares
Filamentos	Ausentes	Presentes
Descarte (planctónicas)	Límpido	Turbio

Biofilm Research

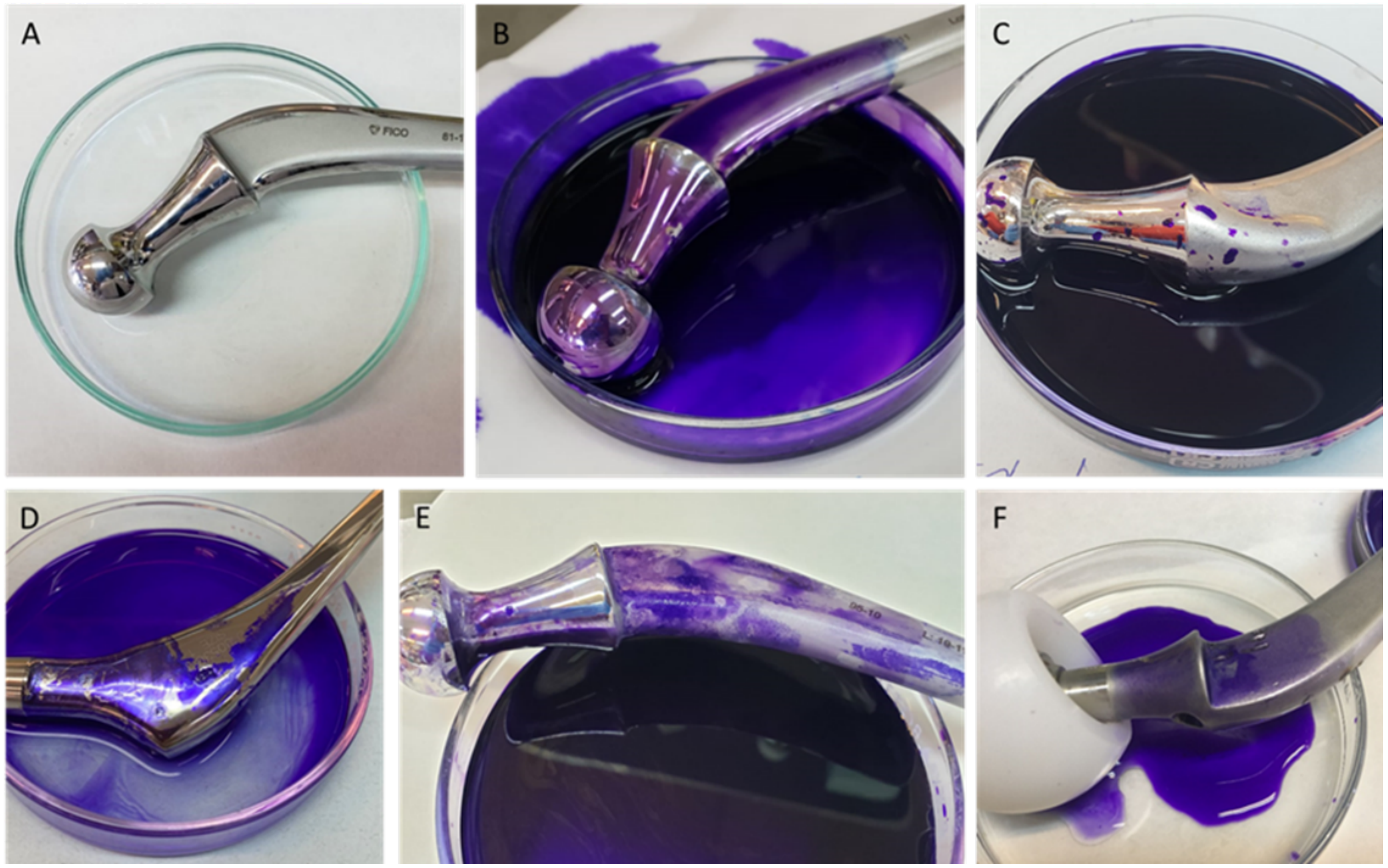
Pathogenesis

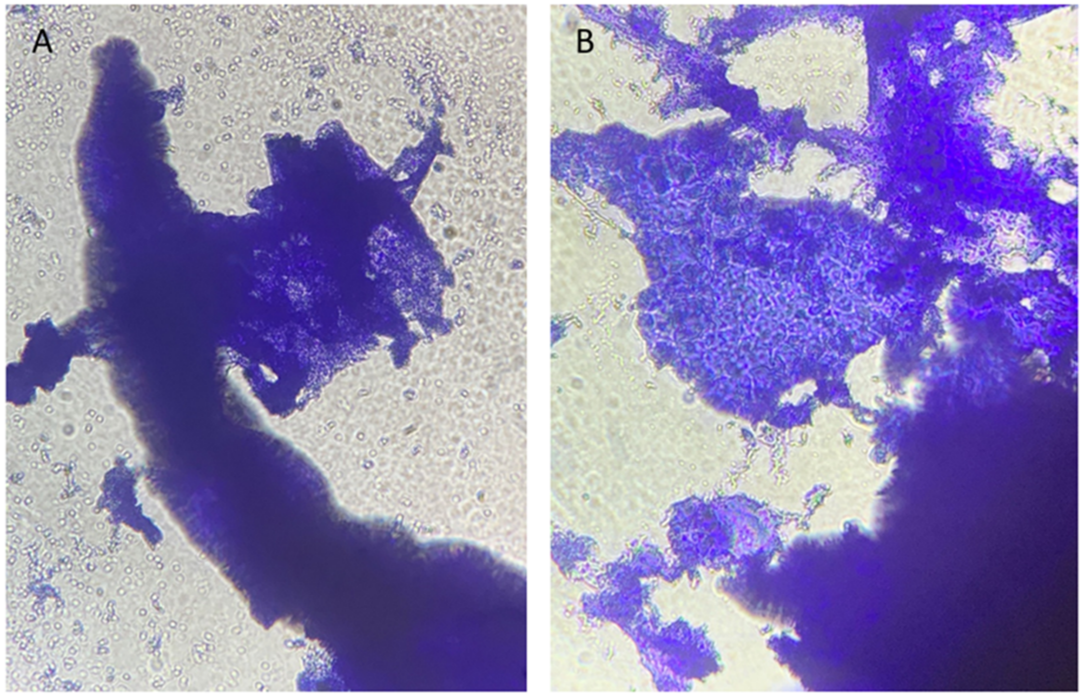
Biofilms on medical implants/devices



BANCO DE PROTESIS

Total Hip Replacement





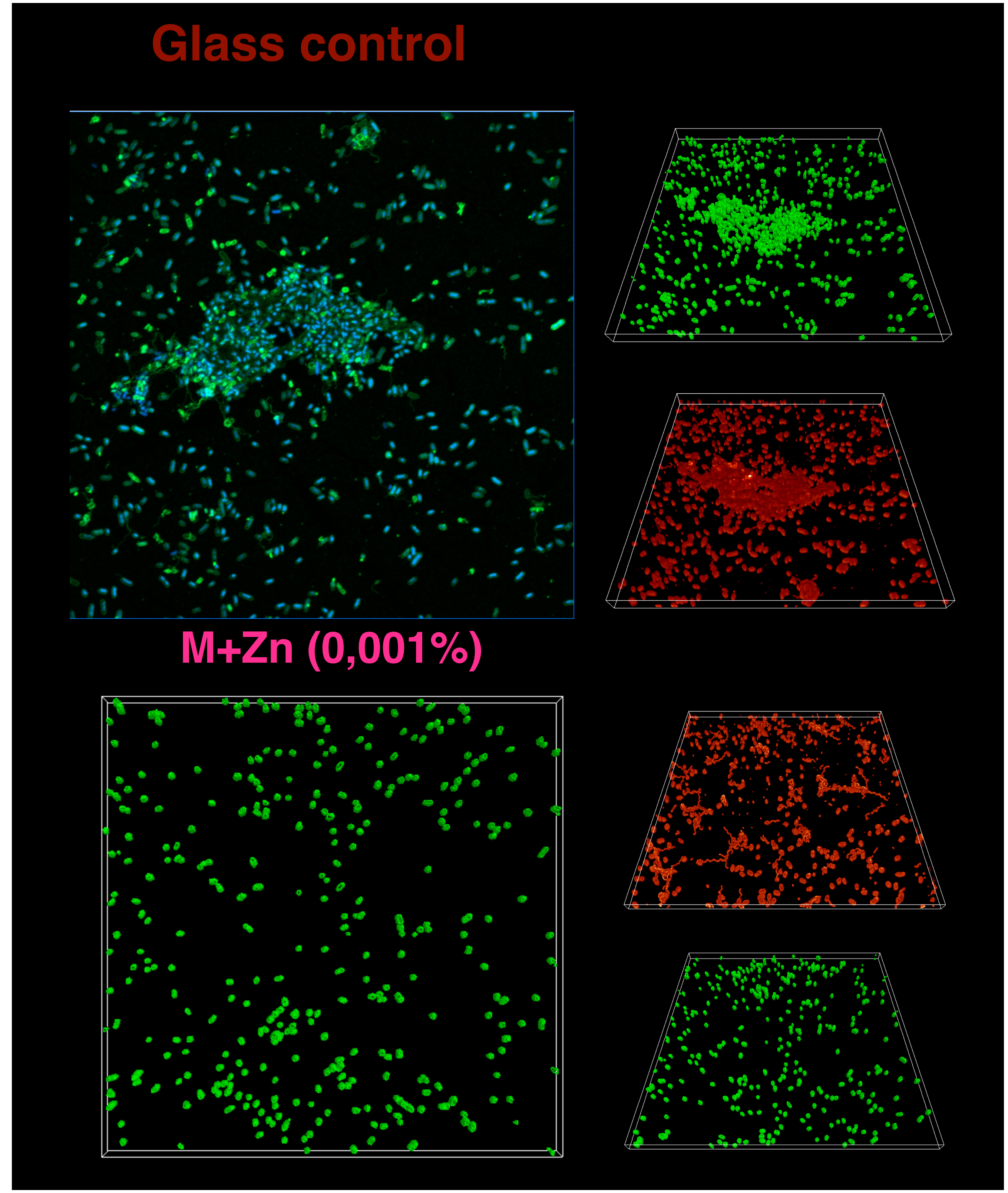
Biofilm Research

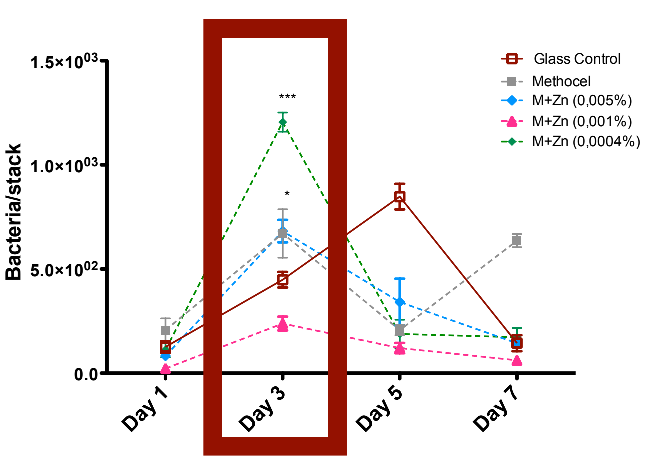
Erradication

Compounds for Prevention & Erradication

Nanotech

Nanoparticles





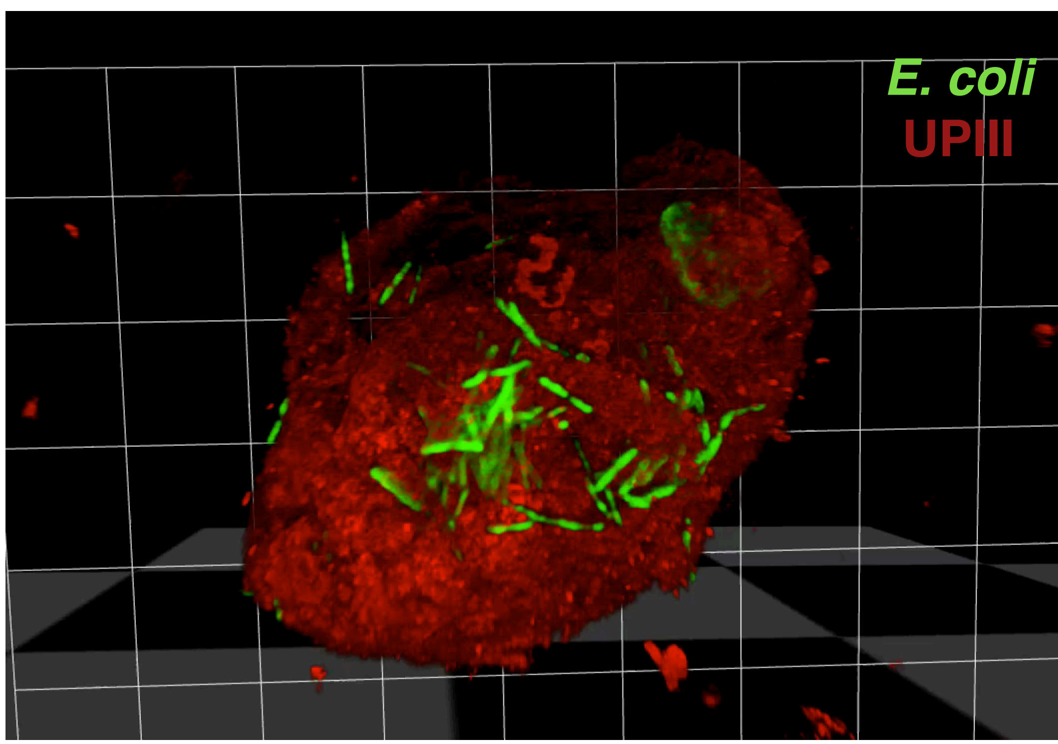
Iribarnegaray et al. 2019
Cruz et al. 2023

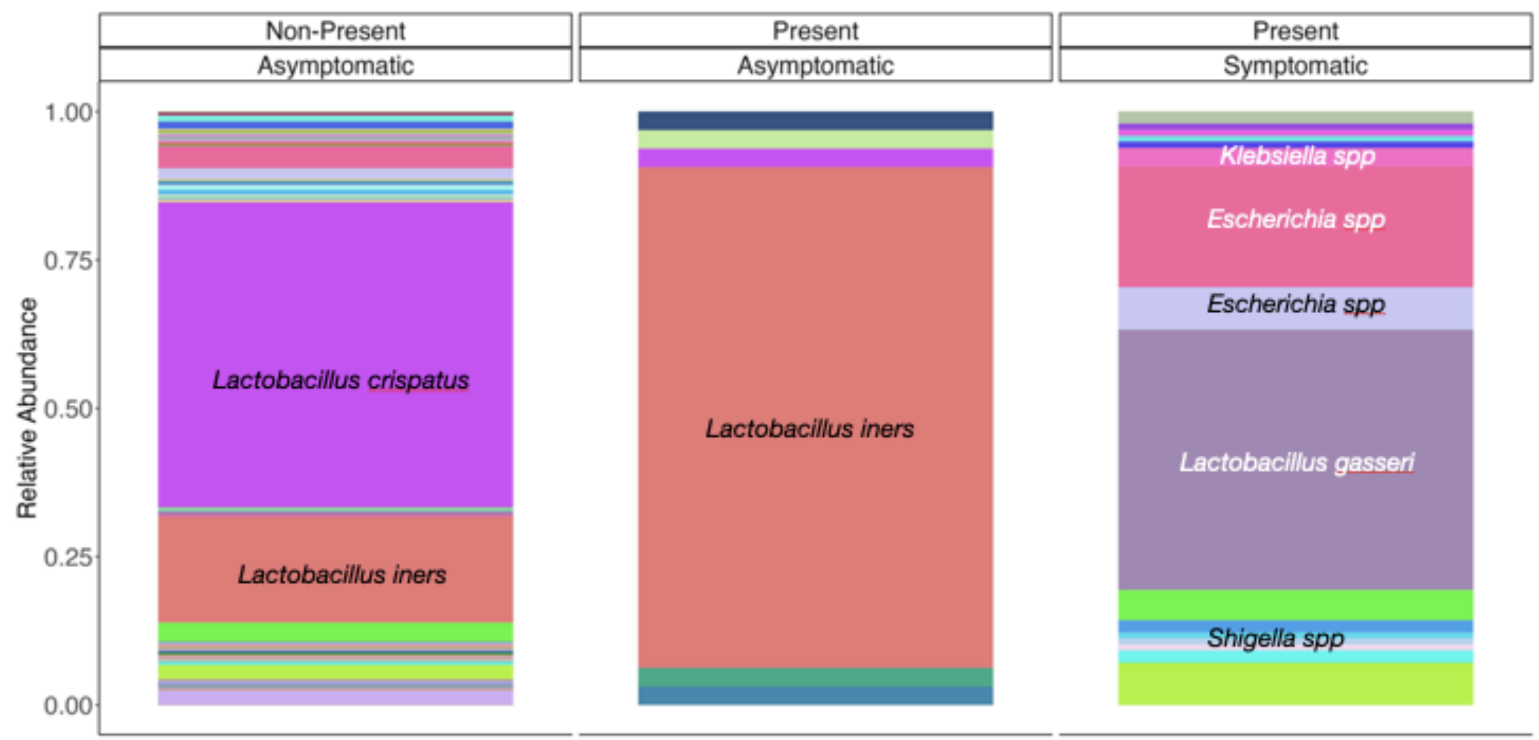
Urinary Tract Infections

Intracellular Bacterial Communirties
Urine Microbiota

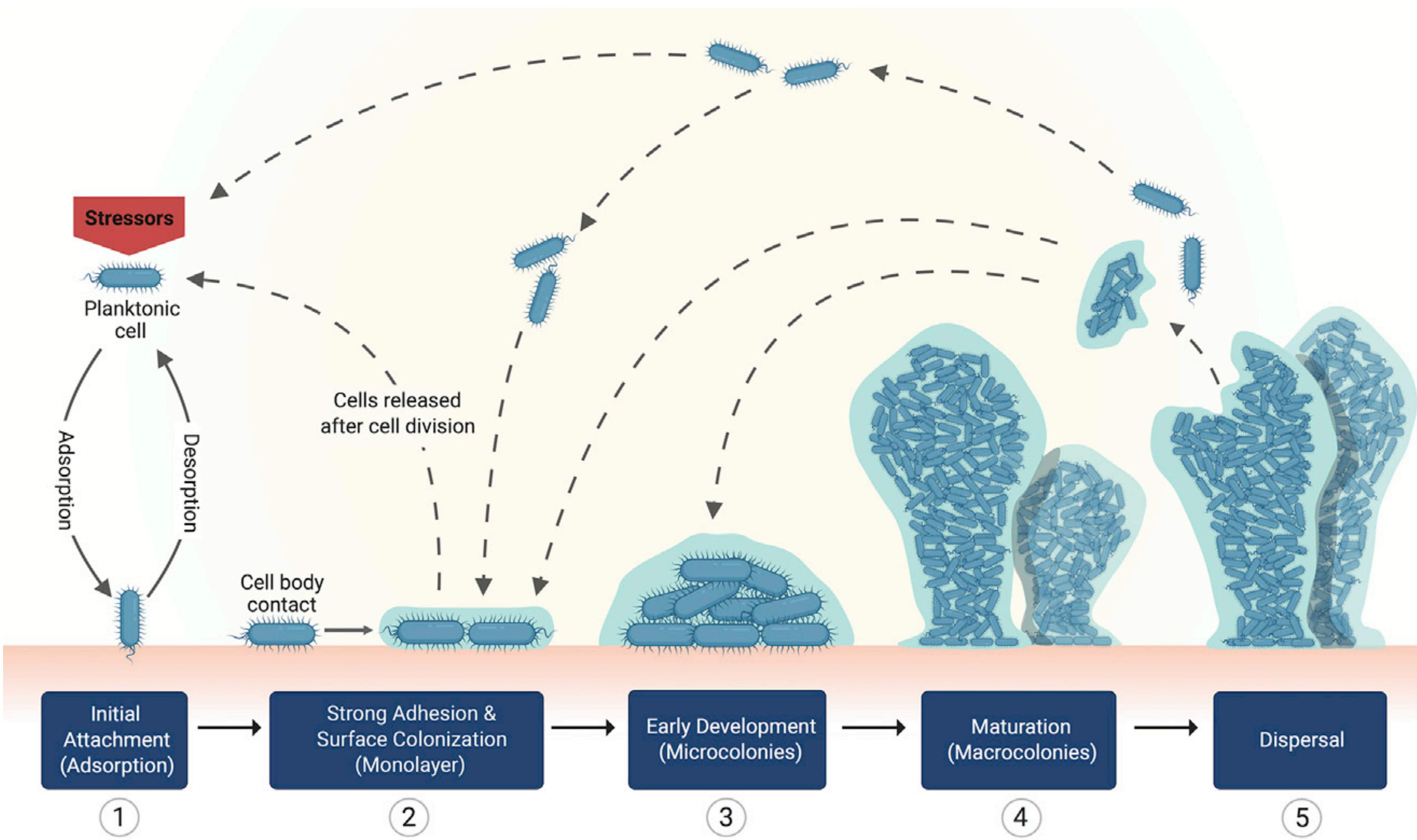
HOSPITAL MACIEL

INSTITUTO DE HIGIENE
Prof. Arnoldo Berta
FACULTAD DE MEDICINA





Robino et al. 2013, 2014
Robino et al 2023 in revision Sci. Rep.


Stressors
Planktonic cell
Adsorption
Desorption
Cell body contact
Cells released after cell division
Initial Attachment (Adsorption)
Strong Adhesion & Surface Colonization (Monolayer)
Early Development (Microcolonies)
Maturation (Macrocolonies)
Dispersal
1
2
3
4
5

Gracias!

Laboratorio de Biofilms Microbianos

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Dra María José González (Posdoc)

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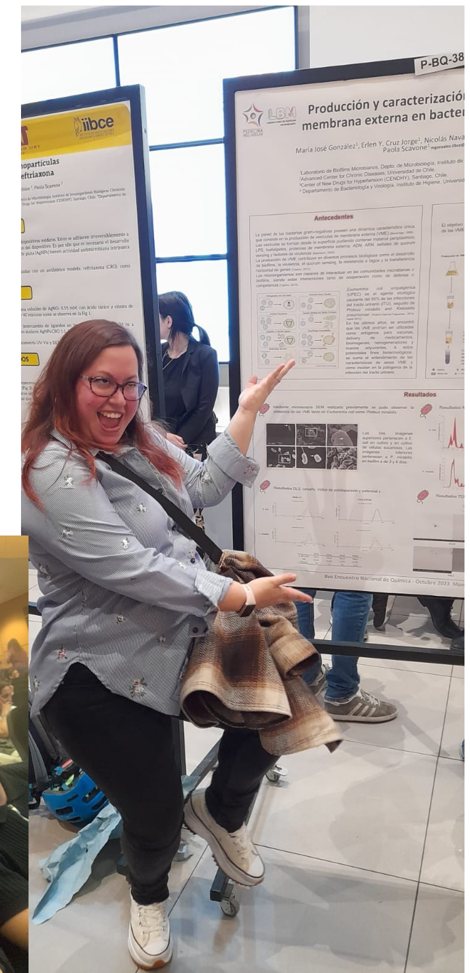
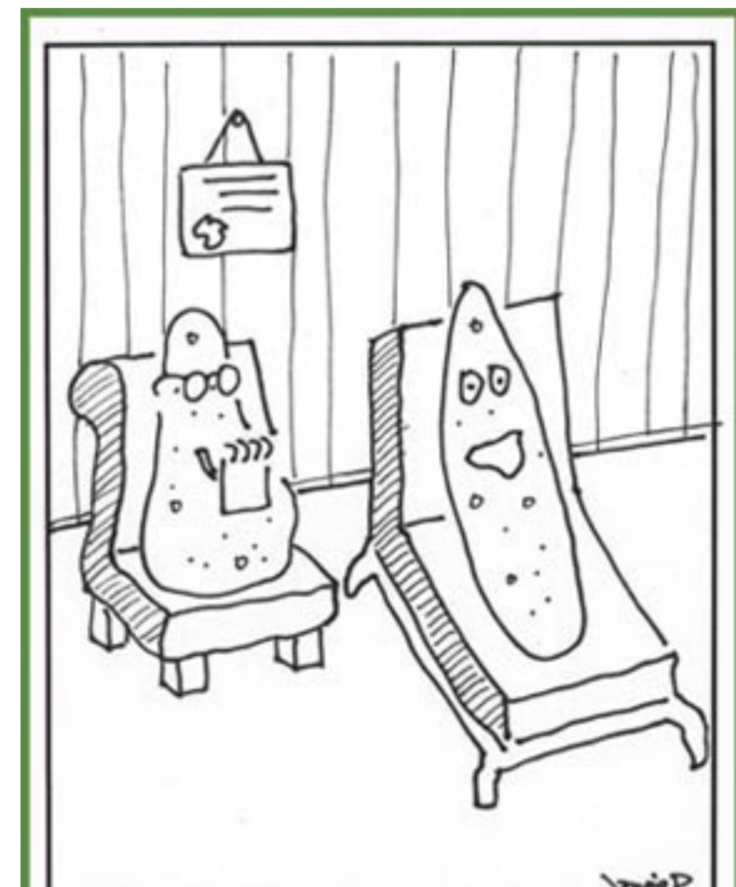
Barbara Miller (est. Grado)

LABORATORIO DE BIOFILMS MICROBIANOS





Acknowledgements

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Banco de Prótesis
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Dra. Cristina Davila
Dr. Nicolás Morales

- Internacionales
  - **Scian-Lab, Universidad de Chile**
    - Dr. Steffen Härtel
  - **Drug Delivery Lab, Ciencia y Tecnología Farmacéutica Universidad de Chile**
    - Dr. Javier O. Morales
  - **Department of Microbiology, School of Pharmacy an Bimolecular Sciences, University of Brighton, UK**
    - Dr. Brian Jones
    - Dr. Jonathan Nzakizwanayo
  - **University of Nottingham, UK**
    - Dr. Vincenzo di Bari
    - Dr. Nikolas Whatson