Relevance of biofilms, control, antimicrobials and disruptive molecules

Pablo Zunino

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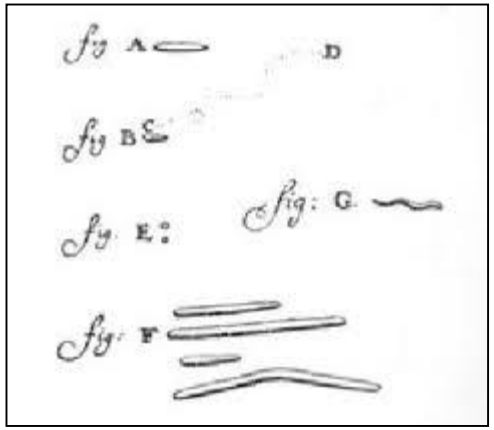
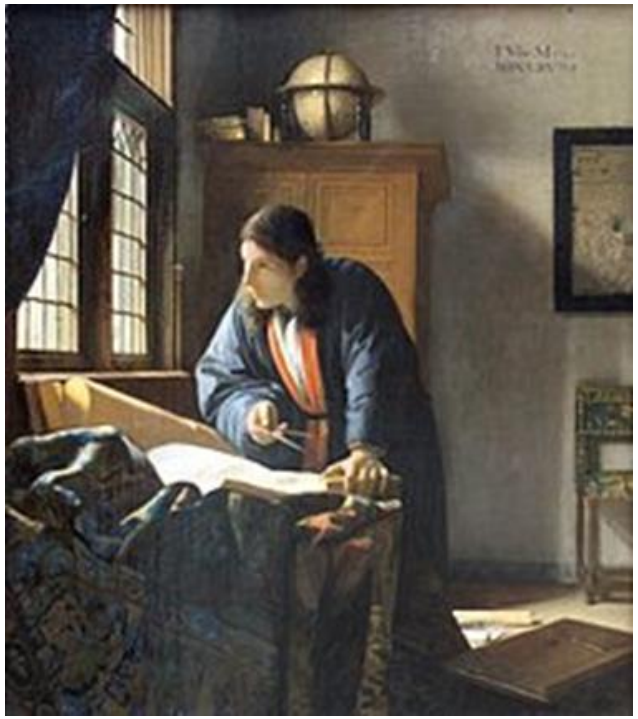
1632

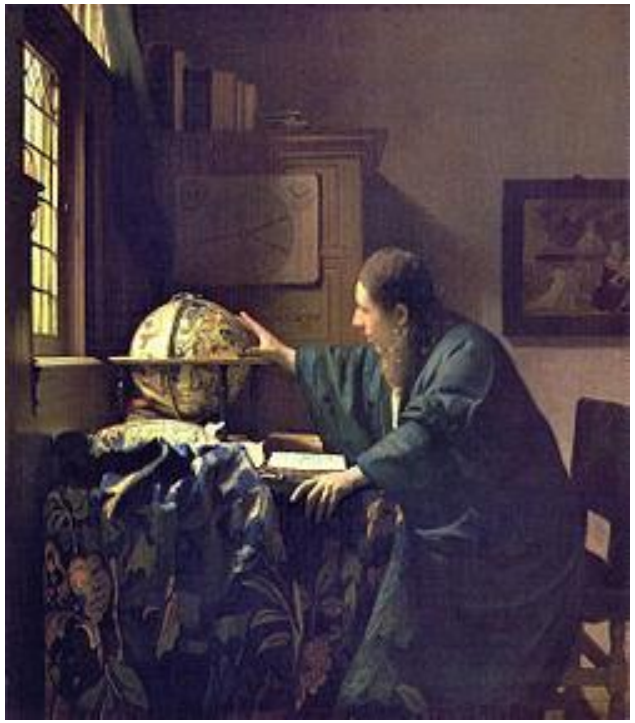


Antonie van Leeuwenhoek (1632-1723)

“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 a la London Royal Society, 1684)



El geógrafo



El astrónomo

Johannes Vermeer, baut. 31 de octubre de 1632

Anton van Leeuwenhoek, n. 24 de octubre de 1632

Rembrandt Harmenszoon van Rijn, n. 15 de julio de 1606

Christiaan Huygens, n. 1629 (La Haya)

Retrato de Christiaan Huygens por Caspar Netscher (1671), famoso retratista holandés

Origin of planet Earth: 4.6 billion years ago

Fossil evidence of microbial life forming biofilms: 3.7 billion years ago (rocks, stromatolites)

Probably fossilized microorganisms 4.28 billion years old in ferruginous sedimentary rocks (Dodd et al., 2017)

Cianobacteria fósil en ámbar (unos 850 millones de años, Museo de Paleontología de California)

Bacteria constitute the first life forms on Earth, playing a critical role in generating conditions for later life forms, fundamentally through the development of photosynthesis and generation of oxygen in the atmosphere (human life genus Homo: 2.5 Ma).

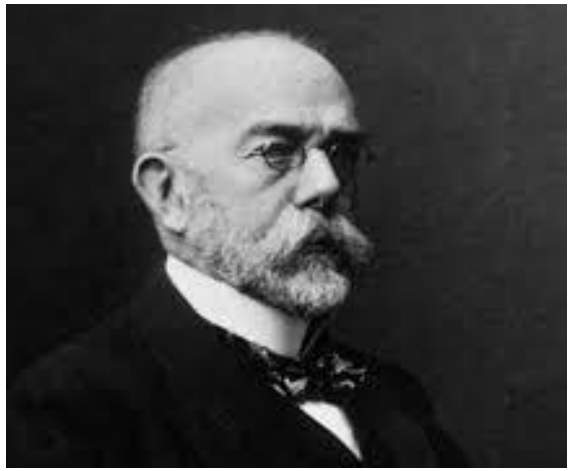
Bacteria: gregarious organisms

Traditional study of microorganisms as free-living planktonic cells in pure liquid media

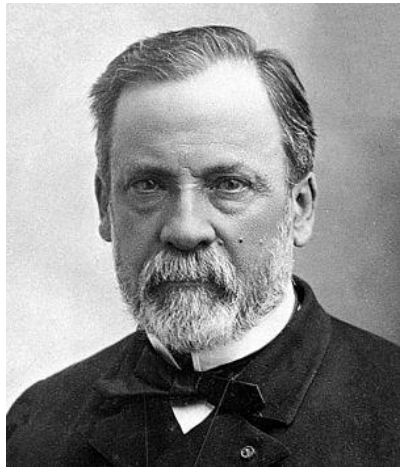
Stromatolites: fossil forms of bacterial biofilms

(dating back up to 3.5 billion years)

Lester Park, Saratoga Springs, New York (cámbrico)



Roberto Koch (1843 - 1910)



Louis Pasteur (1822 - 1895)

Fanny Eilshemius - Hesse (1850 - 1934)

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology

EDITORIAL

Classic Spotlight: Before They Were Biofilms

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Biofilms are surface attached communities of microbes. The term "biofilm," or surface-associated microbial communities, is now familiar to most microbiologists, as is the recognition of the importance of these microbial communities in clinical, environmental, and industrial settings, but the concept that microbes can attach to a surface is much older. While the term "biofilm" would not be coined until the 1970s, two seminal papers published in the 1930s in the *Journal of Bacteriology* (JB) by Arthur Henrici and Claude Zobell established the biofilm concept.

In 1933 Henrici (1) recognized that submerging a clean slide in a variety of aquaria, a lily pond on the University of Minnesota campus, and nearby Lake Alexander resulted in a bacterial community "eventually becoming so thick that individual cells may be distinguished with difficulty. That the cells are actually growing upon the glass is indicated by their occurrence in microcolonies of steadily increasing size." Henrici also observed, "In other cases the groups of cells are evidently surrounded by a sheath of gum which also serves to fasten the colony to the glass"; Henrici was noting the biofilm matrix.

Two years later Zobell, working with Esther Allen (2), reported similar studies in a marine environment. In their paper in JB, they report that "it seems to take several minutes for the bacteria to cement themselves to the glass. . . . But let the slide remained submerged for an hour or two. . .they will be found profusely, so firmly glued to the slide that running water will not detach them." Here Zobell and Allen, though they do not use the terms, observed the well-known phenomena of "reversible" and "irreversible" attachment as well as microcolony formation, the earliest stages in establishing a biofilm.

As Henrici stated in JB in 1933, "It is quite evident that for the most part the water bacteria are not free floating organisms, but grow upon submerged surfaces; they are *benthos* rather than *plankton*." Many years later we are still guided by the keen observations set forth in JB in the 1930s.

REFERENCES

1. **Henrici AT.** 1933. Studies of freshwater bacteria. I. A direct microscopic technique. J Bacteriol 25:277–287.
2. **Zobell CE, Allen EC.** 1935. The significance of marine bacteria in the fouling of submerged surface. J Bacteriol 29:239–251.

Citation O'Toole GA. 2016. Classic spotlight: before they were biofilms. J Bacteriol 198:5. doi:10.1128/JB.00593-15.

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1. **Henrici AT.** 1933. Studies of freshwater bacteria. I. A direct microscopic technique. J Bacteriol 25:277–287.
2. **Zobell CE, Allen EC.** 1935. The significance of marine bacteria in the fouling of submerged surface. J Bacteriol 29:239–251.

Observations of fouling biofilm formation

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Accepted May 29, 1981

McCoy, W. F., J. D. Bryers, J. Robbins, and J. W. Costerton. 1981. Observations of fouling biofilm formation. Can. J. Microbiol. **27**: 910–917.

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

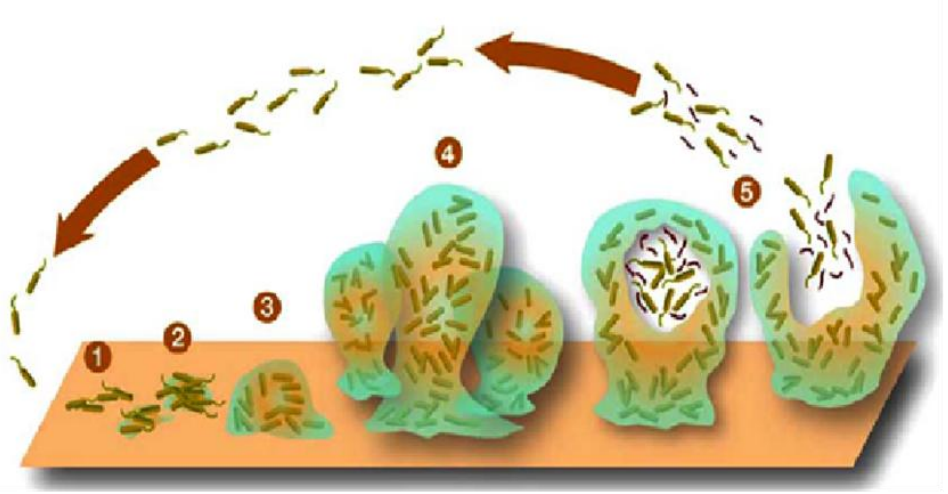
by J. W. Costerton, G. G. Geesey and K.-J. Cheng

SCIENTIFIC AMERICAN
JANUARY 1978
VOL. 238, NO. 1 PP. 86–95

Henrici: “In other cases the groups of cells are evidently surrounded by a sheath of gum which also serves to fasten the colony to the glass” (1933)

Zobel: “It seems to take several minutes for the bacteria to cement themselves to the glass. … But let the slide remained submerged for an hour or two…they will be found profusely, so firmly glued to the slide that running water will not detach them” (1935)

Costerton: a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription (2002, Donlan and 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

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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

"The development of new ways to control bacterial infections will require a much more detailed knowledge than we now have of the polysaccharide constituents of particular pathogens and host tissue cells. That will take time. The basic perception that bacteria colonize their environments by adhering tenaciously and specifically is a first step toward an ability to manipulate and control bacteria more effectively." (1978)

Artículos científicos sobre biofilms (Pubmed - NCBI)

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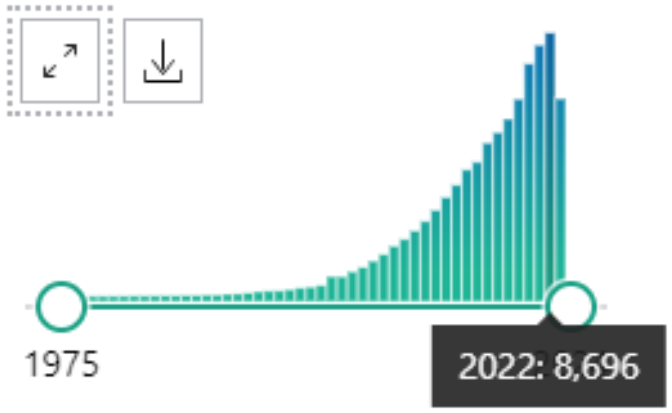

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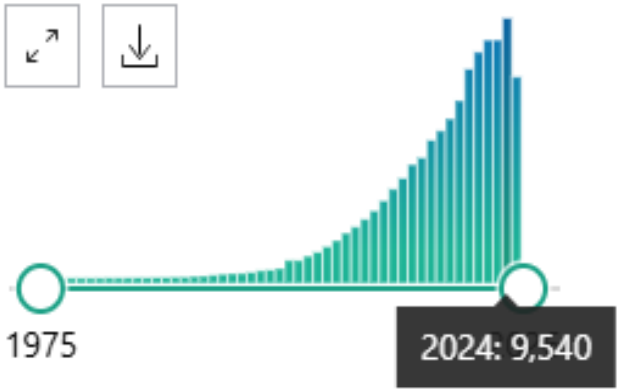


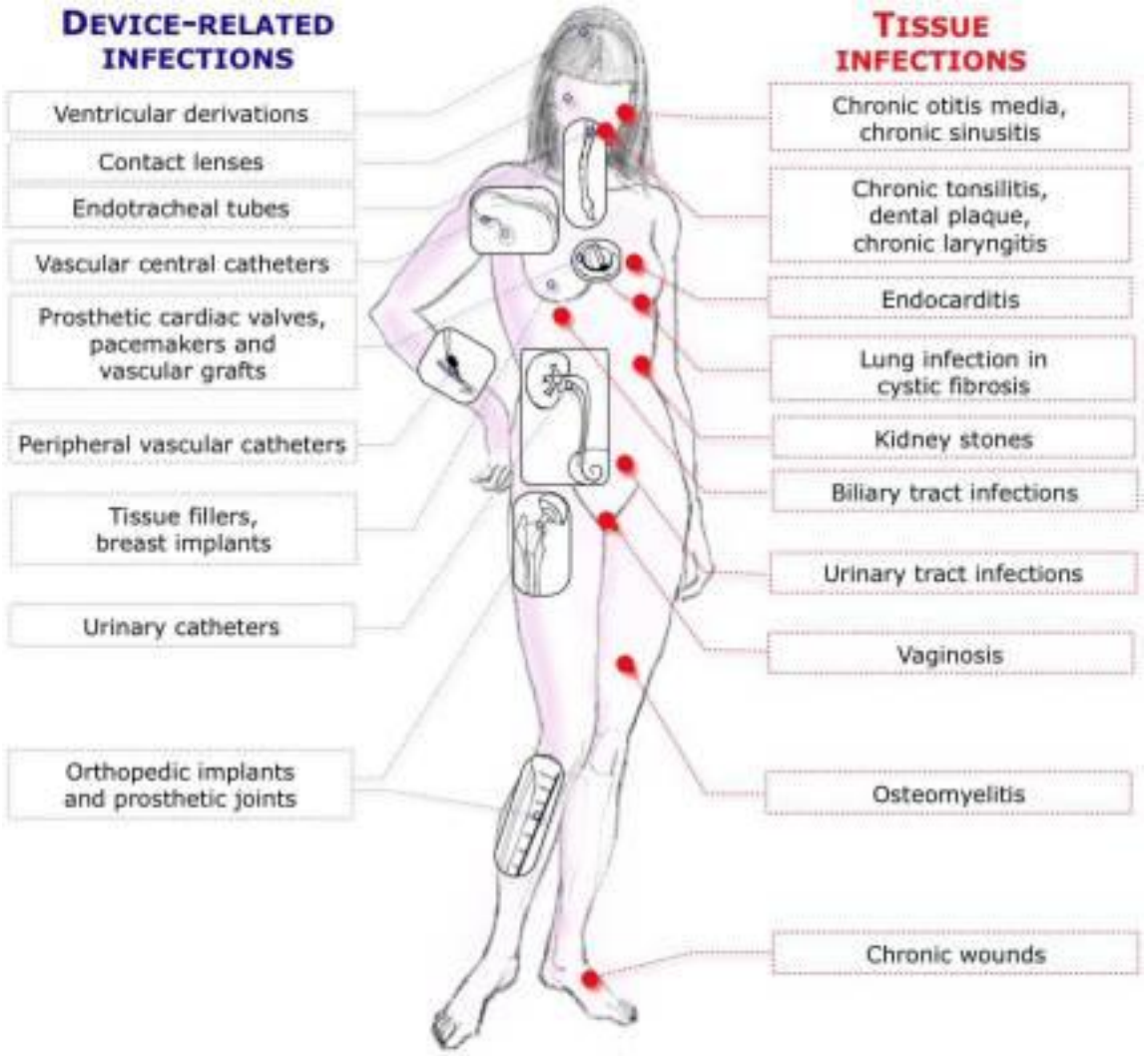
26 por día en 2024!!

Impact of bacterial biofilms on health

The percentages of bacterial infections involving biofilms are estimated to be between 65% (CDC) and 80% (NIH) (Jamal et al., 2018)

E.g. endocarditis, cystic fibrosis, periodontitis, rhinosinusitis, osteomyelitis, wounds, meningitis, urinary tract infections, infections associated with prostheses and other implants, etc.

Infections associated with biofilms



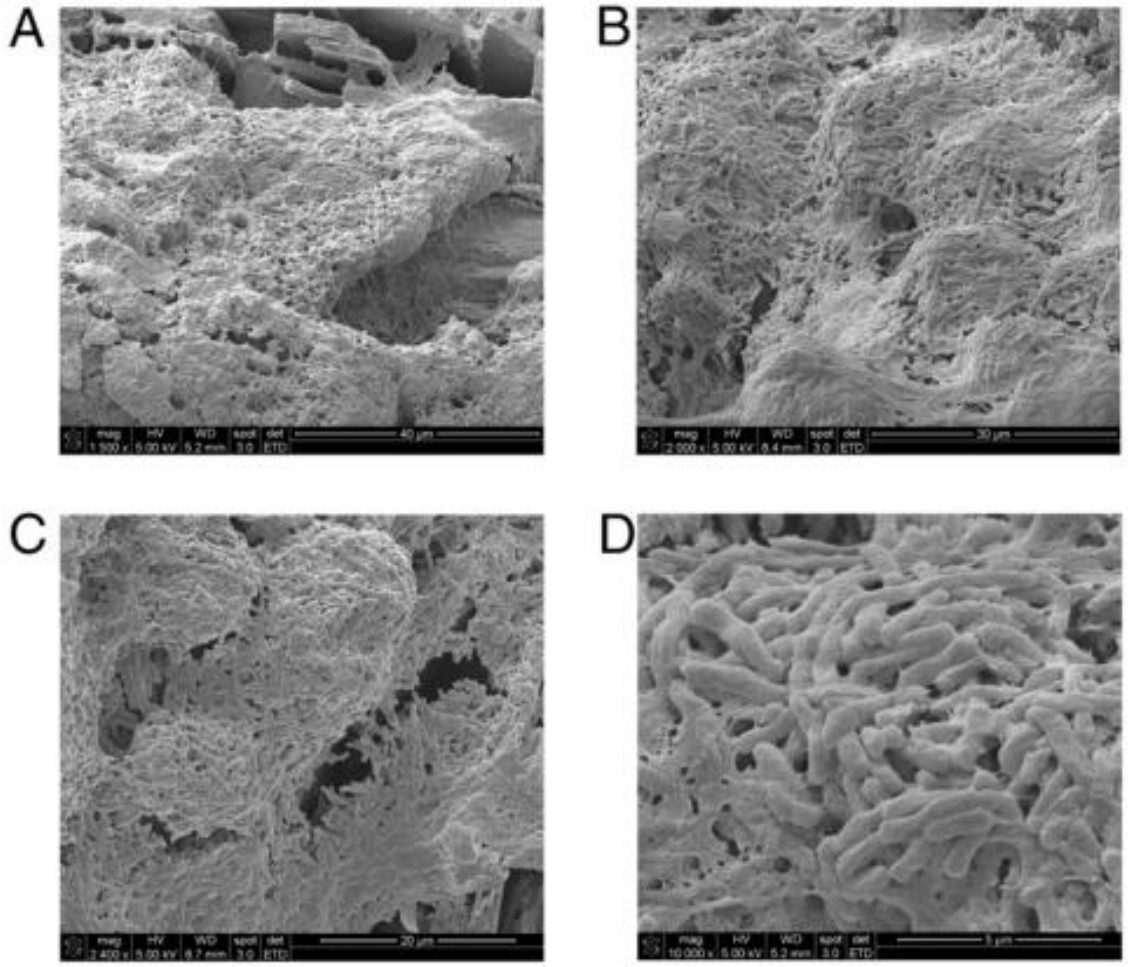
Lebeaux et al., 2013

Infections associated with biofilms on tissues

Table 1. Biofilm-associated diseases of different body systems and their affected organs.

Body System	Affected Organs	Disease
Auditory	Middle ear	Otitis media
Cardiovascular	Cardiac valves	Infective endocarditis
	Arteries	Atherosclerosis
Digestive	Salivary glands	Sialolithiasis (salivary duct stones)
	Gall bladder	Recalcitrant typhoid fever and predisposition to hepatobiliary cancers
	Gastrointestinal tract, especially the small and large intestine	Inflammatory bowel disease and colorectal cancer
Integumentary	Skin and underlying tissue	Wound infections
Reproductive	Vagina	Bacterial vaginosis
	Uterus and fallopian tubes	Chronic endometritis
	Mammary glands (breasts)	Mastitis
Respiratory	Nasal cavity and paranasal sinuses	Chronic rhinosinusitis
	Throat, i.e., pharynx with tonsils and adenoids, and larynx with vocal cords	Pharyngitis and laryngitis
	Upper and lower airways	Pertussis (whooping cough) and other Bordertella infections
	Upper and lower airways	Cystic fibrosis
Urinary	Prostate gland	Chronic bacterial prostatitis
	Urethra, bladder, urethers, kidneys	Urinary tract infections

Vetsby et al., 2020

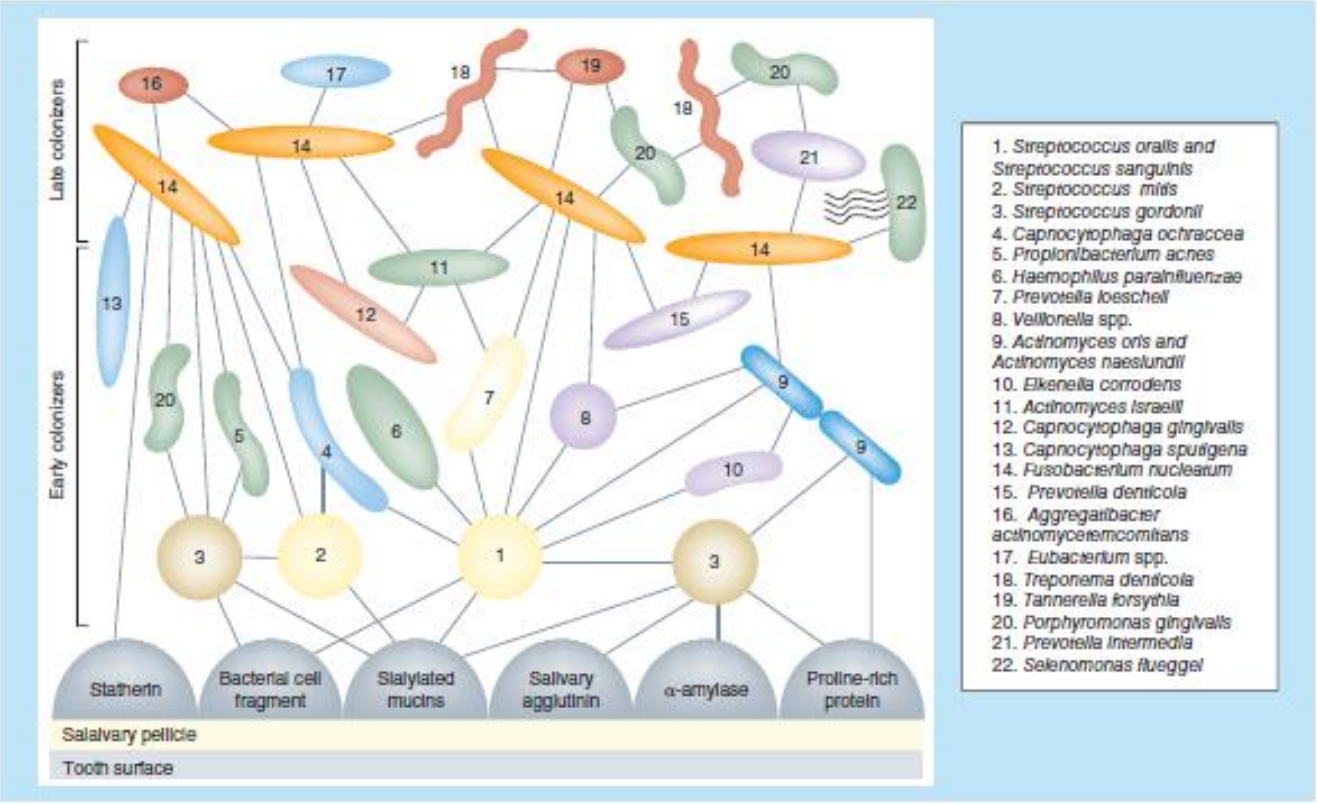


Biofilms in gall bladder (*S*. Typhi, asymptomatic carrier)

Vetsby et al., 2020

Diseases related with biofilms

Dental plaque



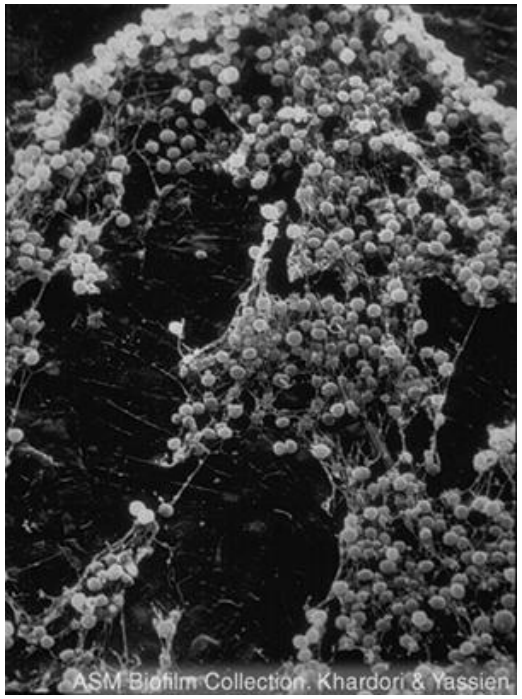
Rabin et al. 2021

Polimicrobial biofilms:
Más de 700 especies de bacterias y arqueas reportadas

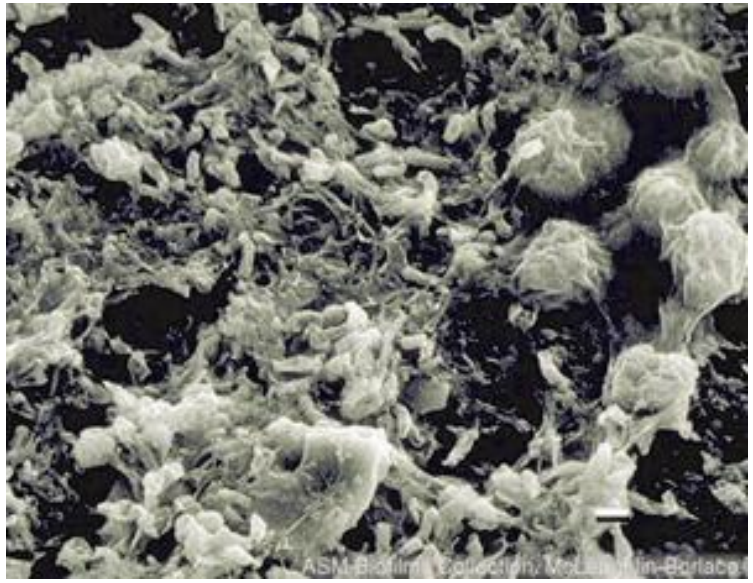
Biofilms on implants

They represent 50 to 70% of nosocomial infections.

They cause infections and can interfere with implant function; removal and replacement can lead to serious medical consequences and economic losses.

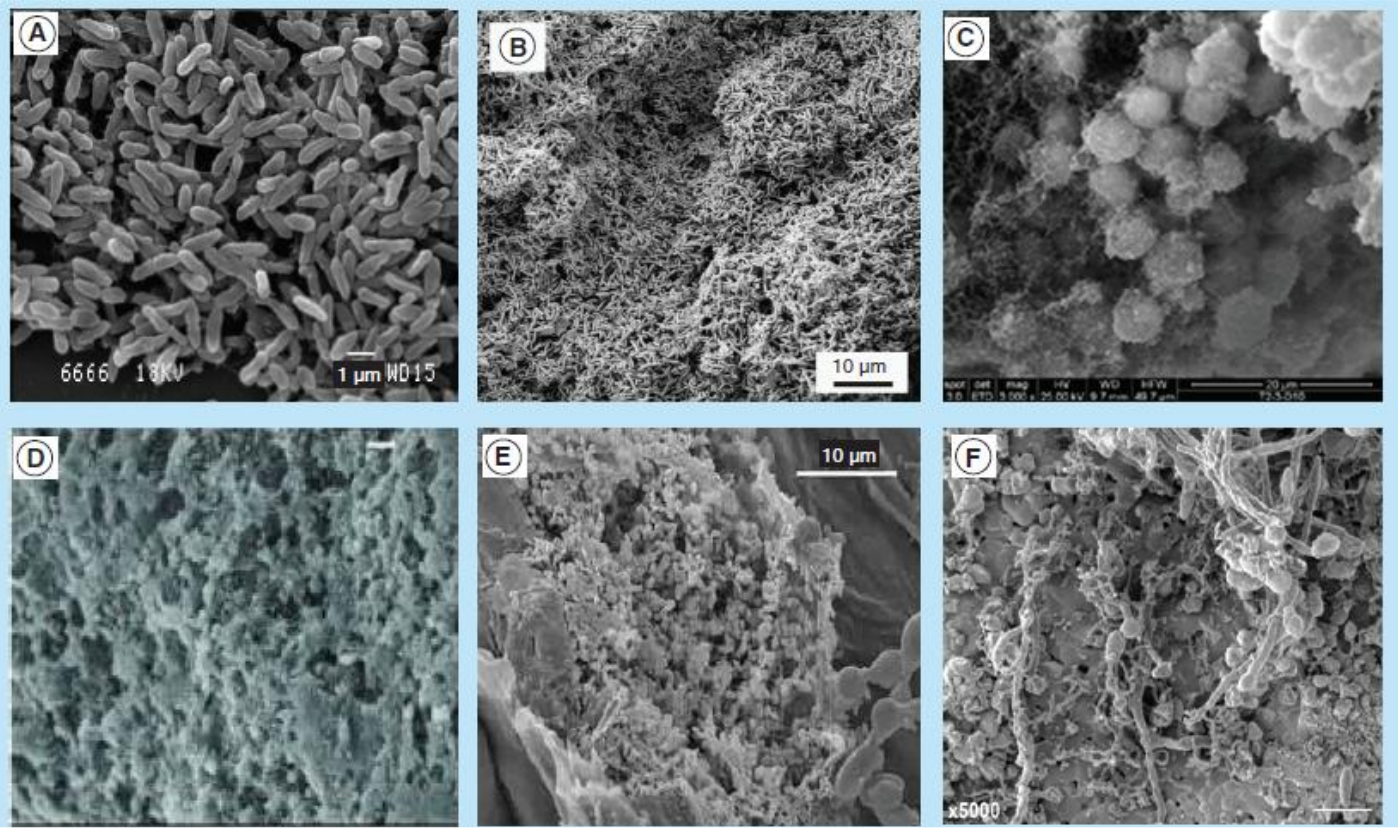


Catéter vascular (MEB)



Lente de contacto (MEB)

Rabin et al. 2021



Rabin et al. 2021

A) *Pseudomonas aeruginosa* adherida a superficies de vidrio

B) *Escherichia coli* en superficie del óxido de titanio

C) *Staphylococcus aureus* asociado a catéter intravenoso *in vivo*

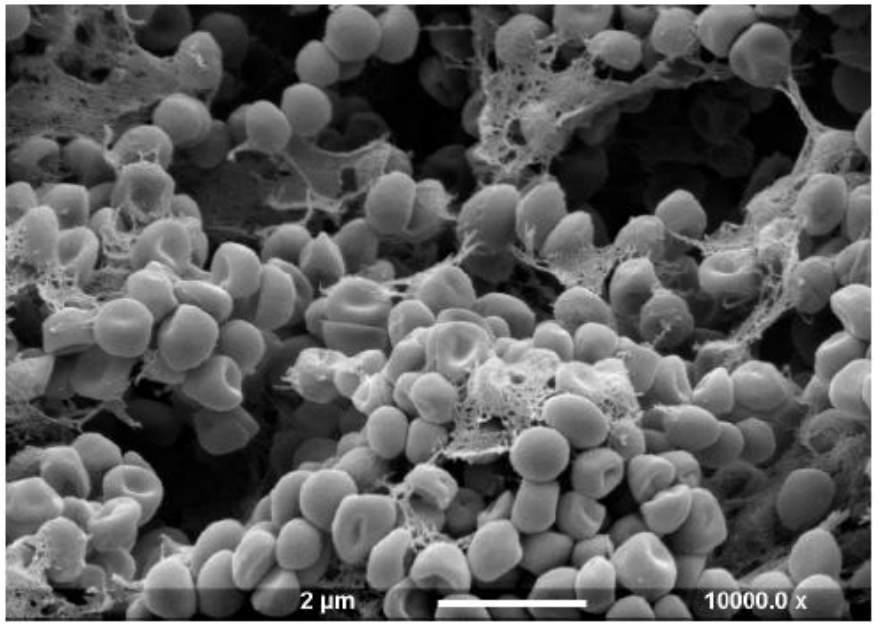
D) *Staphylococcus epidermidis* en un catéter de teflón

E) *Salmonella enterica* serovar Pomona sobre tejidos vegetales

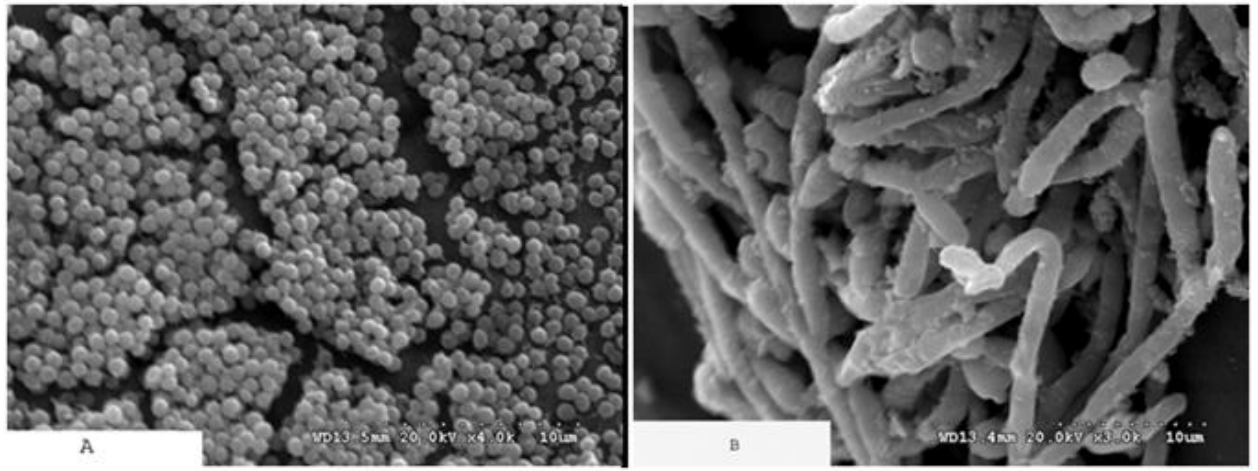
F) *Streptococcus mutans y Candida albicans en* discos de hidroxiapatita

The global medical implants market was estimated at around US$ 114 billion in 2025, a projected US$ 247 billion in 2034.

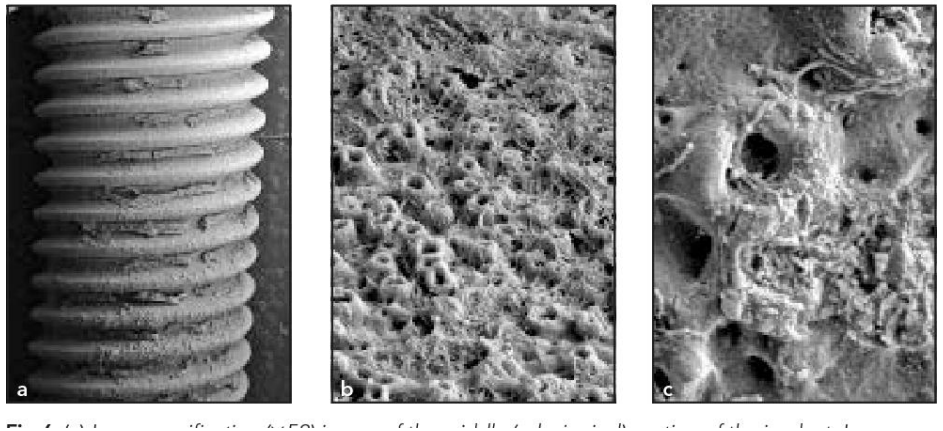
The increasing use of biomaterial-based devices is associated with an aging population, the growing prevalence of diseases, and lifestyle changes (sedentary lifestyle, consumption of unhealthy foods, increased incidence of traumatic accidents, increased demand for grafts and donor organs, etc.)



Biofilm de *S. epidermidis* en implante óseo (Rabin et al. 2021)



S. epidermidis y *C. albicans* formando biofilms en catéteres vasculares (El-Azizi, 2015)



Biofilms polimicrobianos en la superficie rugosa de implante dental (Simion et al., 2016)

Medical Devices Bureau (Canada) recognizes 4 classes of medical devices according to risk

- **Class I:** Low risk to patients and does not require licensing or requires a lower regulatory standard (surgical instruments, dental materials, etc.)

- **Class II:** Requires a manufacturer's declaration of device safety and effectiveness (contact lenses, ultrasound machines, medical catheters, etc.)

- **Class III:** Presents a higher potential risk to the patient (orthopedic implants such as bone cement, hip implants, hemodialysis machines, etc.)

- **Class IV:** Presents the highest potential risk and is subject to in-depth pre-market regulatory review and approval (cardiovascular implants, pacemakers, ventricular assist devices, etc.)

Catheter-associated urinary tract infections (UTI-C)

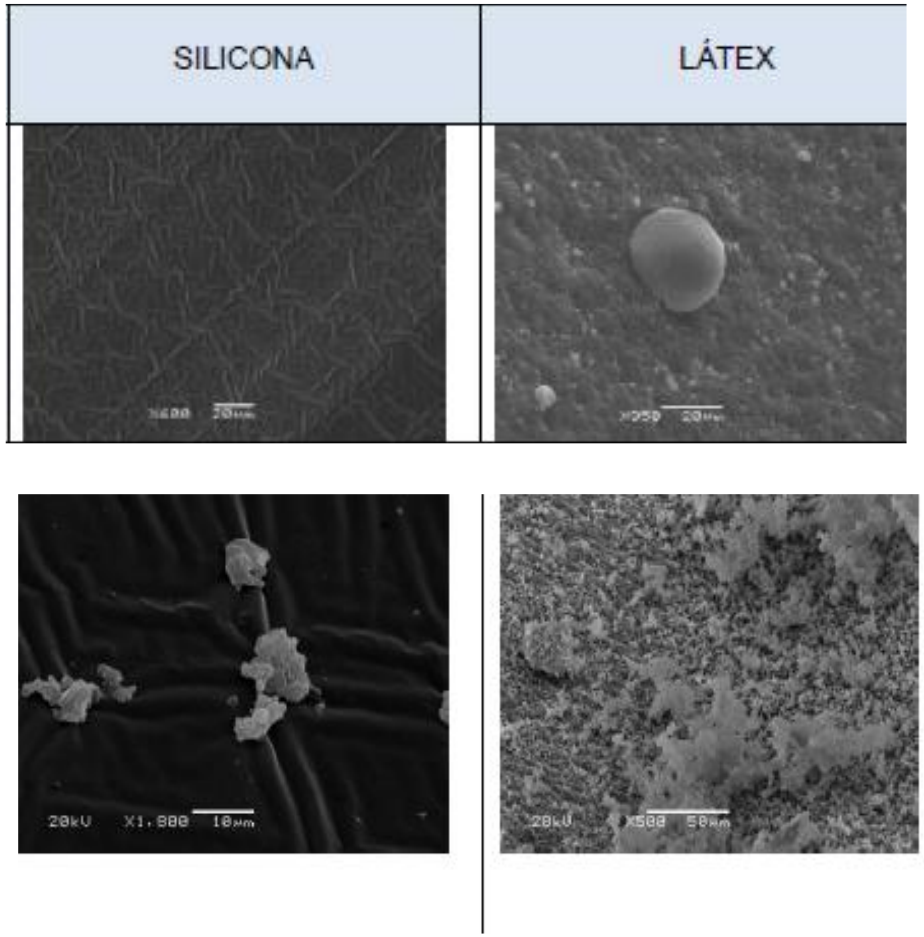
- Linked to the formation of biofilms on the surface of catheters
- Most common nosocomial infections
- Costs (global): US$ 1390 millions in 2025, projected US$ 1960 millions in 2035 (growth rate around 3.5% each year)

Incidence:

Pacientes cateterizados hasta 7 días
→ 10 al 50 % desarrollan ITU-C

Pacientes cateterizados por más de 28 días
→ 100 % desarrollan ITU-C

P. mirabilis biofilms on catheter sections (SEM)



Secciones de catéteres

Biofilms de *P. mirabilis*

Departamento de Microbiología, IIBCE; en colaboración con el Servicio de Microscopía de la Fac. de Ciencias

Magnitude of the problem of biofilms associated with medical implants

Device	Estimated no. inserted in the United States per year	Rate of infection, %	Attributable mortality[a]
Bladder catheters[b]	>30,000,000	10–30	Low
Central venous catheters[b,c]	5,000,000	3–8	Moderate
Fracture fixation devices[b]	2,000,000	5–10	Low
Dental implants[d]	1,000,000	5–10	Low
Joint prostheses[b]	600,000	1–3	Low
Vascular grafts[b]	450,000	1–5	Moderate
Cardiac pacemakers[b,d]	300,000	1–7	Moderate
Mammary implants, in pairs[e]	130,000	1–2	Low
Mechanical heart valves[d]	85,000	1–3	High
Penile implants[b,d]	15,000	1–3	Low
Heart assist devices[d]	700	25–50	High

[a] Semiquantitative scale for attributable mortality: low, <5%; moderate, 5%–25%; high, >25%.
[b] Numbers estimated by analysis of market reports.
[c] Numbers estimated by review of the medical literature.
[d] Numbers estimated by personal communication with personnel from device manufacturing companies.
[e] Numbers estimated by review of data provided by medical associations.

Table 2. Device-related factors that may favor bacterial adherence.

Type of device material
Polyvinyl chloride favors bacterial adherence more than does teflon
Polyethylene favors bacterial adherence more than does polyurethane
Latex favors bacterial adherence more than does silicone
Silicone favors bacterial adherence more than does polytetrafluoroethylene
Stainless steel favors bacterial adherence more than does titanium
Source of device material: synthetic favors bacterial adherence more than does biomaterial
Surface of device
Irregular favors bacterial adherence more than does regular
Textured favors bacterial adherence more than does smooth
Hydrophobic favors bacterial adherence more than does hydrophilic
Shape of device: polymeric tubing favors bacterial adherence more than does wire mesh

Darouiche, 2001

Virulence/biofilm relationship on catheters

P. mirabilis and biofilms

TABLE 1 Characterization of biofilm formation mutants

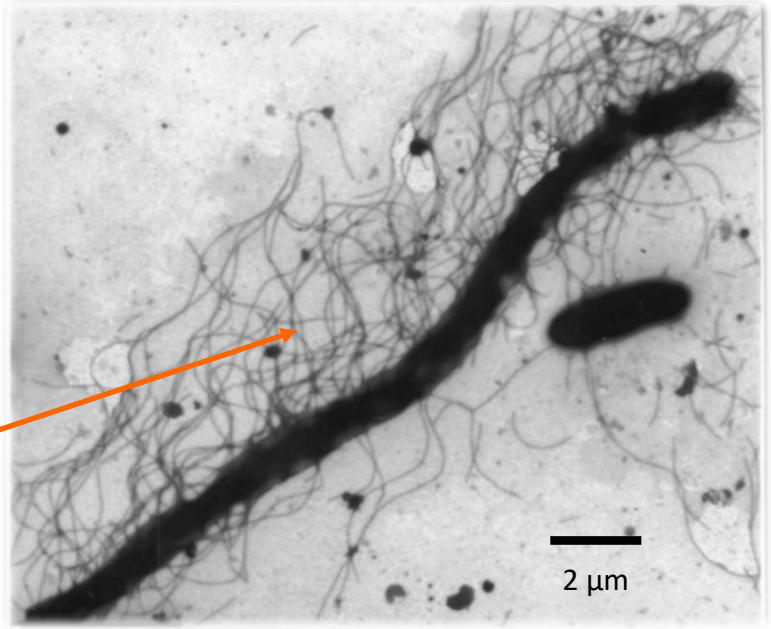
Phenotype and strain	*P. mirabilis* HI4320 locus[a]	Disrupted gene	Putative function/product of disrupted gene[b]	Urease production (mean ± SD)[c]	Motility (% of wild type)	
					Swimming	Swarming
Wild type (B4)				5.7 ± 0.4	100	100
Biofilm enhanced						
NHBFG8	PMI3402		Unknown function; putative MuA-like DNA-binding protein (Pfam 02316) (40/40)	5.9 ± 0.3	0	0
NHBFH4	PMI0262[d]	*mrpI*	Fimbrial recombinase (40/40)[d]	4.3 ± 0.1	36.96	0
NHBFE8	PMI2210		Fimbrial subunit (38/40)	5.3 ± 0.4	70.65	111.34
NHBFH5	PMI2359	*glnE*	Glutamate-ammonia-ligase adenylyltransferase (38/40)	4.8 ± 0.4	51.09	0
NHBFA5	PMI1729	*rsbA*	Regulator of swarming behavior; sensor kinase of two-component system (40/40)	5.5 ± 0.1	116.3	32.99
NHBFF9	PMI0829	*bcr*	Bicyclomycin resistance gene (sulfonamide resistance protein, MFS transporter) (40/40)	4.7 ± 0.3	22.82	3.1
Biofilm deficient						
NHSH1	PMI1608		Unknown function; putative transmembrane protein (Pfam 02659) (39/40)	5.6 ± 0.3	27.17	0
ABBF1.1C8	PMI2867	*gltS*	Sodium/glutamate symport carrier protein (20/20)	5.4 ± 0.5	18.48	0
NHSE5	PMI1551		Unknown function; putative lipoprotein (COG3016, DUF399) (40/40)	5.2 ± 0.5	23.91	0
DLD1A6	PMI2861		Unknown function; putative membrane protein (COG2860, Pfam 03458) (40/40)	5.7 ± 0.7	35.86	84.54
DLD1D11	PMI0696[d]	*lrp*	Leucine-responsive regulator (40/40)[d]	5.2 ± 0.2	77.17	34.02
STS8.1D7	PMI1479[e]	*nirB*	Nitrite reductase (30/30)[e]	4.4 ± 0.1	100	77.32

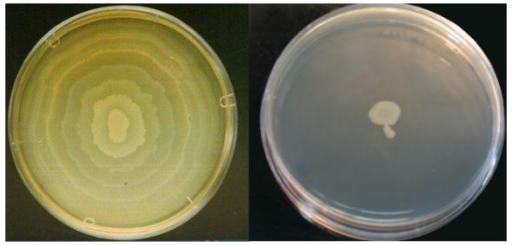
Holling et al., 2014

P. mirabilis flagella and pathogenicity

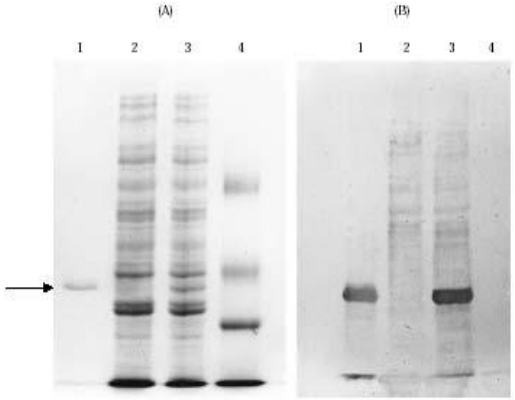
Flagella

(Legnani-Fajardo et al., 1996)



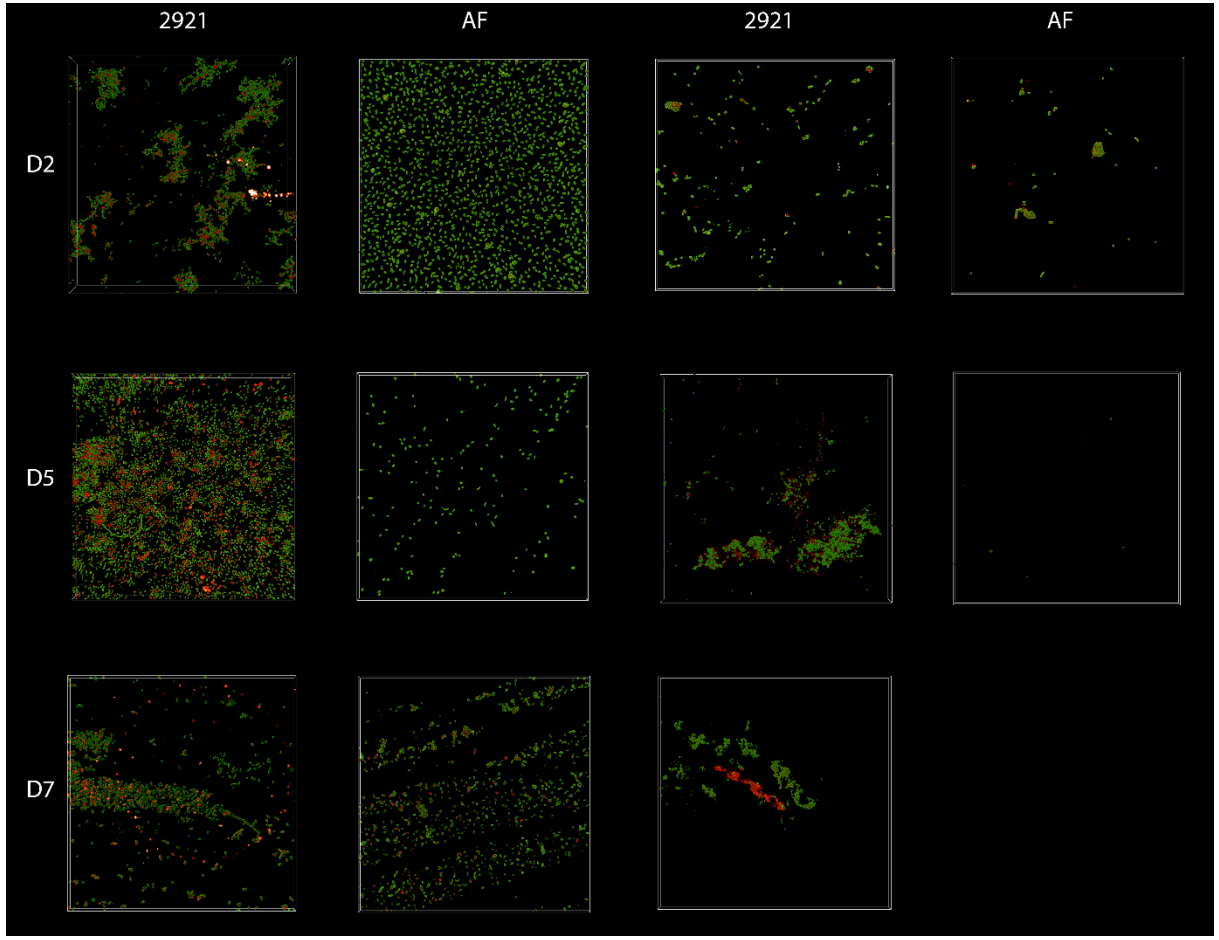


Non-flagellated strains isolated from patients with UTI were infective in experimental models (Zunino et al., 1994)



The isogenic mutant, unable to synthesize flagella, was not attenuated in the different models of experimental UTI

Flagella and biofilms formation

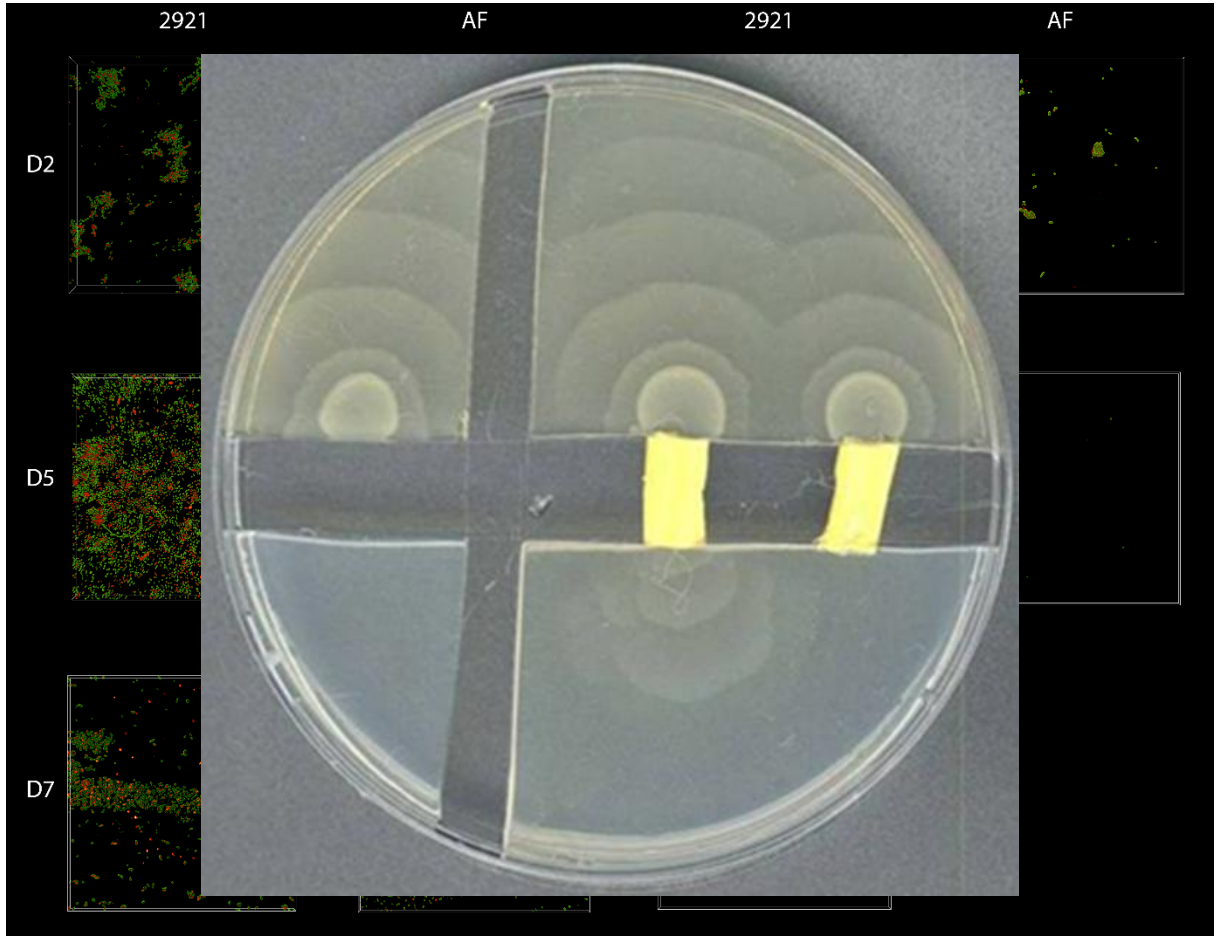


Scavone et al., 2023

3D reconstruction of biofilms formed by the wild-type Pr2921 strain of the aflagellated AF mutant in 2-, 5-, and 7-day cultures in LB broth (left) and artificial urine (right).

Bacteria are represented in green and the extracellular matrix in red.

Flagella and biofilms formation



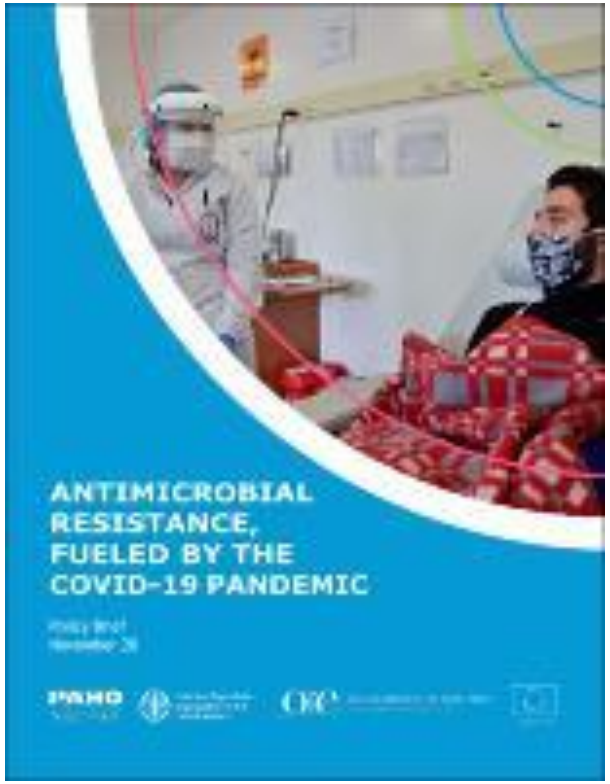
Scavone et al., 2023

3D reconstruction of biofilms formed by the wild-type Pr2921 strain of the aflagellated AF mutant in 2-, 5-, and 7-day cultures in LB broth (left) and artificial urine (right).

Bacteria are represented in green and the extracellular matrix in red.

Each year, approximately 250,000 to 500,000 primary bloodstream infections occur among the 150 million implanted intravascular devices (USA).

Low inoculum for implant-based infection (Nowakowska et al., 2014)



Need for biofilms prevention and eradication strategies!!

Implication of biofilms in industry

- Pathogen reservoirs that can survive sanitization processes
- Alterations of final products (e.g. food industry)
- Corrosion and clogging of equipment, pipes, and surfaces
- Alterations in thermal and refrigeration systems
- Increased antimicrobial resistance (decreased effectiveness of antiseptics, disinfectants, antibiotics, etc.)

https://orapiasia.com/importance-of-biofilm-in-the-food-and-beverage-industry/

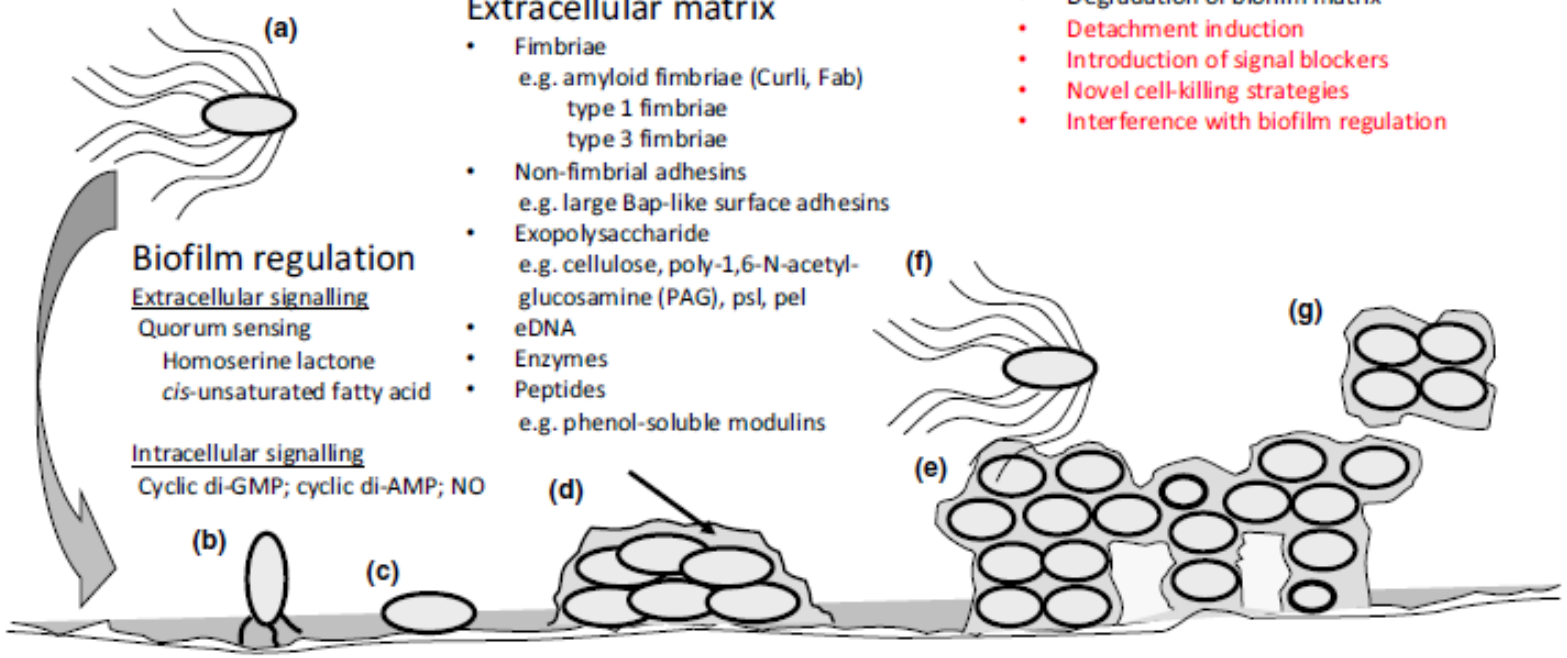
Biofilms: mechanisms and prevention strategies

Biofilm prevention strategies

- Development of antimicrobial surfaces
- Prevention of attachment

Biofilm treatment strategies

- Physical treatment of biofilms
- Photodynamic treatment of biofilms
- Degradation of biofilm matrix
- Detachment induction
- Introduction of signal blockers
- Novel cell-killing strategies
- Interference with biofilm regulation



Extracellular matrix

- Fimbriae
  e.g. amyloid fimbriae (Curli, Fab)
  type 1 fimbriae
  type 3 fimbriae
- Non-fimbrial adhesins
  e.g. large Bap-like surface adhesins
- Exopolysaccharide
  e.g. cellulose, poly-1,6-N-acetyl-glucosamine (PAG), psl, pel
- eDNA
- Enzymes
- Peptides
  e.g. phenol-soluble modulins

Biofilm regulation

Extracellular signalling

Quorum sensing

Homoserine lactone

cis-unsaturated fatty acid

Intracellular signalling

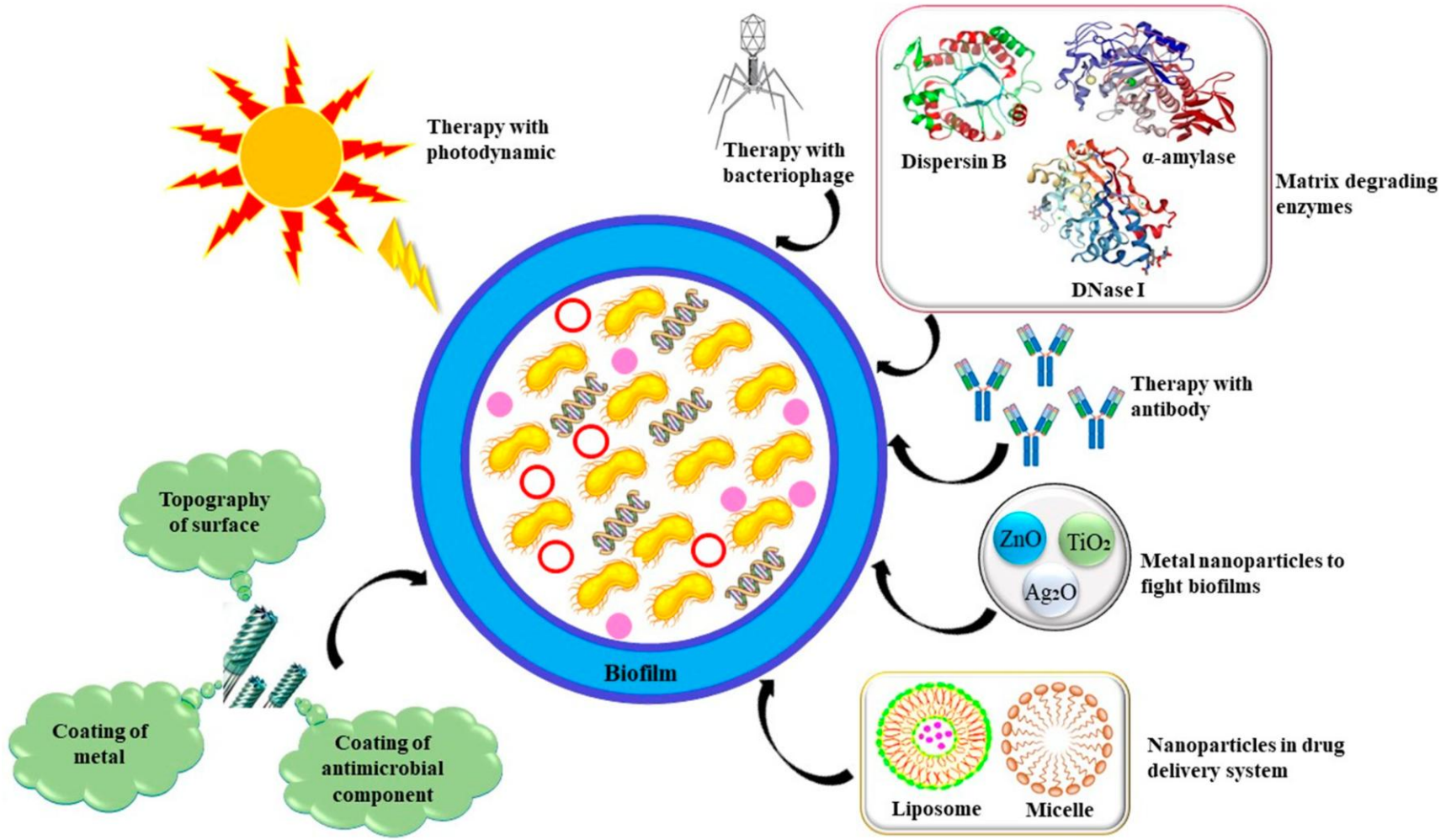
Cyclic di-GMP; cyclic di-AMP; NO

Antibiotic tolerance mechanisms

- Slow growth rate
- Altered metabolism and physiology
- Persister cells
- Oxygen gradient
- Extracellular biofilm matrix
- Upregulated stress response

Romling et al., 2014

Approaches to anti-biofilm research and therapies for their eradication



Mishra et al., 2023

Strategies

Physical methods:

Generally limited to surfaces, equipment, pipes, etc. - environmental advantages, do not generate resistance)

Electrical methods (electroporation)

Magnetic fields

Ultrasound (industry, medical devices, dentistry, etc.)

Plasma

Combinations (chemical, biological, etc.)

Strategies

Chemical methods:

Antisépticos

Antibióticos

Enzimas
(proteinasa K, tripsina, amilasa, lipasa, celulasa, DNase I)

Biosurfactantes
(compuestos de origen biológico que contienen una región hidrófila (polar o no polar) y una región hidrofóbica (lípido o ácido graso)

Quelantes, anticoagulantes
(EDTA, heparina, etc.)

Terapia fotodinámica (ej. fotosensibilizadores: tetrapirroles, colorantes sintéticos, compuestos naturales, etc.).

Table 1 Selected biosurfactants reported in literature with antibiofilm/microbial activities

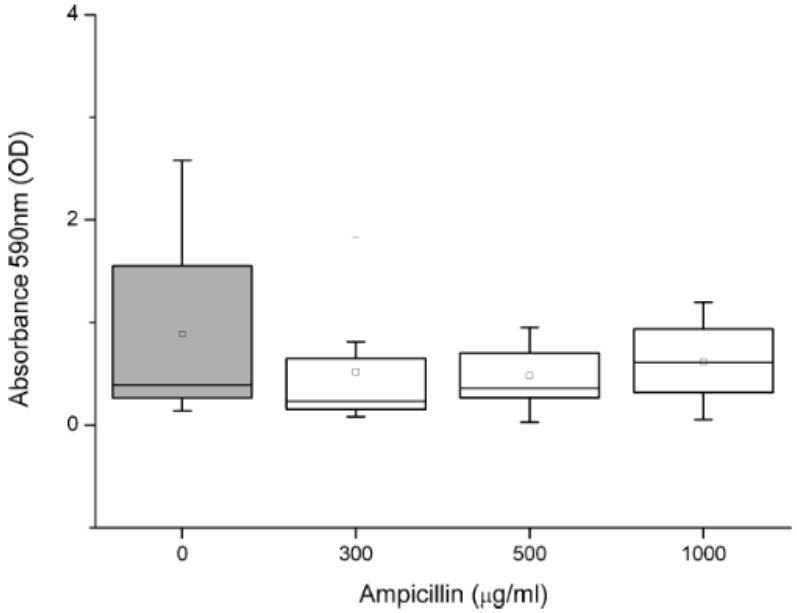
Biosurfactant class	Name	Source	Reference	Effectiveness
Lipopeptide	Putisolvin I and II	*Pseudomonas putida*	Kuiper et al. 2004	Biofilm inhibition of *Pseudomonas* spp.
Lipopeptide	Pseudofactin II		Janek et al. 2010	Effective against *E. coli Enterococcus faecalis Proteus mirabilis* and *Candida sp.*
Lipopeptide	NS	*Bacillus subtilis*	Mireles et al. 2001	Biofilm inhibition of *S. entrica* on urethral catheter
Lipopeptide	Fengycin	*B. subtilis* and *B. licheniformis*	Rivardo et al. 2009	Inhibition of pathogenic *E. coli & S. entrica*
Lipopeptide	NS	Heavy metal tolerant strain of *Bacillus*	Sriram et al. 2011	Inhibits Gram positive and negative bacteria and fungi
Lipopeptide	NS	*Bacillus* sp. strain SW9	Wu et al. 2013	Inhibits biofilm formation in a wide range of bacteria
Lipopeptide	NS	*Bacillus tequilensis*	Pradhan et al. 2013	Biofilm inhibition of *E. coli & Streptococcus mutans*
Lipopeptide	L. fermentum B54	*Lactobacillus*	Velraeds et al. 2000	Inhibits uropathogens
Glycolipids	NS	*Brevibacterium casei*	Kiran et al. 2010	Inhibits mixed pathogenic biofilm bacteria
Mixture of biosurfactants	Lunasan	*Candida sphaerica*	Luna et al. 2011	Inhibition of *P. aeruginosa* and *S. agalactae*
NS	NS	*Lactobacillus paracasei* A20	Gudina et al. 2010	Biofilm inhibition for a range of bacteria, yeasts & filamentous fungi.
Glycolipid	Rhamnolipid	*P. aeruginosa*	Rodrigues et al. 2006b	Inhibits biofilms in *S. aureus Candida tropicalis*
Glycolipid	Rhamnolipid	*P. aeruginosa*	Dusane et al. 2010	Inhibits *B. pumulus*
Mixed biosurfactants	Lunasan	*Lactococcus lactis/Strep thermophilus*	Rodrigues et al. 2004	Effective against *Staphylococcus*, *Streptococcus* , *Rothia and Candida sp.*
NS	NS	*Robinia pseudocacia/Nerium oleander*	Cochis et al. 2012	Effective against *C. albicans*
Glycolipids	Rhamnolipid	*P. aeruginosa*	Dusane et al. 2012	Effective against *Yarrowia sp.*
NS	Rufisan	*Candida lypolytica*	Rufino et al. 2011	Effective against *Streptococcus sp*
Glycolipid	Glucose + palmitic acid	*Serratia marsecens*	Dusane et al. 2011	Effective against *C. albicans* , *P. aeruginosa* and *B. pumilus*

NS not specified

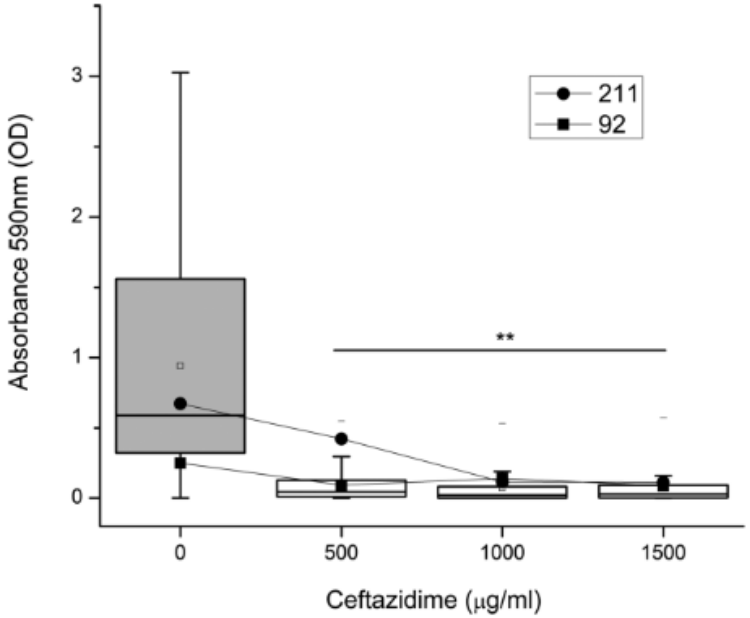
Banat et al., 2014

Effect of different antibiotics on biofilms produced by uropathogenic *Escherichia coli* isolated from children with UTI

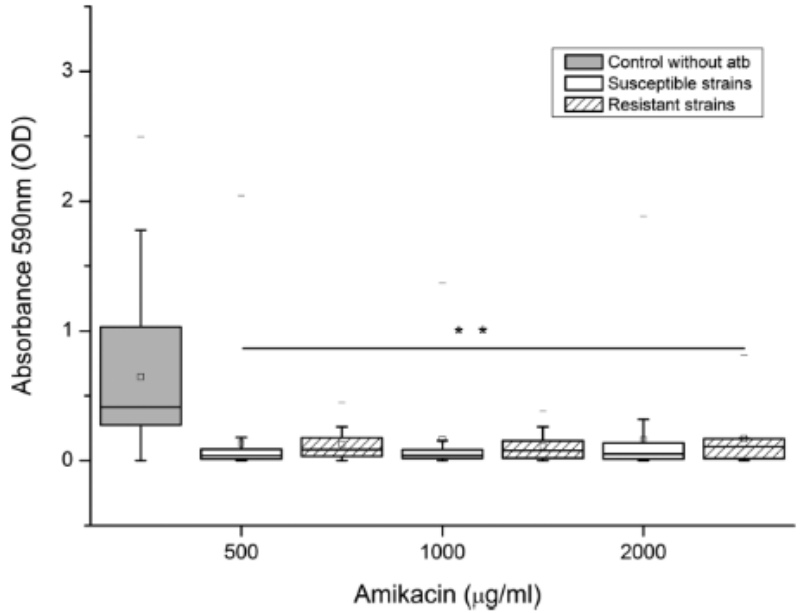
González et al., 2017



Ampicilina: No se observaron diferencias significativas, incluso en cepas susceptibles



Cefalosporina de tercera generación. Indujo una reducción significativa de los biofilms incluso en cepas resistentes



Aminoglucósido. Indujo una reducción significativa de los biofilms incluso en cepas resistentes

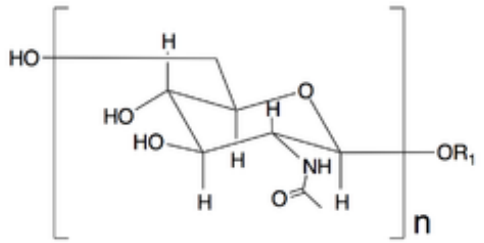
Enzimes (matrix)

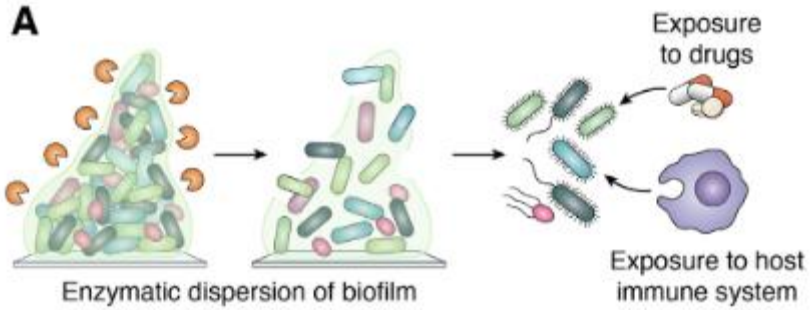
Reported glycoside hydrolases for biofilm dispersion

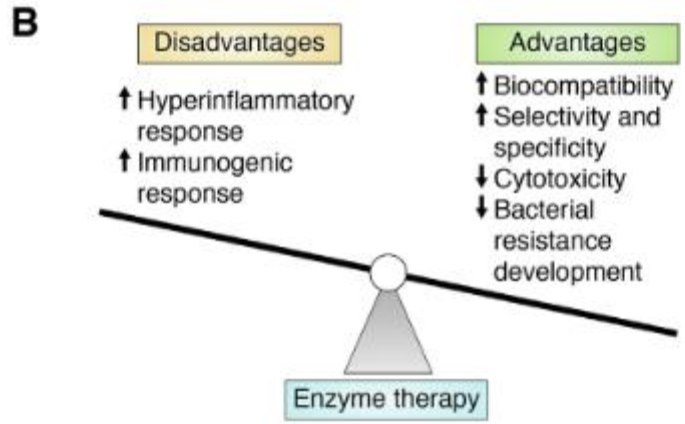
Glycoside hydrolase	Targeting glycosidic linkage	Monosaccharide composition of the polysaccharides
Dispersin B	GlcNAc -(β-1,6)- GlcNAc	*N*-acetyl-D-glucosamine
α-Amylase	Glc-(α-1,4)-Glc	D-glucose
Cellulase	Glc-(β-1,4)-Glc	D-glucose
PslG	Man*p*-(β-1,3)- Man*p*[a]	D-mannose, D-glucose and L-rhamnose
PelA	(1,4)[b]	Partially de-*N*-acetylated *N*-acetylgalactosamine
β-Mannosidase	Man-(β-1,4)-Man	D-mannose
α-Mannosidase	Man-(α-1,3)-Man	D-mannose
PgaB	GlcN-(β-1,6)-GlcN	D-glucosamine
Ega3	GalN-(α-1,4)-GalN	D-galactosamine
Sph3	GalNAc-(α-1,4)-GalNAc	*N*-acetyl-D-galactosamine
CcsZ	Glc-(β-1,4)-Glc	D-glucose
PssZ	ManN-(β-1,4)-ManN	D-mannosamine

[a] The targeting glycosidic linkage of PslG is predicted.
[b] PelA enzyme works on Pel, a linear cationic polysaccharide composed of partially de-N-acetylated 1,4 linked N-acetylgalactosamine.

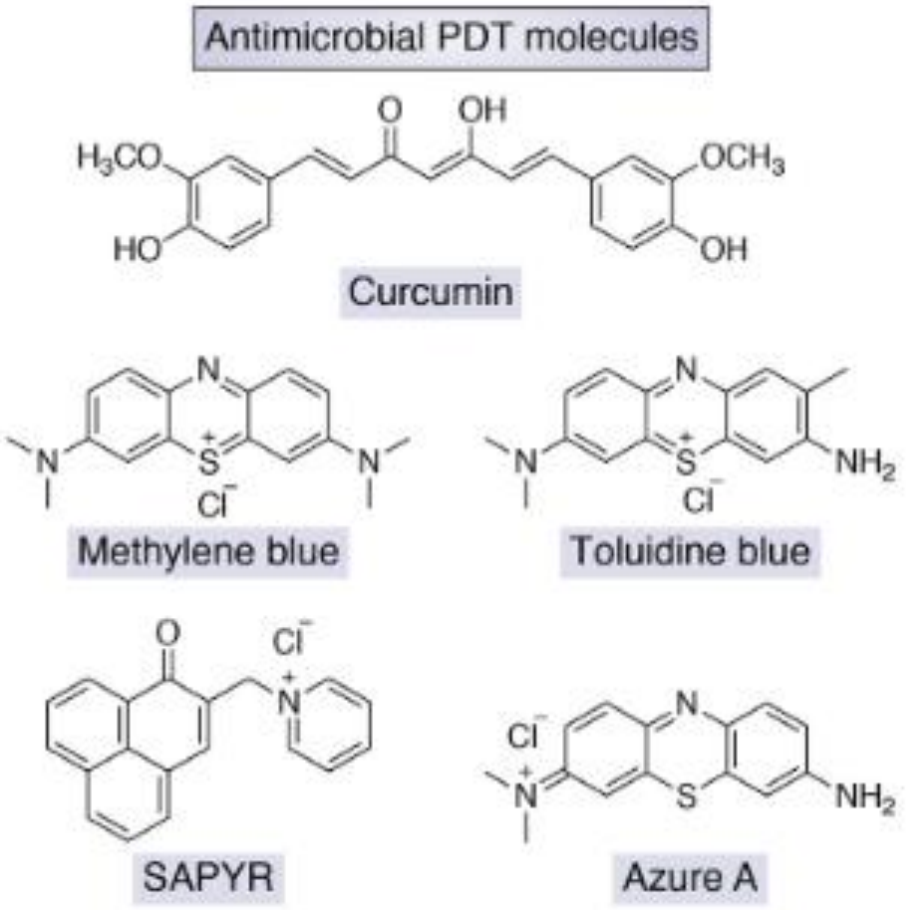
Dispersina B (DspB; glycoside hydrolase *Actinobacillus actinomvcetemcomitans,* 40 kDa)







Antimicrobial photodynamic therapy



Ramakrishnan et al., 2022

Regulation mechanisms

There is no single mechanism responsible for regulation: specificities

•*Quorum sensing*

Main regulatory mechanism

Autoinducers (Gram – y Gram + bacteria)

Adhesion/dispersion modulation:

Most species increase behaviors associated with biofilm formation at high cell concentrations

Mutants affected by QS form weak biofilms – function in extracellular DNA production

Communication between microorganisms: QS in Gram-positive and Gram-negative bacteria

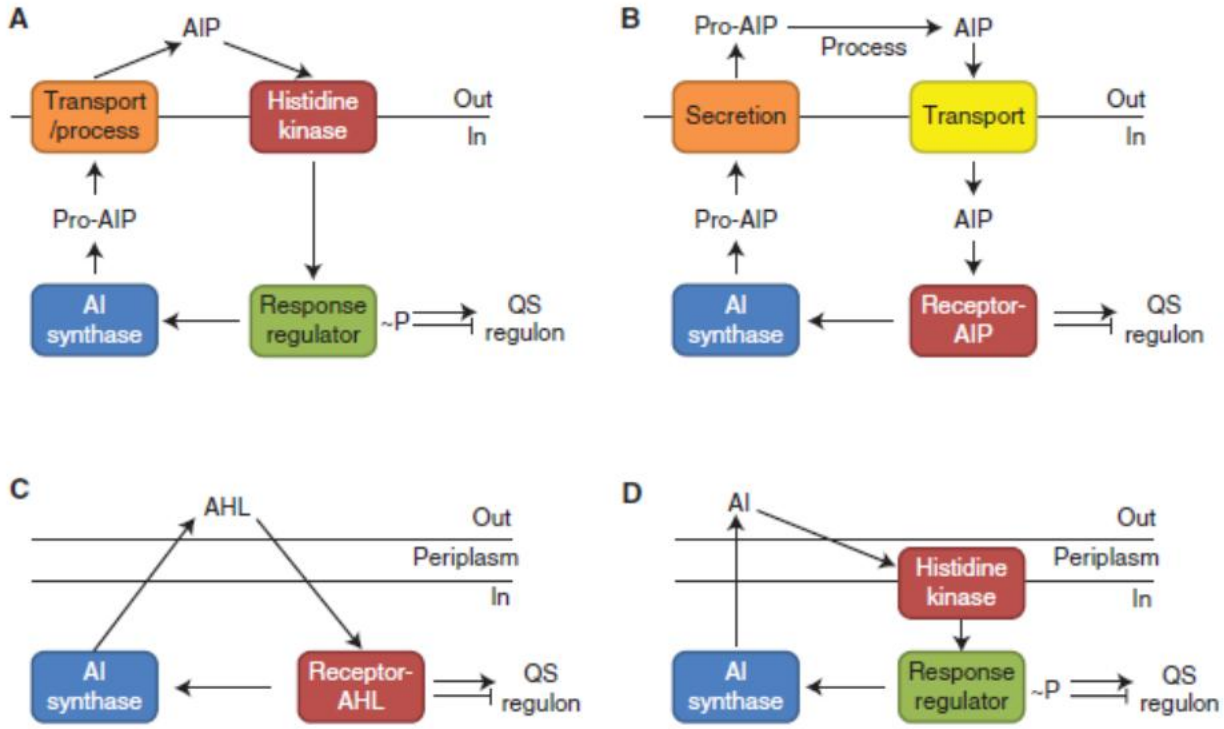
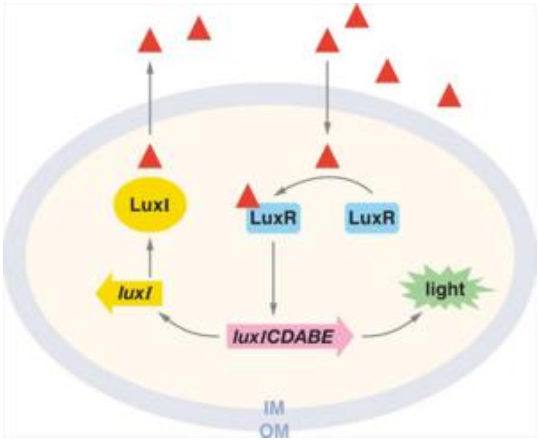


Figure 1. Canonical bacterial quorum-sensing (QS) circuits. Autoinducing peptide (AIP) QS in Gram-positive bacteria by (*A*) two-component signaling, or (*B*) an AIP-binding transcription factor. Small molecule QS in Gram-negative bacteria by (*C*) a LuxI/LuxR-type system, or (*D*) two-component signaling.

Mecanismos generales de QS



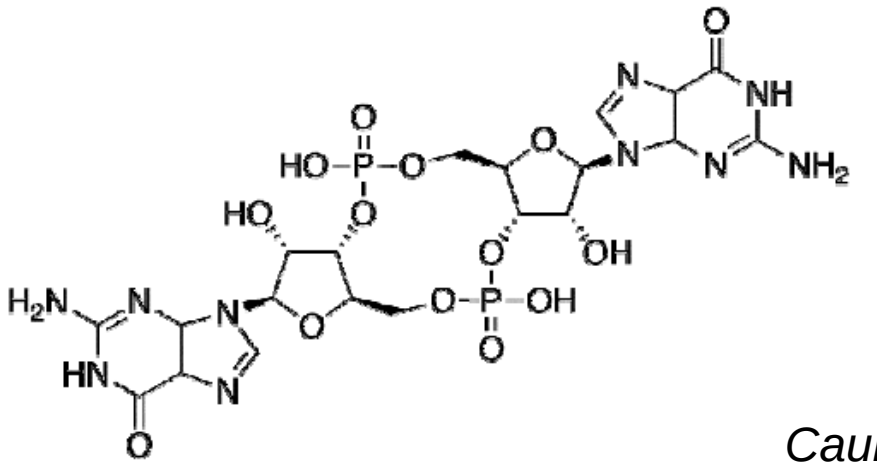
Vibrio fischeri

Euprymna scolopes

Procesos controlados por Quorum Sensing

- Bioluminsencia
- Esporulación
- Competencia
- Producción de antibióticos
- Secreción de factores de vrulencia
- Formación de biofilms

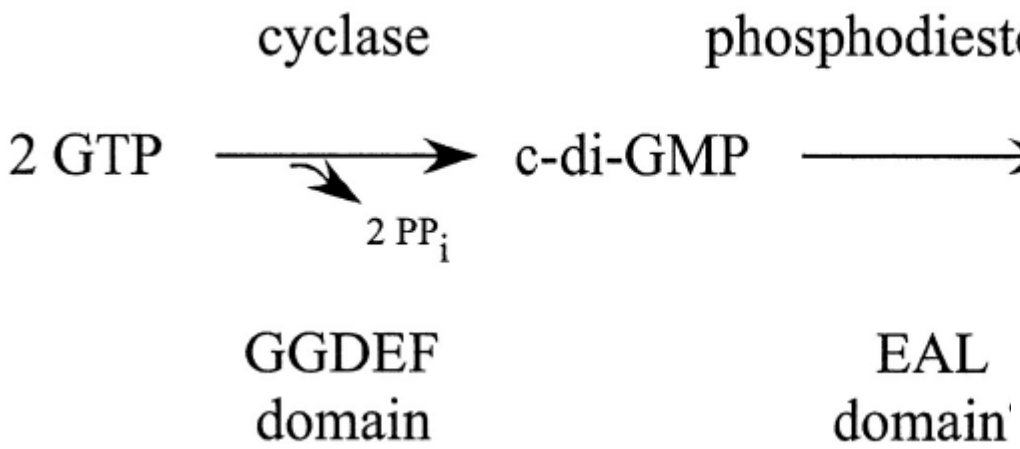
Second messenger: ciclic di-GMP (dimeric guanosine monophosphate)



Caulobacter crecentus, 1995

- Presente en una gran cantidad de especies bacterianas
- Sus niveles intracelulares determinan numerosos comportamientos bacterianos
- Sus niveles intracelulares se regulan por el balance de diguanilato ciclasas (dominio GGDEF) y fosfodiesterasas (dominio EAL o dominio HD-GYP).

Regulación por c-di-GMP



- Comunicación intercelular
- Síntesis fimbrial
- Producción de EPS (matriz)
- Biofilms

- Motilidad
- Virulencia
- Resistencia a metales pesados

Responde a múltiples señales. Descubierto por su papel en la síntesis de celulosa microbiana en *Gluconacetobacter xylinus*

Examples of clinically relevant bacteria that use the second messenger c-di-GMP

Bacteria	Diseases
Vibrio cholerae	Cholera
Pseudomonas aeruginosa	Pulmonary and urinary tracts infections Burn injuries infections Blood infections
Yersinia pestis	Plague of Justinian Black Death Third Pandemic
Klebsiella pneumoniae	Pneumonia Urinary tract Lower biliary tract Wound infections
Legionella pneumophila	Legionnaires' disease
Vibrio vulnificus	Cellulitis Septicemia

Mechanisms of interference with QS (*Quorum quenching*)

- Inhibitors affecting the AI synthesis pathway
- Enzymatic degradation of AI
- AHL analogs and compounds with receptor affinity
- AIP analogs and compounds with receptor affinity
- Action at the level of the signal transduction cascade

Bacha et al. BMC Microbiology (2016) 16:139
DOI 10.1186/s12866-016-0765-9

BMC Microbiology

RESEARCH ARTICLE Open Access

Antimicrobial and anti-Quorum Sensing activities of selected medicinal plants of Ethiopia: Implication for development of potent antimicrobial agents

Ketema Bacha[1*], Yinebeb Tariku[2], Fisseha Gebreyesus[3], Shibru Zerihun[4], Ali Mohammed[5], Nancy Weiland-Bräuer[6], Ruth A. Schmitz[6] and Mulugeta Mulat[1,7]

Table 1 Summary of traditional medicinal plants and their extracts evaluated for antimicrobial and Quorum Quenching (anti-Quorum Sensing) activities

Sample No	Code	Scientific name of plant with brief description	Type of Extract
1	ACFA1	*Aframomum corrorima* mature semi-ripe fruit acetone extract	Oleo resin
2	ACFA2	*Aframomum corrorima* mature unripe fruit acetone extract	Oleo resin
3	ACFA3	*Aframomum corrorima* mature ripe fruit acetone extract	Oleo resin
4	ACFO	*Aframomum corrorima* mature unripe fruit oil	Essential oil
5	ACHO	*Aframomum corrorima* mature ripe fruit husk oil	Essential oil
6	ASRM	*Albiza schimperiana* root methanol extract	Crude extract
7	CLRA1	*Curcuma longa* finger rhizome acetone extract	Oleo resin
8	CLRO1	*Curcuma longa* finger rhizome oil	Essential oil
9	CLRO2	*Curcuma longa* main rhizome oil	Essential oil
10	CLRA2	*Curcuma longa* main rhizome acetone extract	Oleo resin
11	EBBP	*Erythrinia brucei* stem bark petroleum ether extract	Crude extract
12	JSSP	*Justica schimperiana* seed petroleum ether extract	Crude extract
13	NSSO	*Nigella sativa* seed oil	Essential oil
14	NSSP	*Nigella sativa* seed petroleum ether extract	Crude extract
15	OSLC	*Ocimum sauve* leaf chloroform extract	Crude extract
16	VALC	*Vernonia amygdalina* leaf chloroform extract	Crude extract
17	VALM	*Vernonia amygdalina* leaf methanol extract	Crude extract
18	VALP	*Vernonia amygdalina* leaf petroleum ether extract	Crude extract

Bacha et al., 2016

Table 4 Quorum Quenching activities of selected medicinal plants of Ethiopia

Extracts	AHL-QQ activity in *E. coli* based reporter strain AI1-QQ.1	Antimicrobial activity against *E. coli* K12 DSM 498
ACFA1	-	-
ACFA2	-	-
ACFA3	-	-
ACFO	-	+++
ACHO	-	+
ASRM	+	+
CLRA1	-	-
CLRA2	-	-
CLRO1	-	-
CLRO2	-	++
EBBP	-	-
JSSP	+	-
NSSO	-	+
NSSP	-	-
OSLC	-	-
VALC	-	-
VALM	-	-
VALP	-	-

Uso de cepa de *E. coli* reportera: contiene un gen que codifica una proteína letal fusionada a un promotor inducido en presencia de la molécula señal de Quorum Sensing AHL

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Short communication

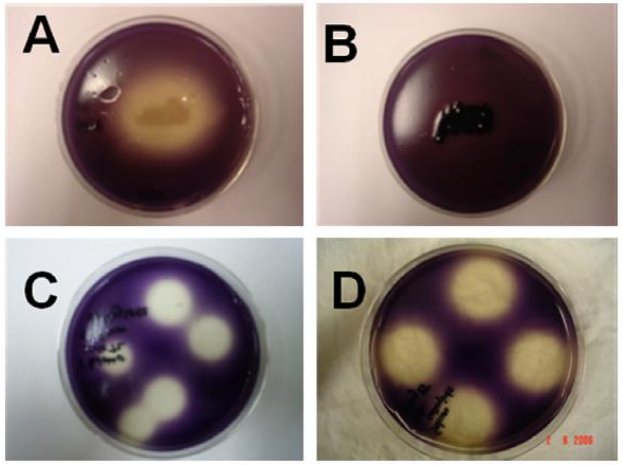
Use of quorum sensing antagonists to deter the formation of crystalline *Proteus mirabilis* biofilms

Steven M. Jones*, Tammy T. Dang, Robert Martinuzzi

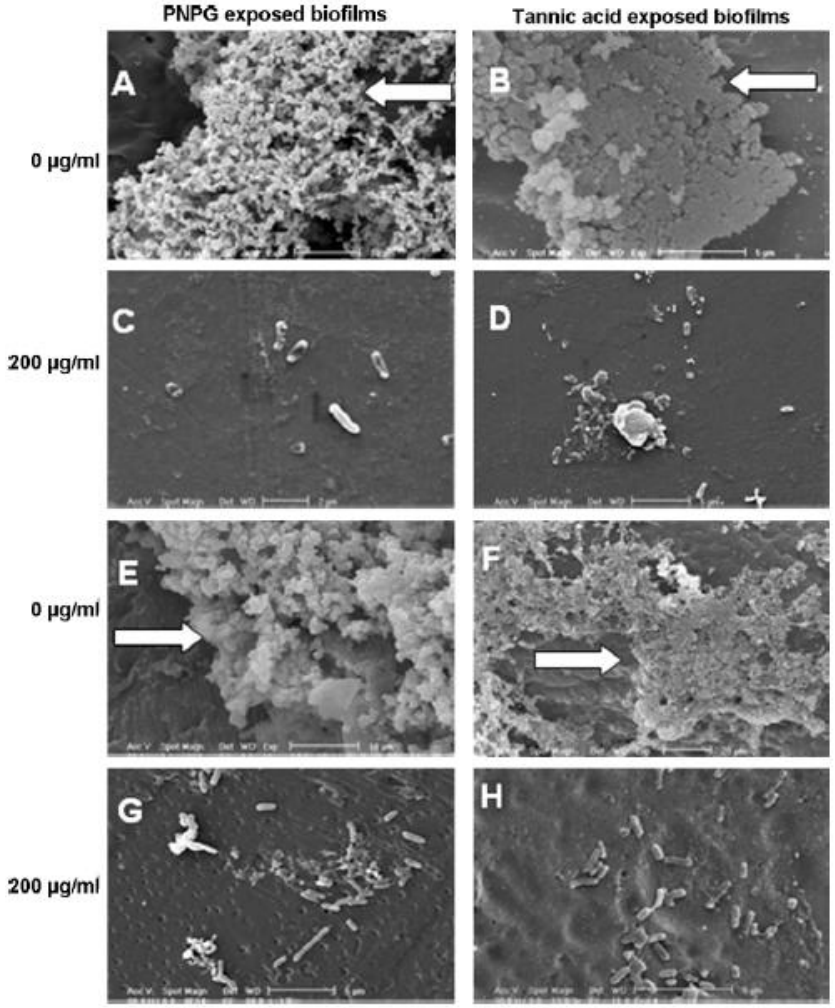
Evaluation of the antibiofilm effect of QS inhibitors:

- p-nitrofenilglicerol
- taninos

Evaluation: effect on pigment production of *Chromobacterium violaceum*



Preventive and disruptive effect on biofilms



Micrografías electrónicas de barrido: (A, B) biofilms de *Proteus mirabilis* sin tratar y biofilms (C, D) cultivadas en presencia de 200 g / ml de p-nitrofenil glicerol (PNPG) o ácido tánico durante un período de 24 h; (E, F) biofilms de *P. mirabilis* de 24 h sin tratar y (G, H) biofilms de 24 h expuestos a 200 g / ml de PNPG o ácido tánico durante 24 h. Las flechas representan la presencia de material cristalino en biofilms de *P. mirabilis* sin tratar.

Nuevas tecnologías de superficies

Materiales comúnmente empleados (ej. implantes ortopédicos): cerámica, cromo-cobalto, polietileno, polimetilmetacrilato, aleaciones de titanio, etc.

Estrategias:

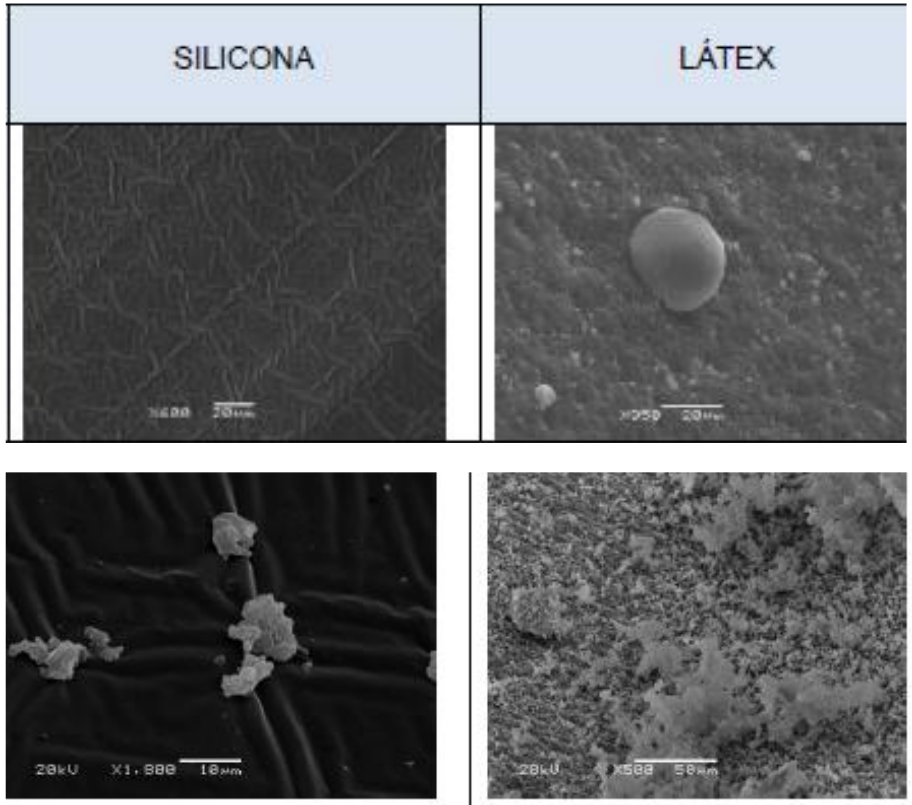
- **Modificaciones pasivas de las superficies**

Reducción de la adhesión bacteriana mediante la alteración de la química de la superficie del implante y/o modificación de la estructura de la superficie, sin liberación local o captura superficial de agentes bactericidas

Ej. Metales
Cerámicas
Nanopatrones
Biosurfactantes (ej. lipopéptido producido por *B. subtilis* ATCC 19659)

Surfaces - Materials

ITU-C Biofilms de *P. mirabilis* sobre secciones de catéteres (SEM)



Secciones de catéteres

Biofilms de *P. mirabilis*

Departamento de Microbiología, IIBCE; en colaboración con el Servicio de Microscopía de la Fac. de Ciencias

- **Modificaciones activas de las superficies**

Recubrimientos que se caracterizan farmacológicamente como agentes bactericidas activos

Ejemplos:

- Moléculas inorgánicas (ej. Ag, actividad antimicrobiana, Cu, Zn)
- Ingredientes no metálicos (por ejemplo, yodo, selenio),
- Antibióticos (ej. vancomicina, gentamicina)
- Péptidos antimicrobianos (12 a 50 aa)
- Aceites esenciales
- Esfingosina (aminoalcohol)
- Nanocoatings (nanopartículas, sales de plata, ZnO, etc.)

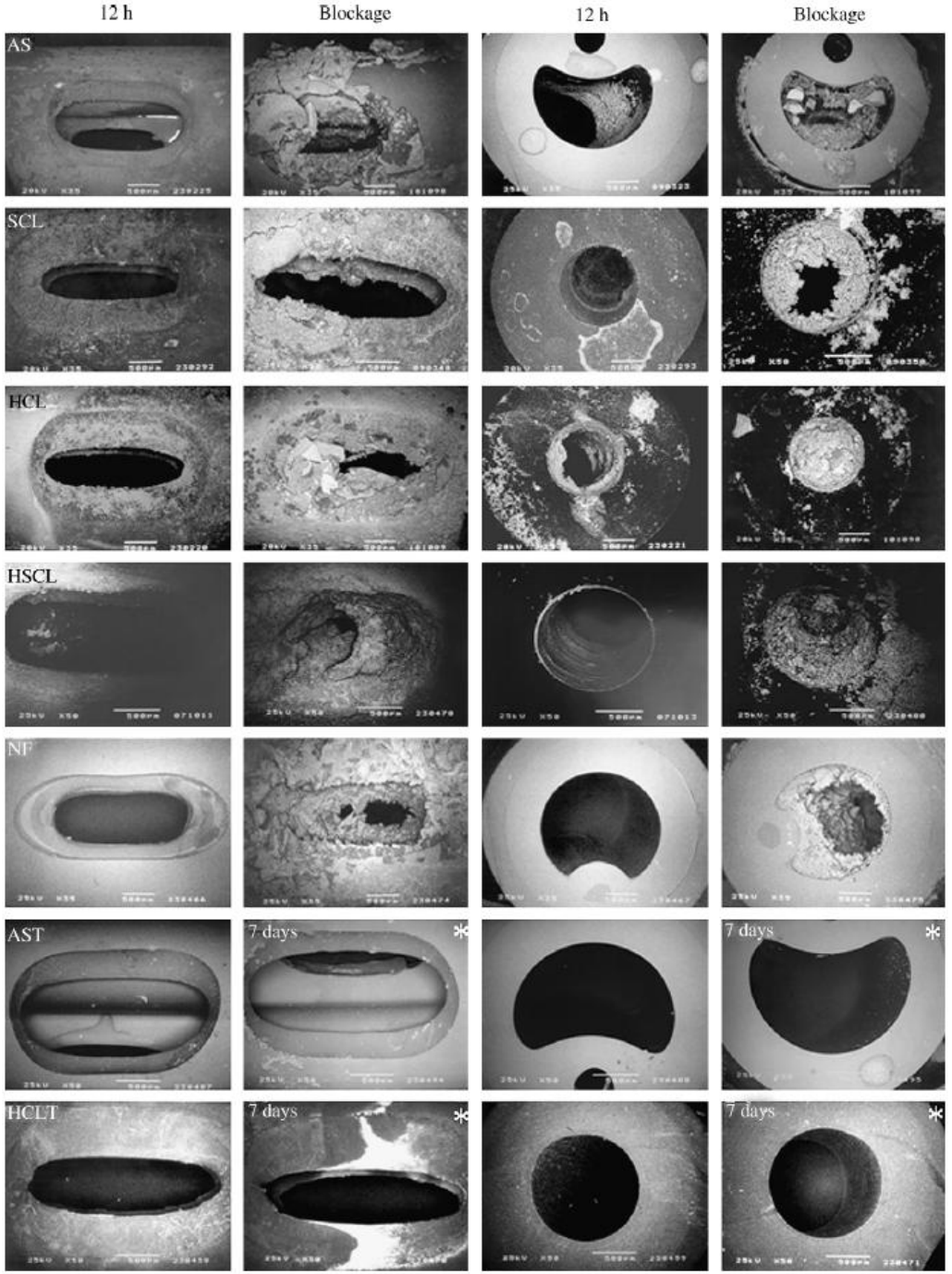
- **Coberturas**

Ej. Albúmina
Elastina
Heparina

Modificado de Rodríguez-Merchán et al. 2021

Estrategias de prevención de biofilms cristalinos de *P. mirabilis* en la luz de catéteres urinarios

AS - silicona
SCL - látex cubierto de silicona
HCL - látex cubierto de hidrogel
HSCL - látex cubierto de hidrogel/oro
NF - silicona nitrofurazona
AST - silicona/triclosan
HCLT - látex cubierto de hidrogel/triclosan



Stickler y Morgan, 2008

Nanoparticles

Generally smaller than 100 nm

Research Article

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Nanomedicine

Magnesium-doped zinc oxide nanoparticles alter biofilm formation of *Proteus mirabilis*

Victoria Iribarnegaray[1], Nicolas Navarro[2,3], Luciana Robino[4], Pablo Zunino[1], Javier Morales[†,2,3] & Paola Scavone*[,†,1]

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[†]Authors share senior authorship

NANOMEDICINE, VOL. 18, NO. 10 | RESEARCH ARTICLE

Gold-, silver- and magnesium-doped zinc oxide nanoparticles prevents the formation of and eradicates bacterial biofilms

Erlen Cruz Jorge, Nicolás Navarro Martínez, María José González, Sofía V Sánchez, Luciana Robino, Javier O Morales & Paola Scavone

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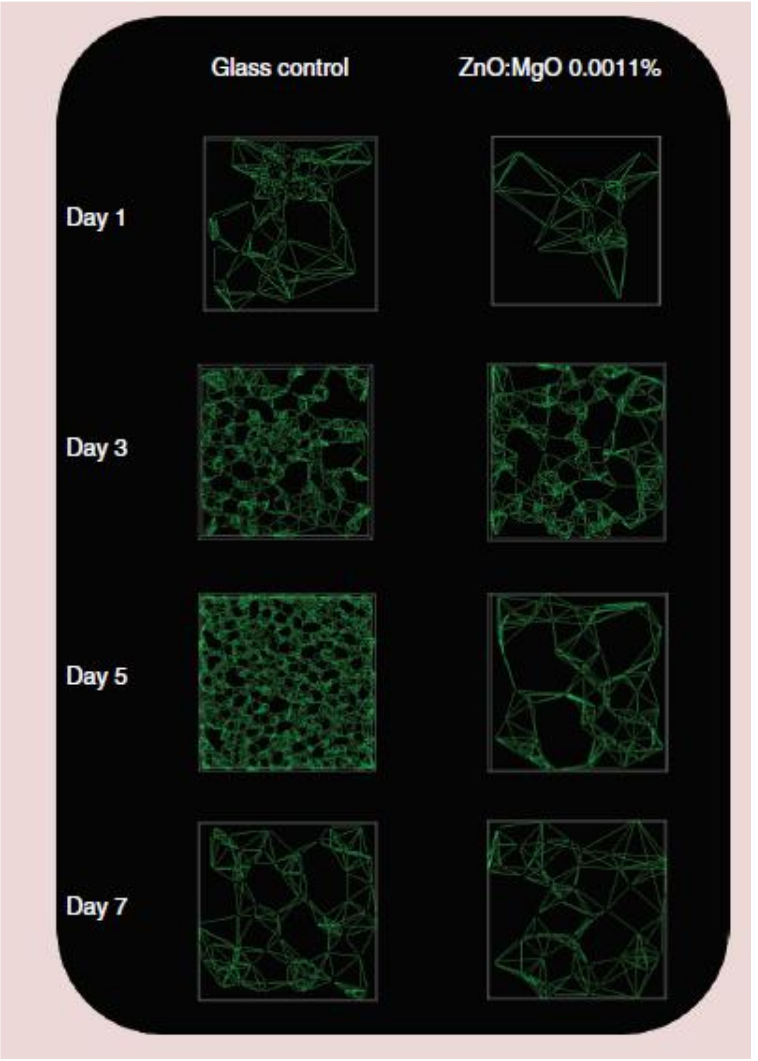


“Small molecules”

Organic compound of low molecular weight (no more than 800 Da), which enables rapid diffusion through the cell membrane

Different biological actions

Agent	Mechanism	Effect	Reference
Anti-virulence compounds	Inhibition of gene expression of virulence factors	Inhibition of biofilm formation by *S. aureus*	[23]
Anti-biofilm compounds	Unknown	Inhibition of biofilm formation by *S. epidermidis*	[25]
ABC-1	Inhibition of c-di-GMP-inducible transcription	Inhibition of biofilm formation by multiple Gram-negative and Gram-positive bacterial pathogens	[26]
Aryl rhodanines	Unknown	Inhibition of biofilm formation by *S. aureus* and *S. epidermidis*	[29]
Cis-2-decenoic acid	Unknown	Dispersion of biofilms by *E. coli*, *K. pneumoniae*, *P. mirabilis*, *S. pyogenes*, *B. subtilis*, *S. aureus*, and *C. albicans*	[30]
D-amino acids	Unknown	Inhibition of biofilm formation by *S. aureus* and *P. aeruginosa*	[31]
N-acetylcysteine	Interference with exopolysaccharide formation in biofilms	Inhibition of biofilm formation by *S. epidermidis*	[32]
Chelators	Interference with metal ion’s function in biofilm formation	Inhibition of biofilm formation by *S. aureus*	[33]

Examples of small molecules

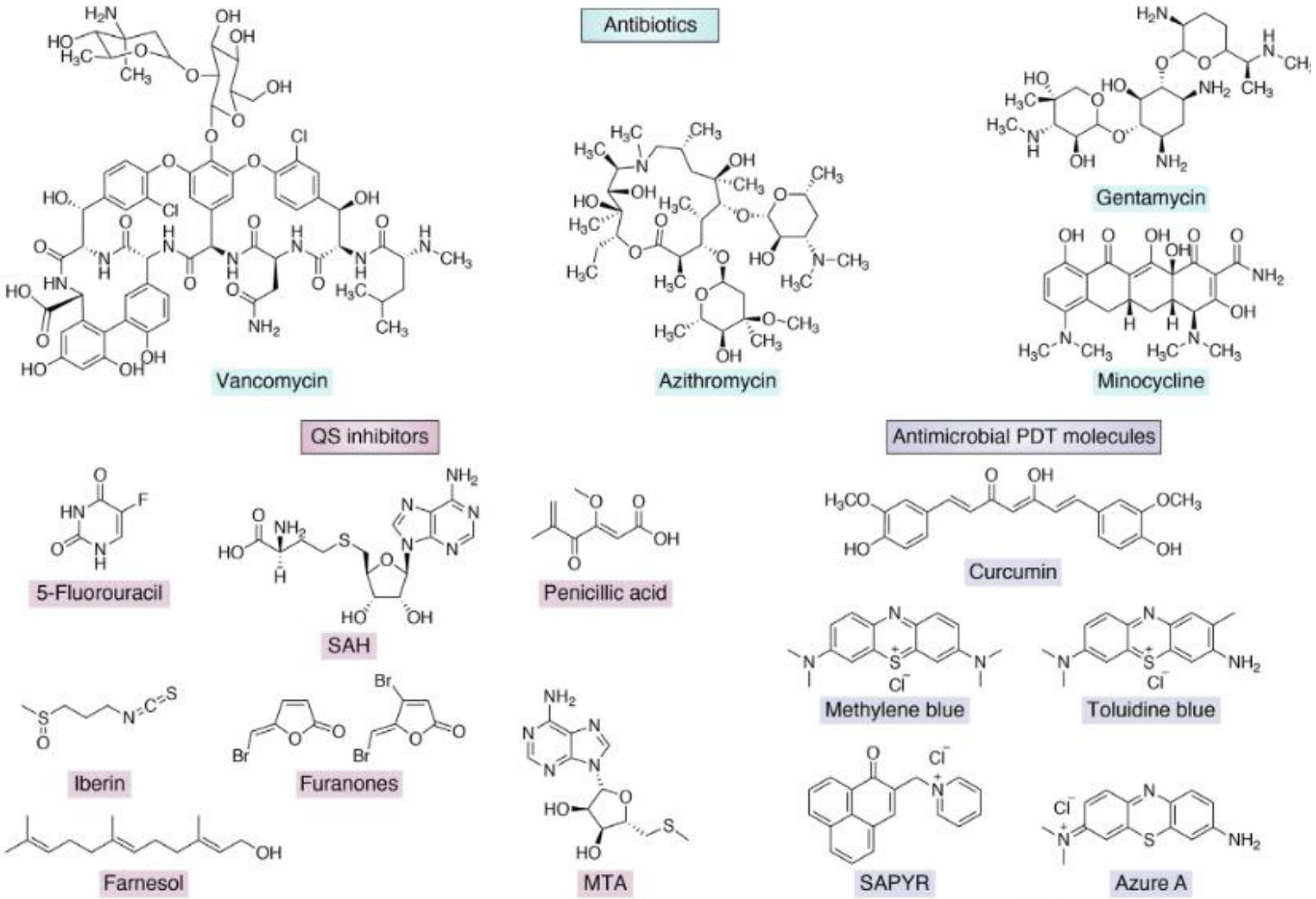


Figure 3. Examples of small molecules used for the treatments of biofilm-associated infections.

Ramakrishnan et al., 2022

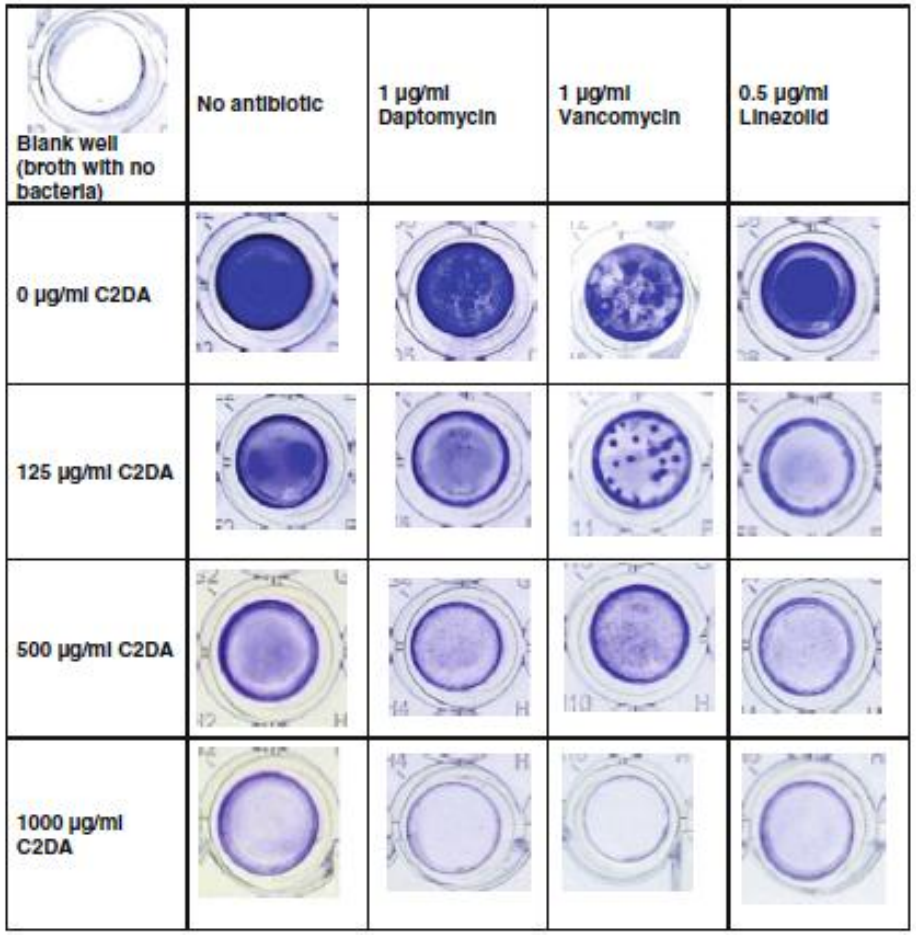
Clin Orthop Relat Res (2012) 470:2663–2670
DOI 10.1007/s11999-012-2388-2

Clinical Orthopaedics and Related Research®
A Publication of The Association of Bone and Joint Surgeons®

SYMPOSIUM: 2011 MUSCULOSKELETAL INFECTION SOCIETY

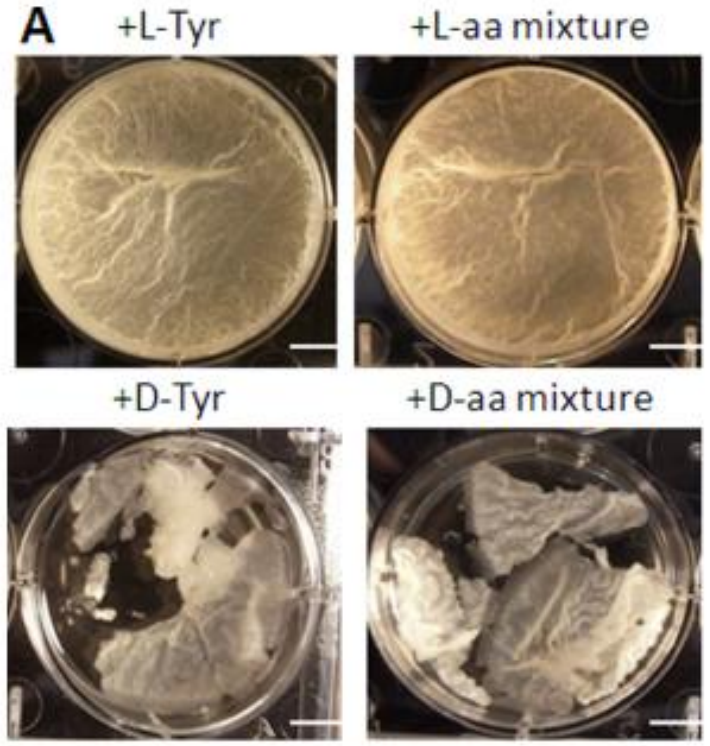
Cis-2-decenoic Acid Inhibits S. aureus Growth and Biofilm In Vitro: A Pilot Study

Jessica Amber Jennings PhD, Harry S. Courtney PhD, Warren O. Haggard PhD



Tinción con CV de biofilms sometidos a distintas concentraciones de C2DA (ácido graso insaturado) y antibióticos

Disruption: D-amino acids



Science. 2010 April 30; 328(5978): 627–629. doi:10.1126/science.1188628.

D-Amino Acids Trigger Biofilm Disassembly

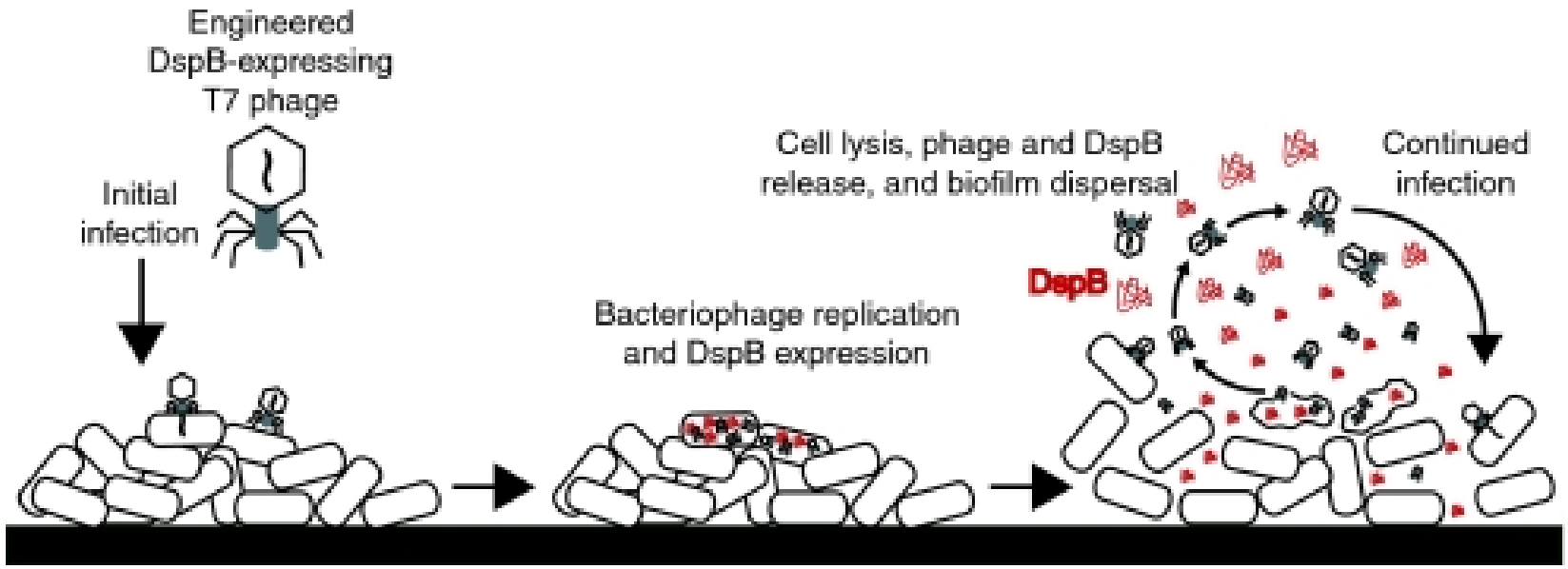
Illana Kolodkin-Gal[1], Diego Romero[2], Shugeng Cao[3], Jon Clardy[3], Roberto Kolter[2], and Richard Losick[1,*]

D-leucina, D-metionina, D-tirosina, D-triptofano: disrupción de fibras amiloides que intervienen en la cohesión del biofilm (*B. subtilis*, *S. aureus, P. aeruginosa*)

Interferencia con la estructura normal de la capa de peptidoglicanos

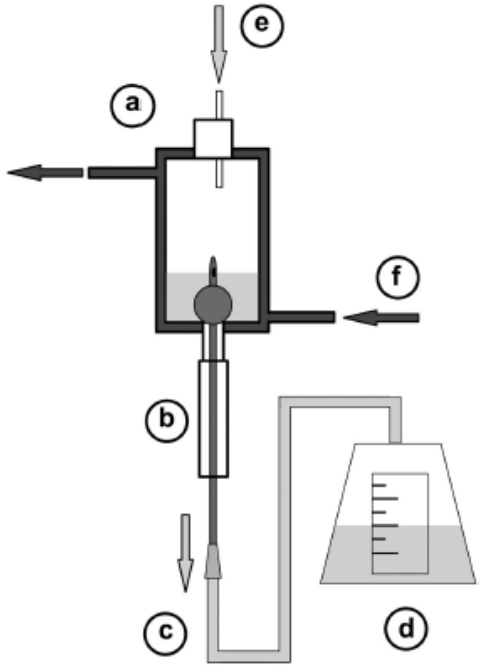
Phage therapy

- Wild phages
- Modified phages

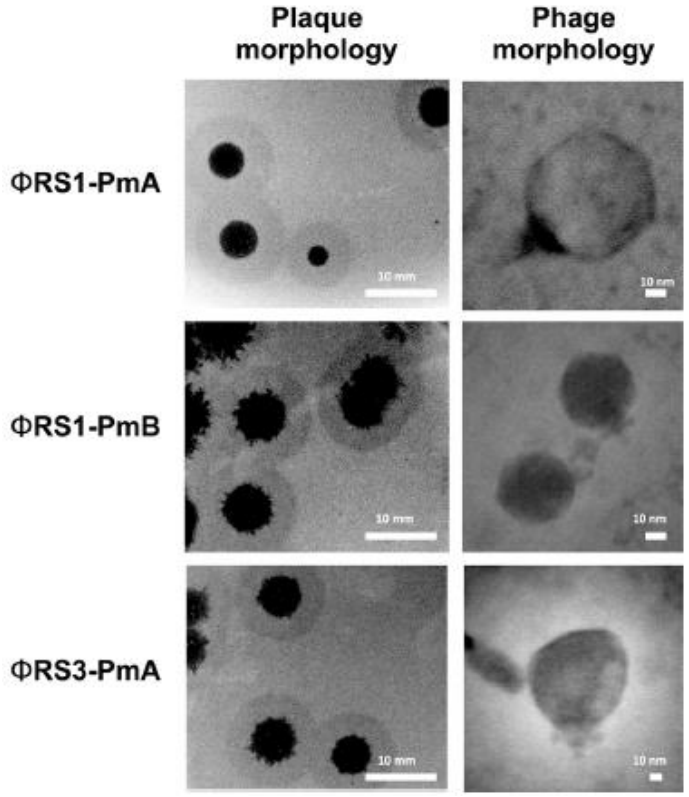


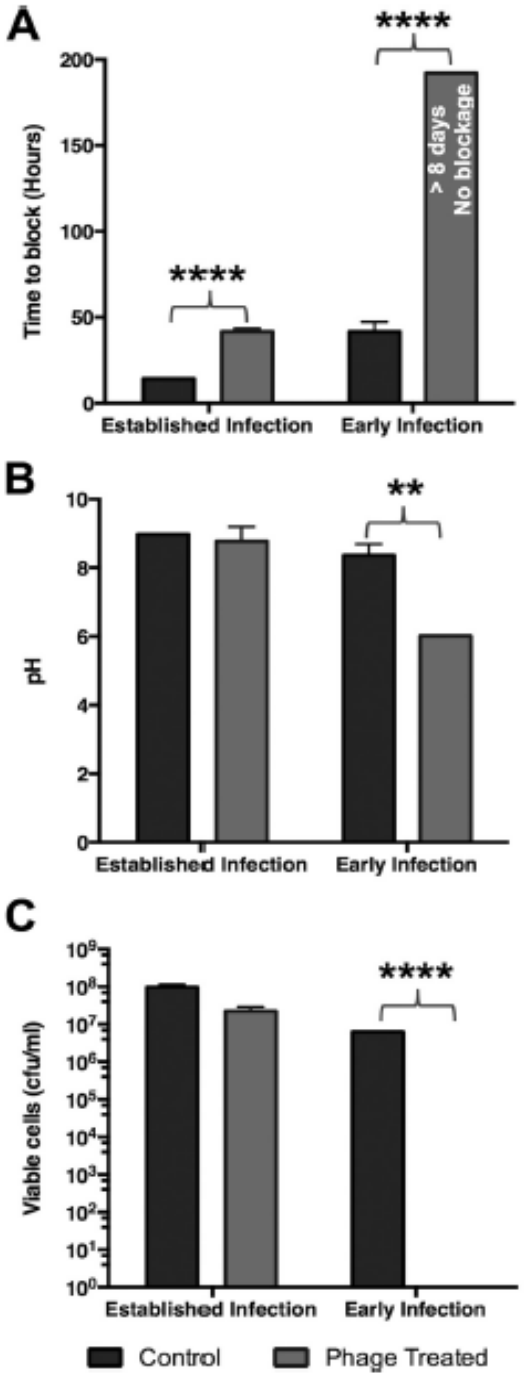
Phages and *P. mirabilis* biofilms

Nzakizwanayo *et a*l., 2015



Modelo de vejiga *in vitro*

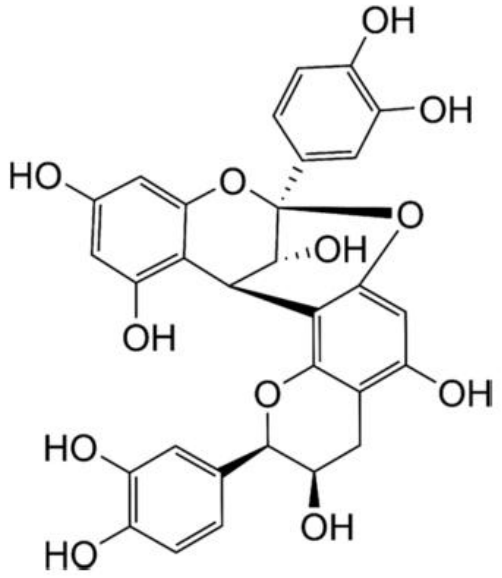




Natural products

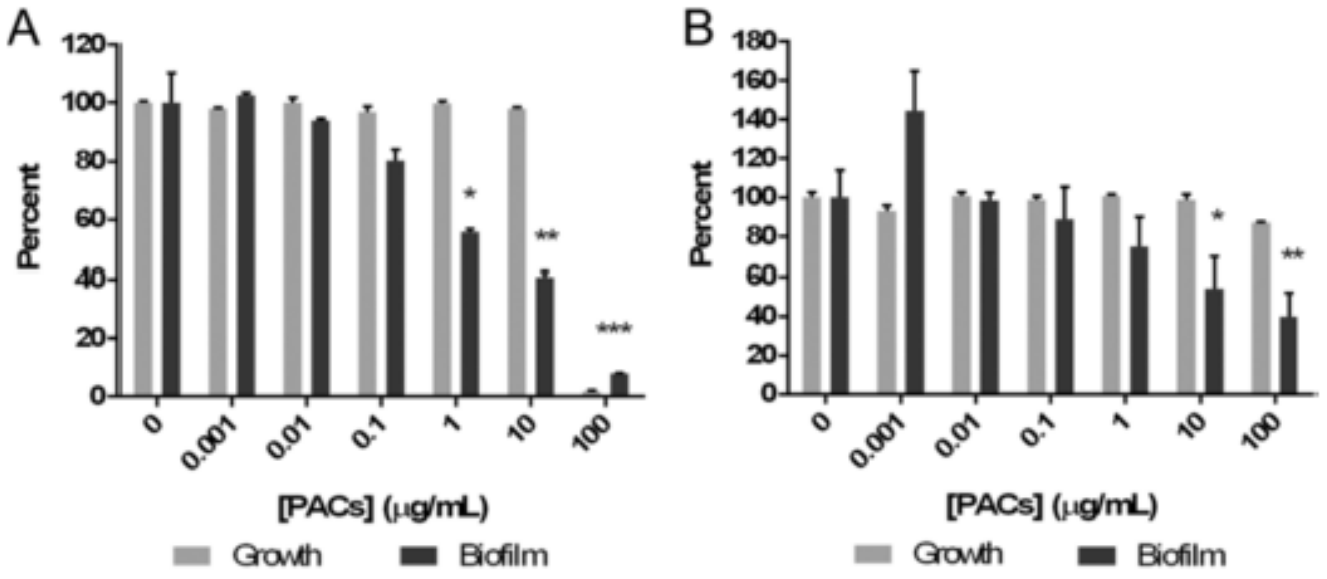
Arándanos

(*Vaccinium macrocarpon*)



Proantocianidina de arándano

Efecto de proantocianidina de *V. macrocarpum* sobre *P. aeruginosa*



Prevención

Disrupción

Ulrey et al., 2014

Original Article

Effect of *Ibicella lutea* on uropathogenic *Proteus mirabilis* growth, virulence, and biofilm formation

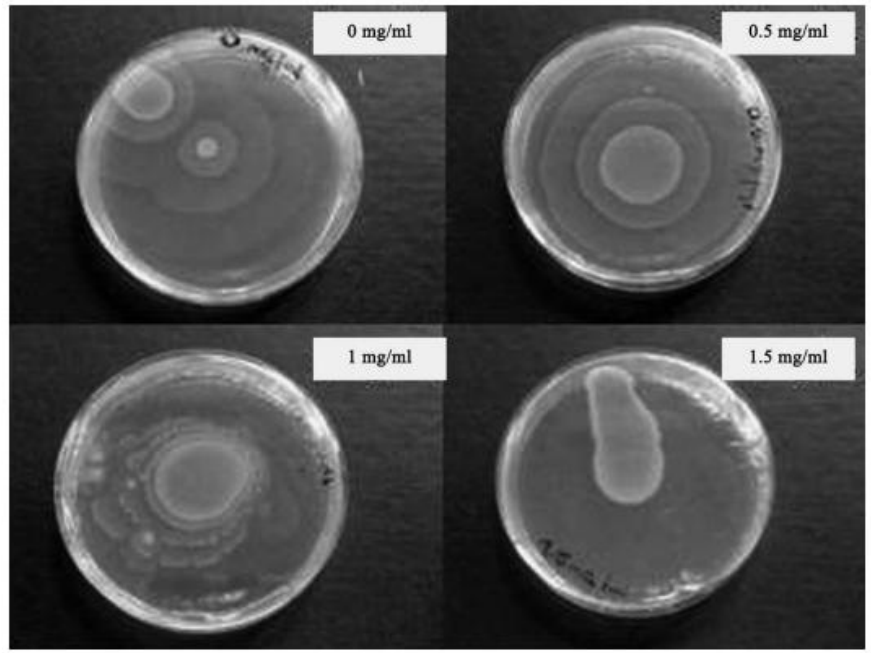
Vanessa Sosa and Pablo Zunino

J Infect Dev Ctries 2009; 3(10):762-770.

Table 2. Effect of *I. lutea* extract on biofilm formation by Pr2921 in polystyrene and glass and on auto-aggregation.

Concentration of *I. lutea*	Biofilm quantification		Auto-aggregation
	Polystyrene	Glass	
0 mg ml^{-1}	1.529 ± 0.600	0.623 ± 0.294	8.95 ± 6.34
1 mg ml^{-1}	0.236 ± 0.020*	0.007 ± 0.003*	7.31 ± 2.90
2 mg ml^{-1}	0.450 ± 0.163*	0.045 ± 0.035*	11.96 ± 2.59

Results are presented as the means ± standard deviations of three independent assays. The *I. lutea* extract inhibited biofilm formation but not affected the auto-aggregation.
*significantly different from control at $P < 0.05$ assessed by Mann-Whitney non-parametric analysis.
No significant differences between the two concentrations of extract *I. lutea* were observed.



Lithraea molleoides (“aruera” o “molle dulce”)

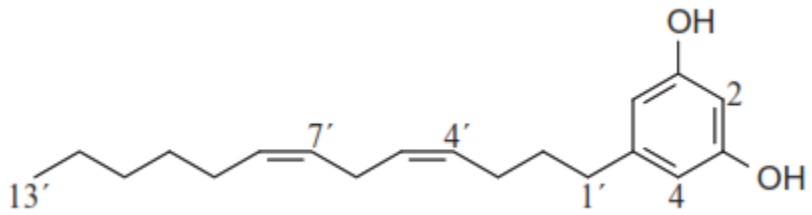


Table 1
Swarming inhibition of *Proteus mirabilis* by *Lithrea molleoides* extract and (Z,Z)-5-(trideca-4',7'-dienyl)-resorcinol (**1**).

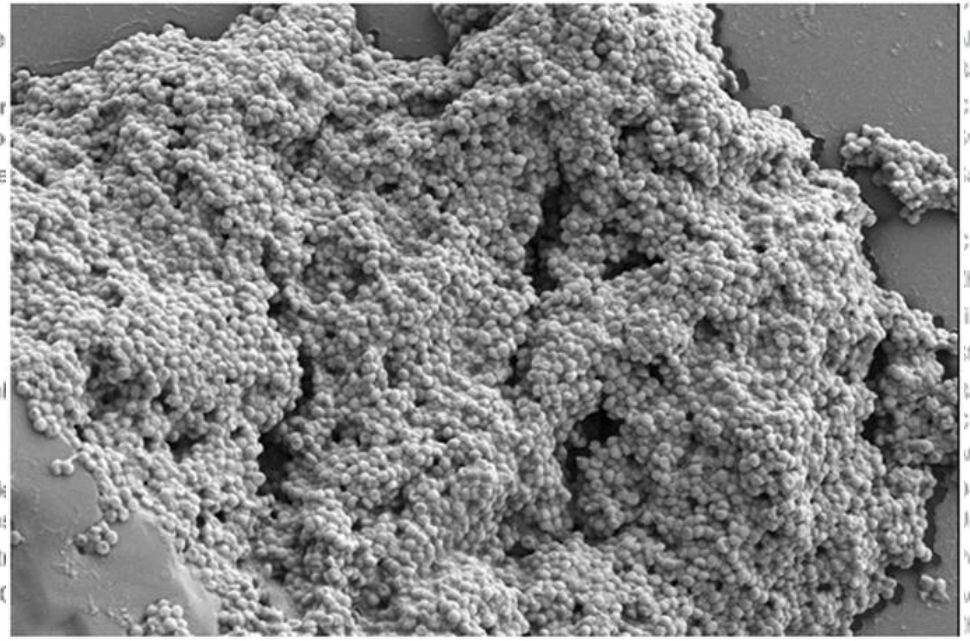
Time (h)	Distance (mm)[a]										
	Extract (μg/ml)				Compound **1** (μg/ml)					Control	Control EtOH
	1000	500	250	125	125	62	31	16	8		
6	–	1.0 ± 0	1.7 ± 0.1	2.0 ± 0	–	–	–	–	–	1.7 ± 0.1	1.5 ± 0
8	–	2.0 ± 0	6.0 ± 0	8.0 ± 0	–	–	–	–	–	2.0 ± 0	2.0 ± 0
24	7 ± 1.0	6.0 ± 0	9.0 ± 0	8.0 ± 0	–	1.0 ± 0	3.0 ± 0	3.0 ± 0	6.0 ± 0	10.3 ± 0.3	9.0 ± 0

[a] Data represent the mean ± standard error of the parameter evaluated. Control ETOH: control ethanol; –: no growth.

Carpinella et al., 2011

Control of biofilms in food industry

Methodology	Examples	Mechanism of action	Reference
Chemical treatments	Sanitizers (NaOCl, peracetic acid, NaOH, H_2O_2)	Cell structures oxidation	Rosenberg et al., 2008; Bayoumi et al., 2012; Schmidt, 2012; Bang et al., 2014; Nam et al., 2014; Ban and Kang, 2016; Techaruvichit et al., 2016; Yang et al., 2016; Møretrø et al., 2017
Enzymatic disruption	Cellulases	Extracellular matrix disruption	Wang et al., 2012; Coughlan et al., 2016; Stiefel et al., 2016
	Proteases		Qvlahal-Lansir et al., 2003;]7; Boels, 2011; Huang an et al., 2016;
	Glycosidases		Boels, 2011; Huang et al., 2014; Coughlan et al., 2016
	DNAses		Coughlan et al., 2016
Steel coatings	Nanoparticle CuO, MgO		Jexander, 2009; Beyth et al., 2015; lai et al., 2015
	Repelling sur modified top		Campoccia et al., 2013; Jindal et al., 2016; Swartjes and Veeregowda, 2016
	Functionalize nisin)		Sandreschi et al., 2016; Gu et al., 2017
Biosurfactants	Lichenysin		Coronel-León et al., 2016
	Surfactin		Zhang et al., 2017; Zhao et al., 2017
Bacteriophages	P100		lister et al., 2016; Iacumin et al., 2016
Bacteriocins	Nisin		Strempel et al., 2015
QS inhibition	Binding of inl (lactic acid)		Rasmussen et al., 2005; Brackman and Coenye, 2015; Coughlan et al., 2015; Amrutha et al., 2017
	Eznymatic d (paroxonase		Dong et al., 2001; Yang et al., 2005; Afroz et al., 2008; Koh et al., 2013
	sRNA post-t		Perez-Martinez and Haas, 2011
	Inhibition of		Adonizio et al., 2008; Chung et al., 2011; Zhu et al., 2015; Al-Shabib et al., 2016
	Furanones	Motility inhibition	Keskinen and Annous, 2011; Vestby et al., 2014
Essential oils	Citral	QS inhibition, motility inhibition	Shi et al., 2017
	Carvacrol	Bactericidal	Friedman, 2014
High hydrostatic pressure	H_2O	Bactericidal (also endospores)	Evelyn and Silva, 2015; Santos et al., 2017
Non-thermal plasma	UV plus O_2, N_2, O_3, H_2O and He	Bactericidal	Scholtz et al., 2015
Photocatalysis		Bactericidal	Chorianopoulos et al., 2011; Priha et al., 2011; Nica et al., 2017; Ishwarya et al., 2018



Galie et al. 2018

Methodology	Examples	Mechanism of action	Reference
Chemical treatments	Sanitizers (NaOCl, peracetic acid, NaOH, H_2O_2)	Cell structures oxidation	Rosenberg et al., 2008; Bayoumi et al., 2012; Schmidt, 2012; Bang et al., 2014; Nam et al., 2014; Ban and Kang, 2016; Techaruvichit et al., 2016; Yang et al., 2016; Møretrø et al., 2017
Enzymatic disruption	Cellulases	Extracellular matrix disruption	Wang et al., 2012; Coughlan et al., 2016; Stiefel et al., 2016
	Proteases		Oulahal-Lagsir et al., 2003; Chaignon et al., 2007; Boels, 2011; Huang et al., 2014; Coughlan et al., 2016; Stiefel et al., 2016
	Glycosidases		Boels, 2011; Huang et al., 2014; Coughlan et al., 2016
	DNAses		Coughlan et al., 2016
Steel coatings	Nanoparticles (Ag^{2+}, Fe_3O_4, TiO_2, ZnO, CuO, MgO	Alteration of bacterial membrane	Alexander, 2009; Beyth et al., 2015; Rai et al., 2015
	Repelling surfaces (monolayers, hydrogels, modified topography)	Inhibition of bacterial binding	Campoccia et al., 2013; Jindal et al., 2016; Swartjes and Veeregowda, 2016
	Functionalized surfaces (with lisozyme or nisin)	Bactericidal	Sandreschi et al., 2016; Gu et al., 2017
Biosurfactants	Lichenysin	Inhibition of bacterial adhesion	Coronel-León et al., 2016
	Surfactin		Zhang et al., 2017; Zhao et al., 2017
Bacteriophages	P100	Cell lysis	Fister et al., 2016; Iacumin et al., 2016
Bacteriocins	Nisin	Cell membrane alteration	Strempel et al., 2015
QS inhibition	Binding of inhibitors to QS receptors (lactic acid)	Downregulation of adhesion and virulence mechanisms	Rasmussen et al., 2005; Brackman and Coenye, 2015; Coughlan et al., 2015; Amrutha et al., 2017
	Eznymatic degradation of QS signals (paroxonases)		Dong et al., 2001; Yang et al., 2005; Uroz et al., 2008; Koh et al., 2013
	sRNA post-transcriptional control		Perez-Martinez and Haas, 2011
	Inhibition of QS signals biosynthesis		Adonizio et al., 2008; Chung et al., 2011; Zhu et al., 2015; Al-Shabib et al., 2016
	Furanones	Motility inhibition	Keskinen and Annous, 2011; Vestby et al., 2014
Essential oils	Citral	QS inhibition, motility inhibition	Shi et al., 2017
	Carvacrol	Bactericidal	Friedman, 2014
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Non-thermal plasma	UV plus O_2, N_2, O_3, H_2O and He	Bactericidal	Scholtz et al., 2015
Photocatalysis		Bactericidal	Chorianopoulos et al., 2011; Priha et al., 2011; Nica et al., 2017; Ishwarya et al., 2018

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Gracias!

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