

Relevancia de los biofilms, control, antimicrobianos y moléculas disruptoras



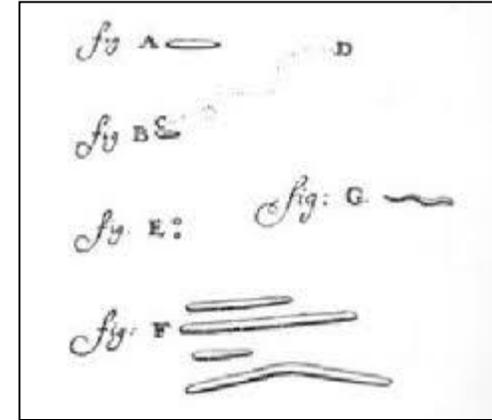
Pablo Zunino

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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)

Origen del planeta Tierra: 4600 millones de años

Evidencia fósil de vida microbiana formando biofilms: 3700 millones de años (rocas, estromatolitos)

Probablemente, microorganismos fosilizados de 4280 millones de años en rocas sedimentarias ferruginosas (Dodd et al., 2017, Nature)



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

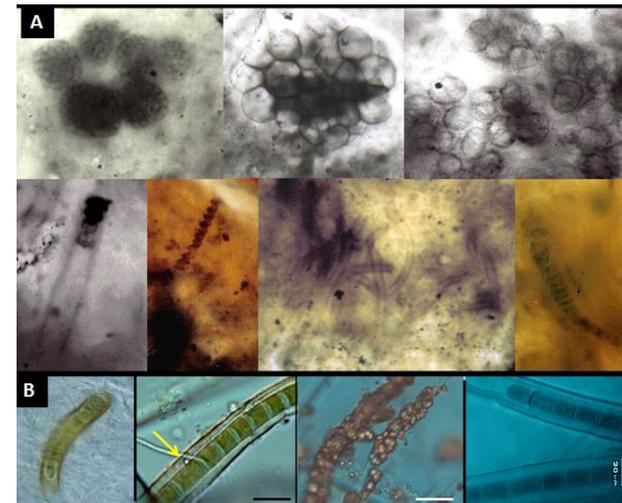
Las bacterias constituyen las primeras formas de vida en la Tierra, teniendo un rol crítico en la generación de condiciones para formas de vida posteriores, fundamentalmente a través del desarrollo de la fotosíntesis y generación de oxígeno en la atmósfera (vida humana aprox. 2,5 Ma)

Bacterias: organismos gregarios

Estudio “tradicional” de los microorganismos

- células planctónicas, de vida libre
- medios líquidos puros

Estromatolitos: formas fósiles de biofilms bacterianos
(datan de hasta 3.700 millones de años)



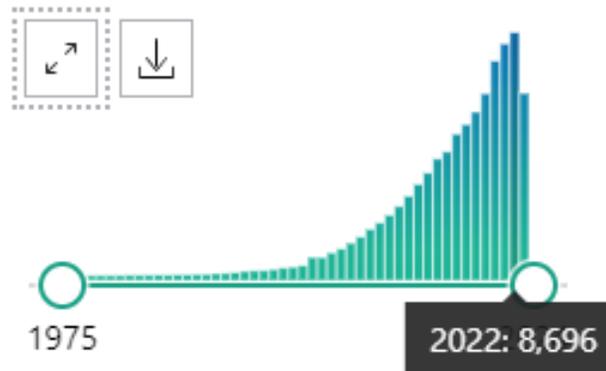
Lester Park, Saratoga Springs, New York (cámbrico)

Artículos científicos sobre biofilms (Pubmed - NCBI)



MY NCBI FILTERS 

RESULTS BY YEAR



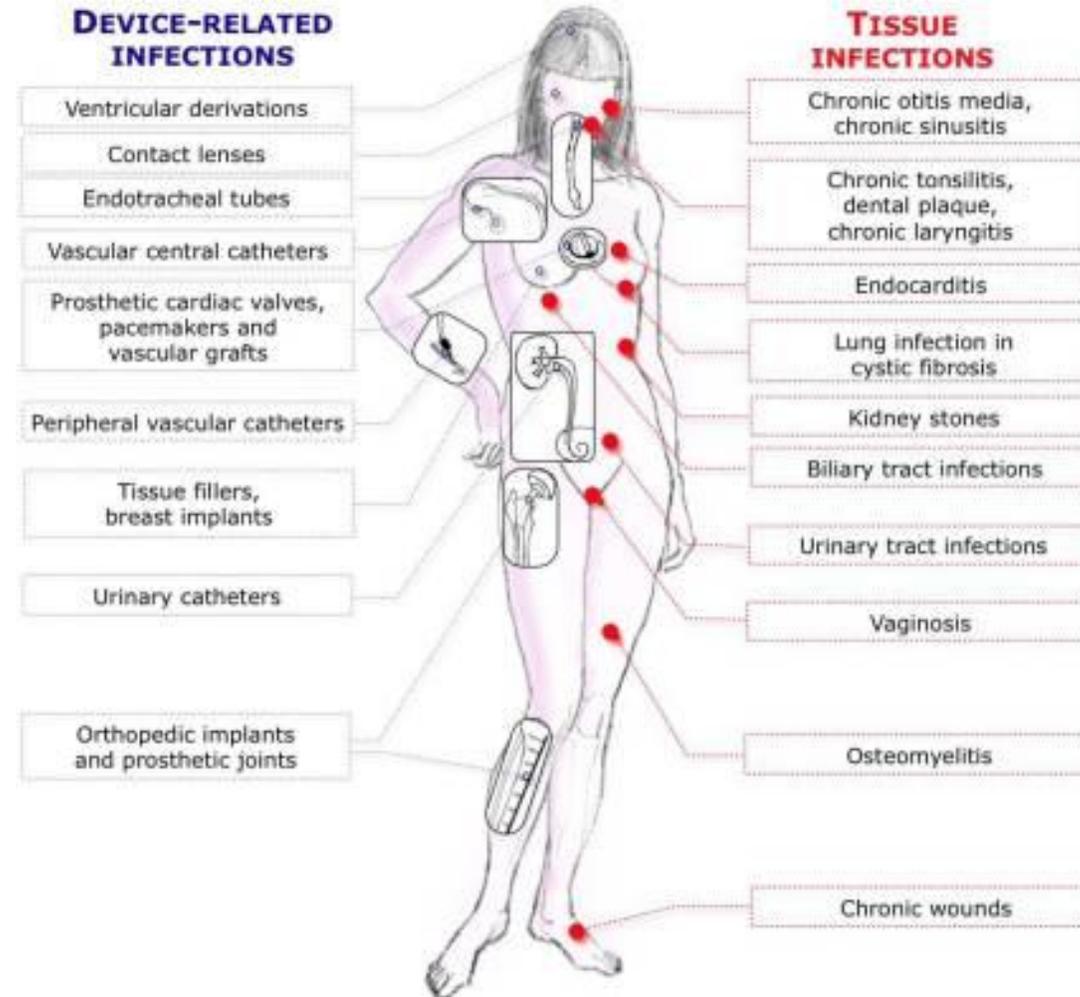
25 por día en 2022!!

Impacto de los biofilms bacterianos en la salud

Los porcentajes de infecciones bacterianas que involucran biofilms se estiman entre un 65% (CDC) y un 80% (NIH) (Jamal et al., 2018)

Ej. endocarditis, fibrosis quística, periodontitis, rinosinusitis, osteomielitis, heridas, meningitis, infecciones urinarias, infecciones asociadas a prótesis y otros implantes, etc.

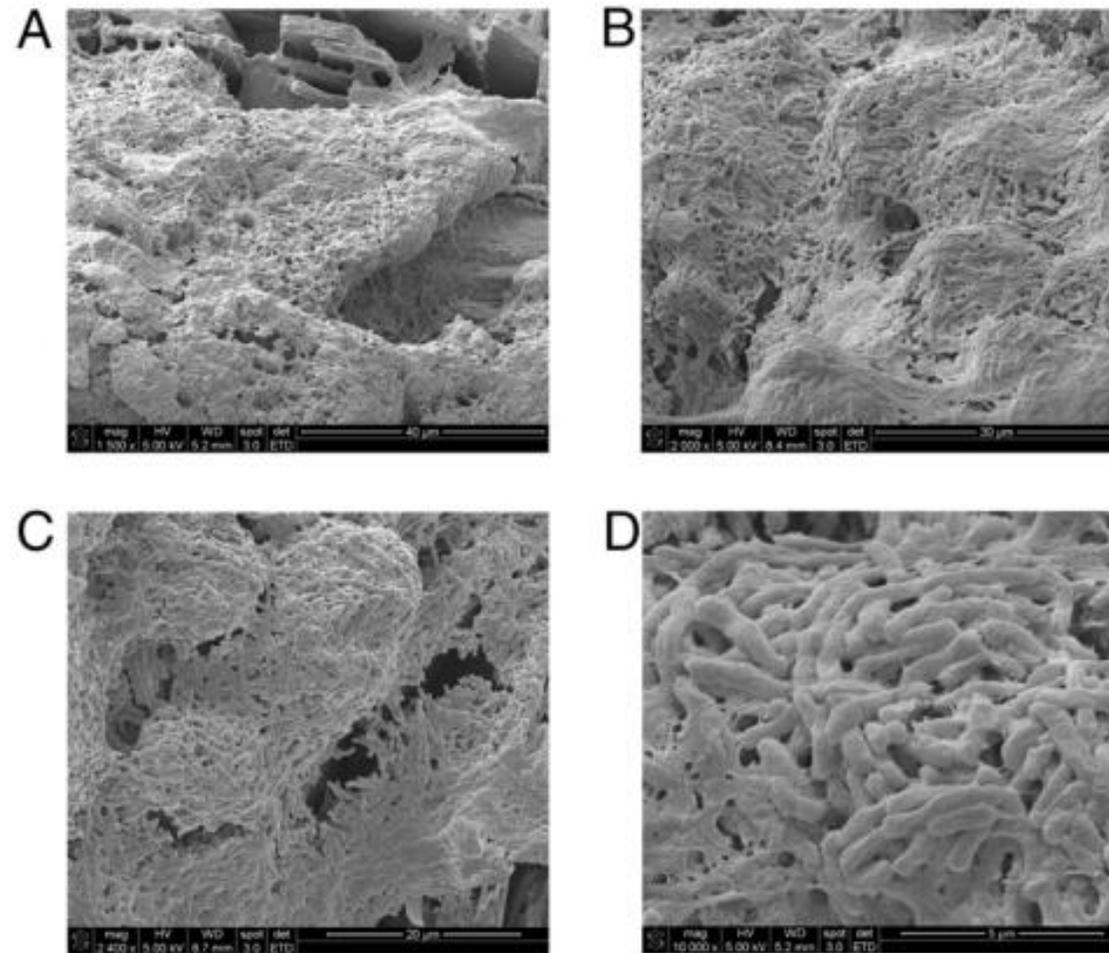
Infecciones asociadas a biofilms



Infecciones asociadas a biofilms en tejidos

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

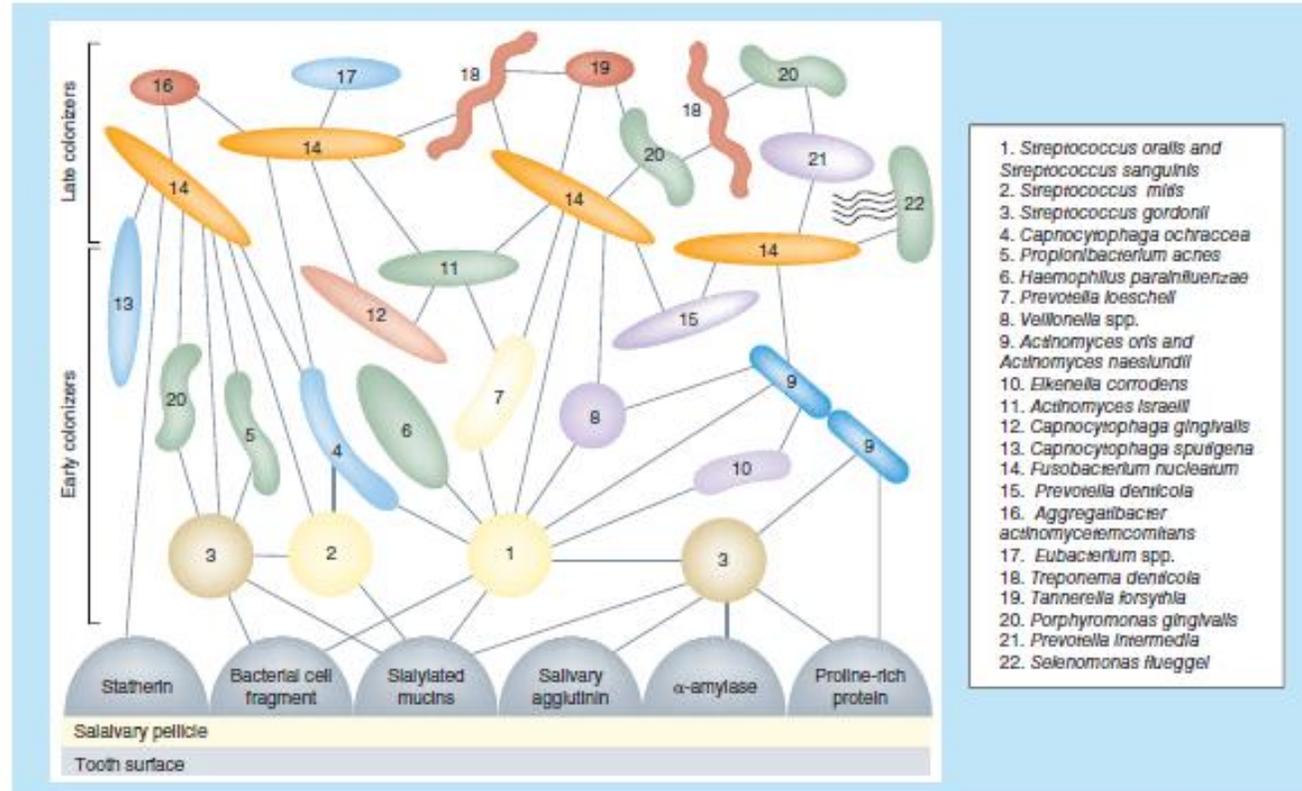
Body System	Affected Organs	Disease
Auditory	Middle ear	Otitis media
Cardiovascular	Cardiac valves	Infective endocarditis
	Arteries	Atherosclerosis
Digestive	Salivary glands	Sialolithiasis (salivary duct stones)
	Gall bladder	Recalcitrant typhoid fever and predisposition to hepatobiliary cancers
	Gastrointestinal 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 Bordetella infections
	Upper and lower airways	Cystic fibrosis
Urinary	Prostate gland	Chronic bacterial prostatitis
	Urethra, bladder, ureters, kidneys	Urinary tract infections



Biofilms en vesícula biliar (*S. Typhi*, portador asintomático)

Enfermedades relacionadas con biofilms

Placa dental



Rabin et al. 2021

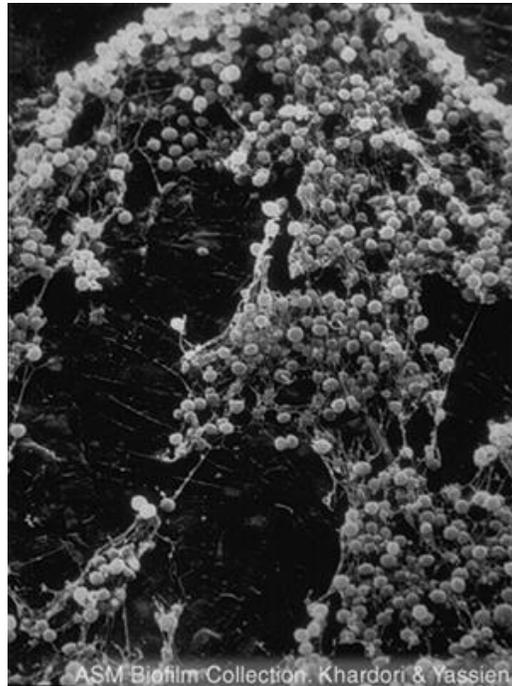
Biofilms polimicrobianos

Más de 700 especies de bacterias y arqueas reportadas

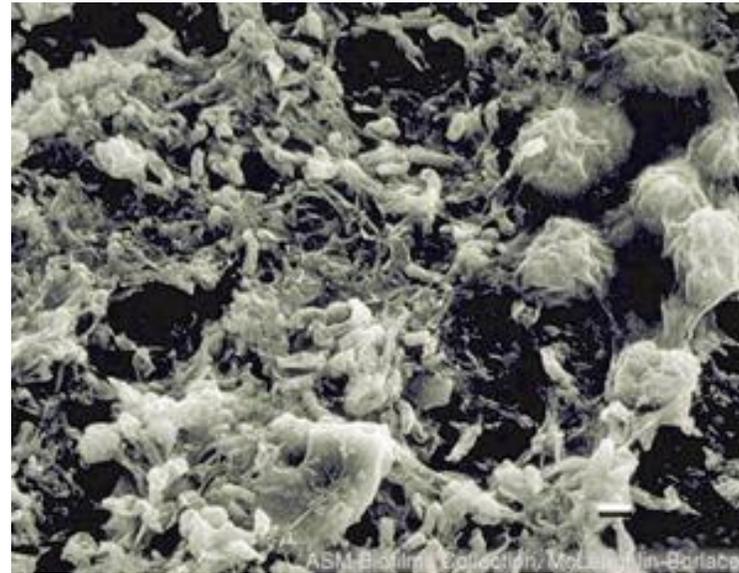
Biofilms en implantes

Representan 50 al 70 % de infecciones nosocomiales

Causan infecciones y pueden interferir con la función del implante; remoción y recambio, serias consecuencias médicas y pérdidas económicas

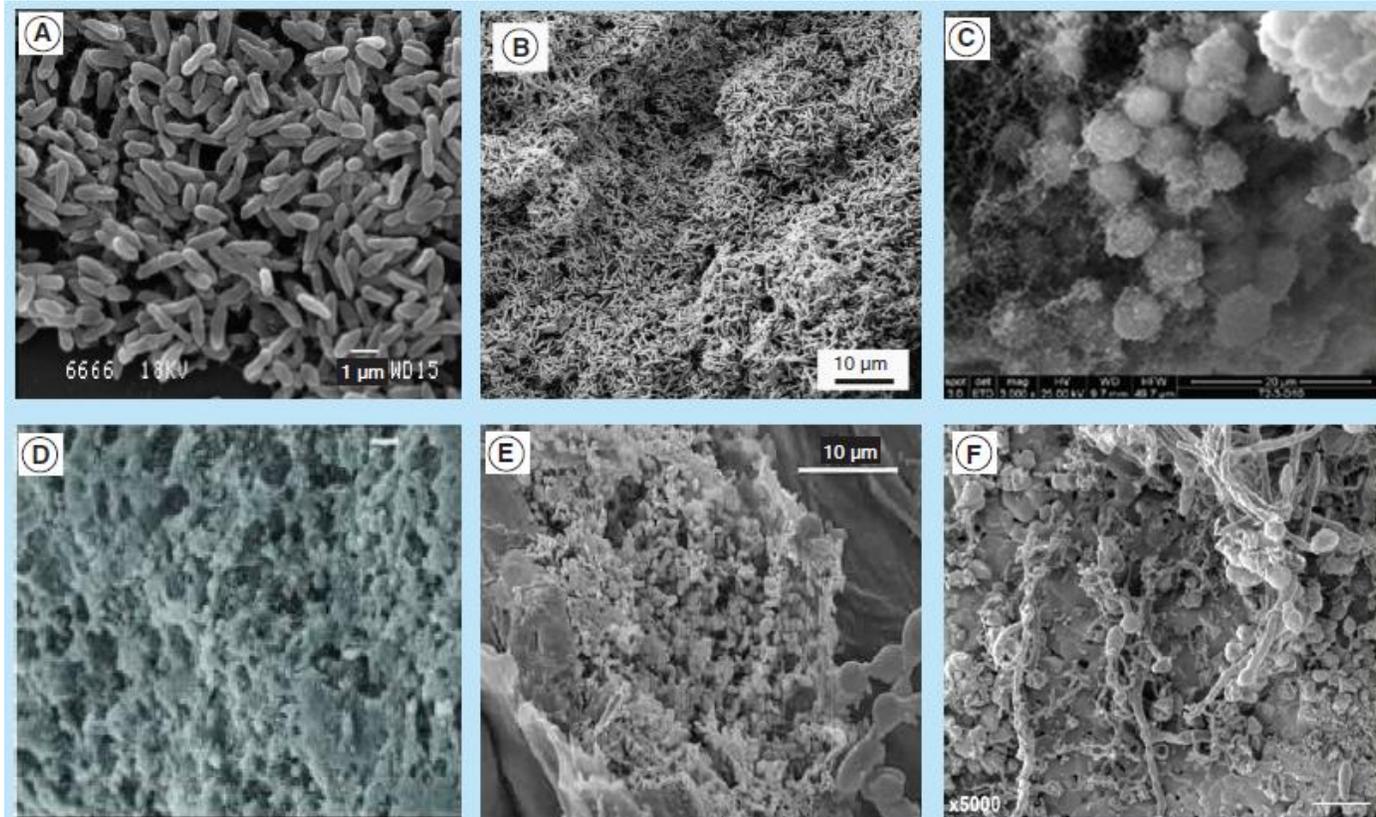


Catéter vascular (MEB)



Lente de contacto (MEB)

Rabin et al. 2021

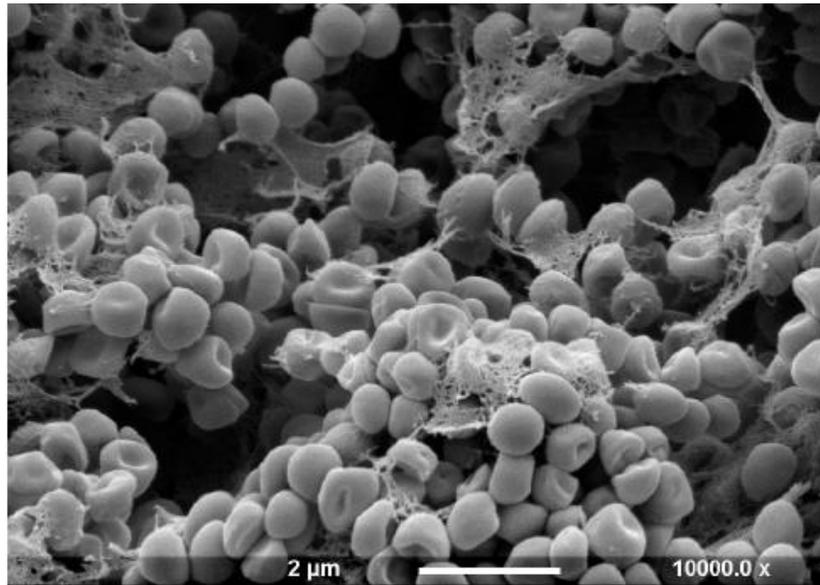


Rabin et al. 2021

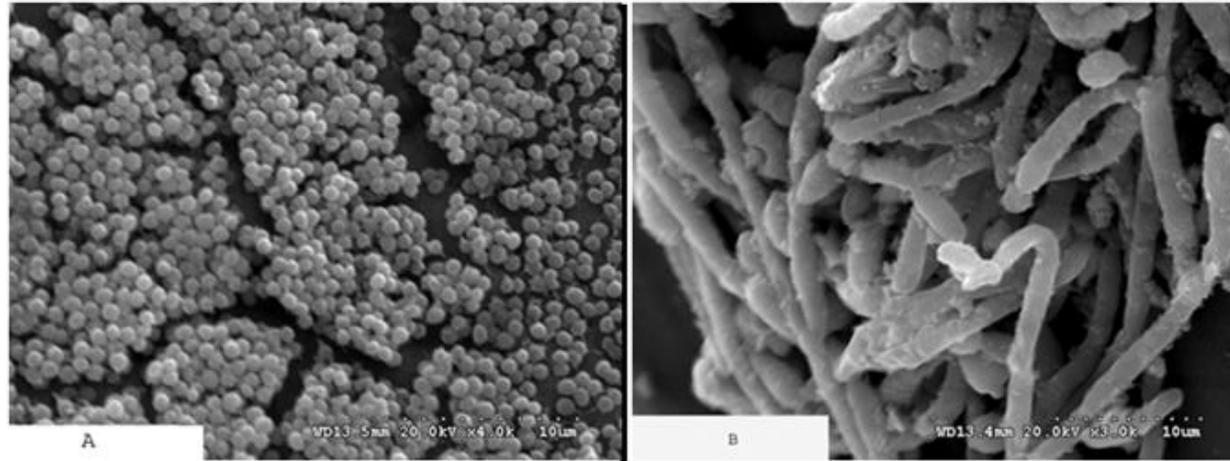
- A) *Pseudomonas aeruginosa* adherida a superficies de vidrio
- B) *Escherichia coli* en superficie del óxido de titanio
- C) *Staphylococcus aureus* asociado a catéter intravenoso *in vivo*
- D) *Staphylococcus epidermidis* en un catéter de teflón
- E) *Salmonella enterica* serovar Pomona sobre tejidos vegetales
- F) *Streptococcus mutans* y *Candida albicans* en discos de hidroxiapatita

El mercado global de los implantes médicos se estimó en unos U\$S 79.1 mil millones en 2014, unos 133 mil millones en 2022

El aumento en el uso de dispositivos basados en biomateriales se asocia con el envejecimiento de la población, la creciente prevalencia de enfermedades y modificación de estilos de vida (sedentarismo, consumo de alimentos poco saludables, aumento de accidentes traumáticos, aumento de la demanda de injertos y órganos de donantes, 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)

organized in a biofilm.

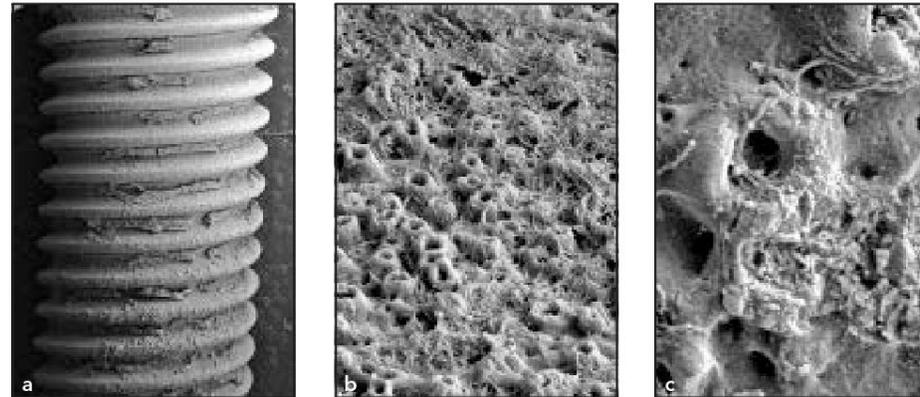


Fig. 2. SEM images of the biofilm formed on the surface of the dental implant. (a) Low magnification, (b) medium magnification, (c) high magnification.

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

Medical Devices Bureau (Canadá) reconoce 4 clases de dispositivos médicos de acuerdo al riesgo

- **Clase I:** bajo riesgo para los pacientes y no requiere una licencia o requiere norma normativa más baja (instrumentos quirúrgicos, material dental, etc.)
- **Clase II:** exigir la declaración del fabricante de seguridad y efectividad del dispositivo (lentes de contacto, máquinas de ultrasonido, catéteres médicos, etc.)
- **Clase III:** presenta un mayor riesgo potencial para el paciente (implantes ortopédicos como cemento óseo, implantes de cadera, máquinas de hemodiálisis, etc.)
- **Clase IV:** presenta el mayor riesgo potencial y sujeto a una revisión en profundidad y aprobación reglamentaria previa a la comercialización (implantes cardiovasculares, marcapasos, dispositivos de asistencia ventricular, etc.)

Infecciones urinarias asociadas a catéteres (ITU-C)

- Vinculadas a la formación de biofilms en la superficie de los catéteres
- Infecciones nosocomiales más comunes
- Costos (USA): U\$S 450 millones/año

Incidencia:

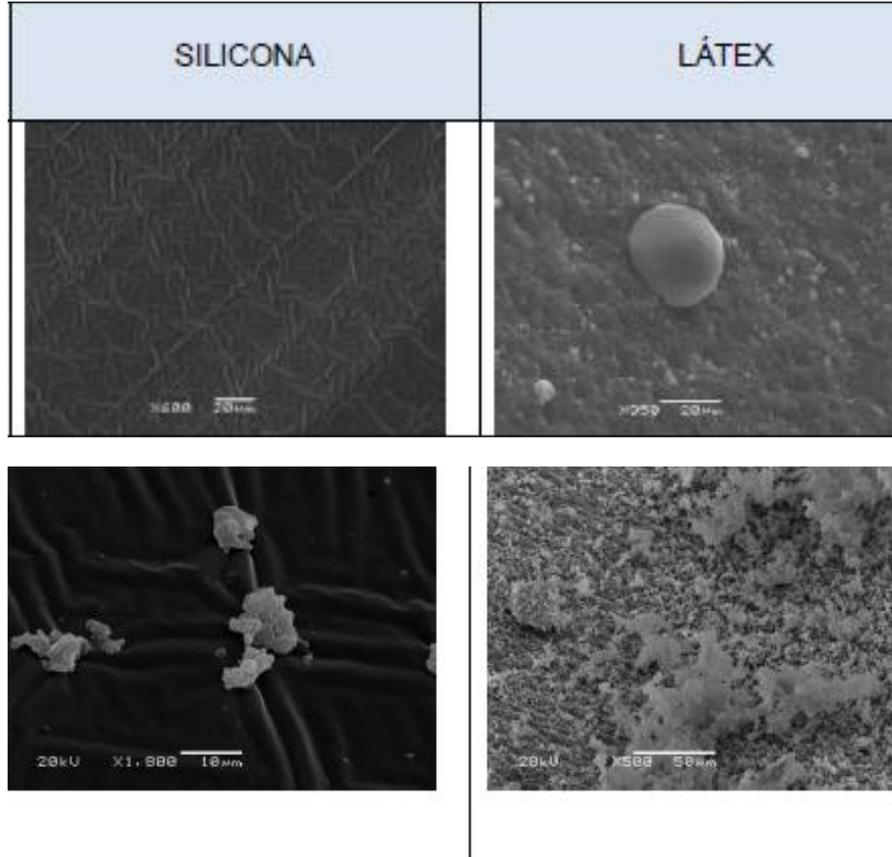
Pacientes cateterizados hasta 7 días

→ 10 al 50 % desarrollan ITU-C

Pacientes cateterizados por más de 28 días

→ 100 % desarrollan ITU-C

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

Magnitud del problema de los biofilms asociados a implantes médicos

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

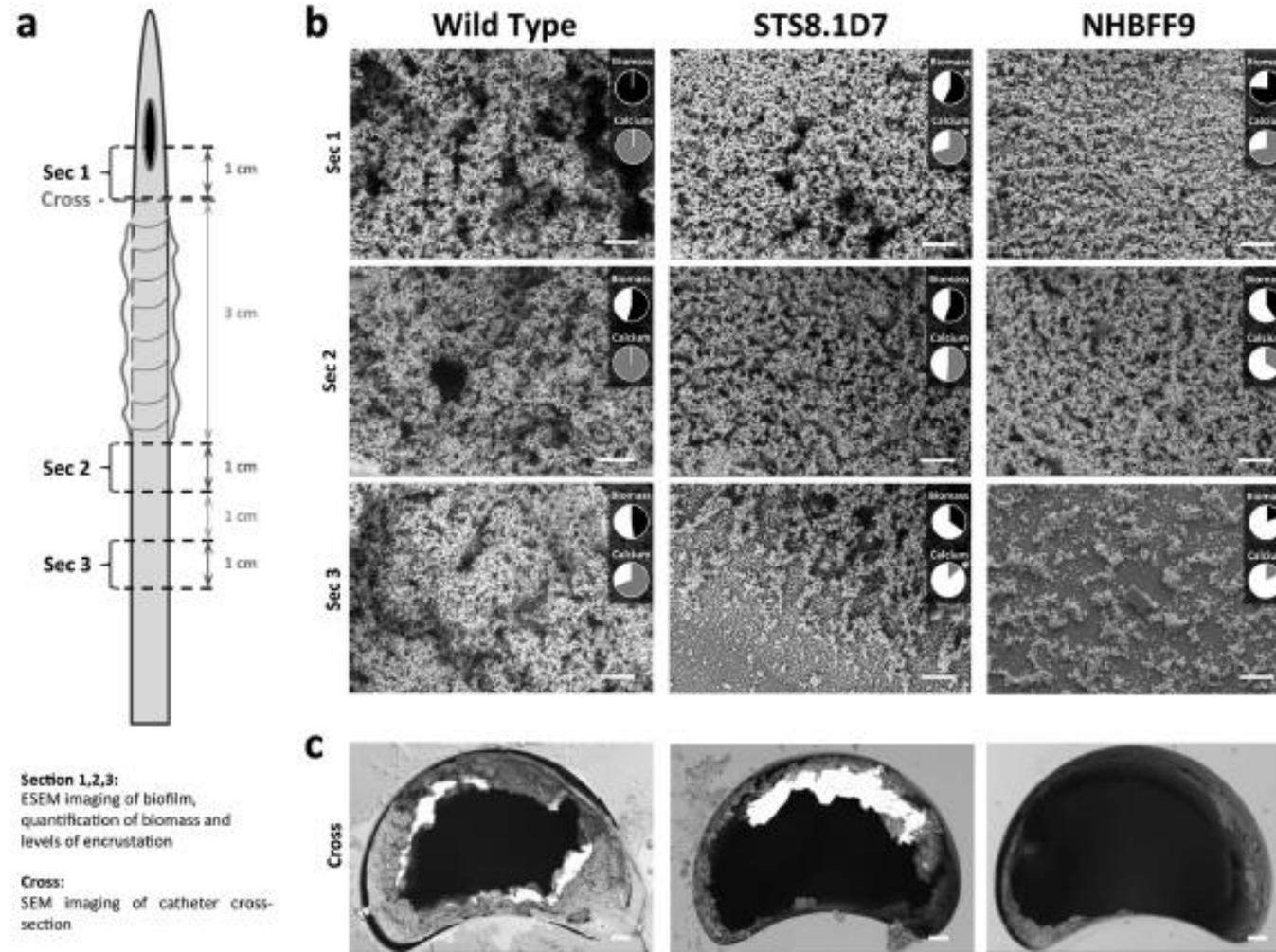
Relación virulencia/biofilms en catéteres

P. mirabilis y biofilms

TABLE 1 Characterization of biofilm formation mutants

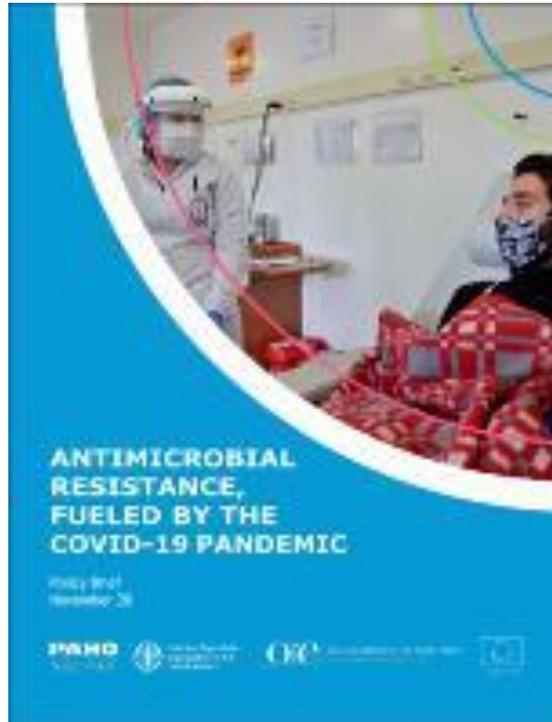
Phenotype and strain	<i>P. mirabilis</i>		Putative function/product of disrupted gene ^b	Urease production (mean ± SD) ^c	Motility (% of wild type)	
	HI4320 locus ^d	Disrupted gene			Swimming	Swarming
Wild type (B4)				5.7 ± 0.4	100	100
Biofilm enhanced						
NHBF8	PMI3402		Unknown function; putative MuA-like DNA-binding protein (Pfam 02316) (40/40)	5.9 ± 0.3	0	0
NHBFH4	PMI0262 ^d	<i>mrpI</i>	Fimbrial recombinase (40/40) ^d	4.3 ± 0.1	36.96	0
NHBF8	PMI2210		Fimbrial subunit (38/40)	5.3 ± 0.4	70.65	111.34
NHBFH5	PMI2359	<i>glnE</i>	Glutamate-ammonia-ligase adenylyltransferase (38/40)	4.8 ± 0.4	51.09	0
NHBF5	PMI1729	<i>rsbA</i>	Regulator of swarming behavior; sensor kinase of two-component system (40/40)	5.5 ± 0.1	116.3	32.99
NHBF9	PMI0829	<i>bcr</i>	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	<i>gltS</i>	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	<i>lrp</i>	Leucine-responsive regulator (40/40) ^d	5.2 ± 0.2	77.17	34.02
STS8.1D7	PMI1479 ^e	<i>nirB</i>	Nitrite reductase (30/30) ^e	4.4 ± 0.1	100	77.32

Mutantes *nirB* y transportador MFS



Cada año alrededor de 250.000 a 500.000 infecciones primarias de la corriente sanguínea ocurren entre los 150 millones de dispositivos intravasculares implantados (USA)

Bajo inóculo para la infección a partir de implantes (Nowakowska et al., 2014)

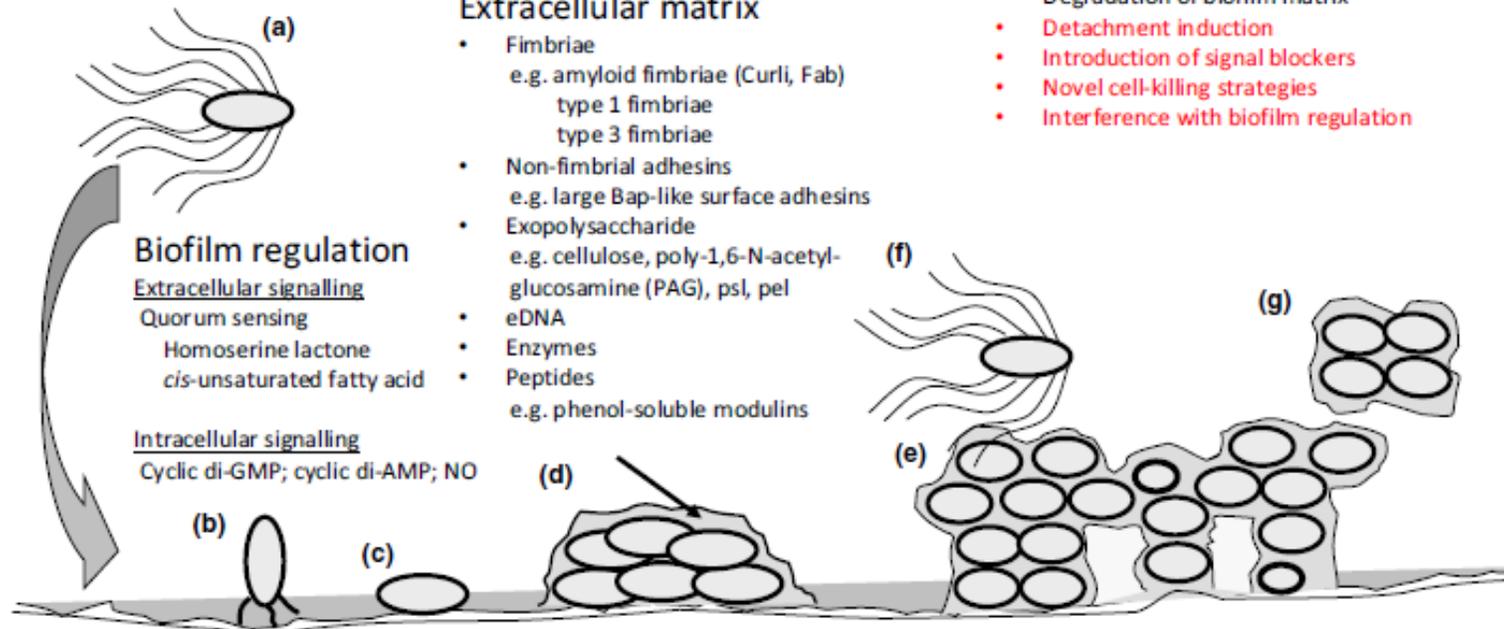


Necesidad de estrategias de prevención y eliminación!!

Biofilms: mecanismos y estrategias de prevención

Biofilm prevention strategies

- Development of antimicrobial surfaces
- Prevention of attachment



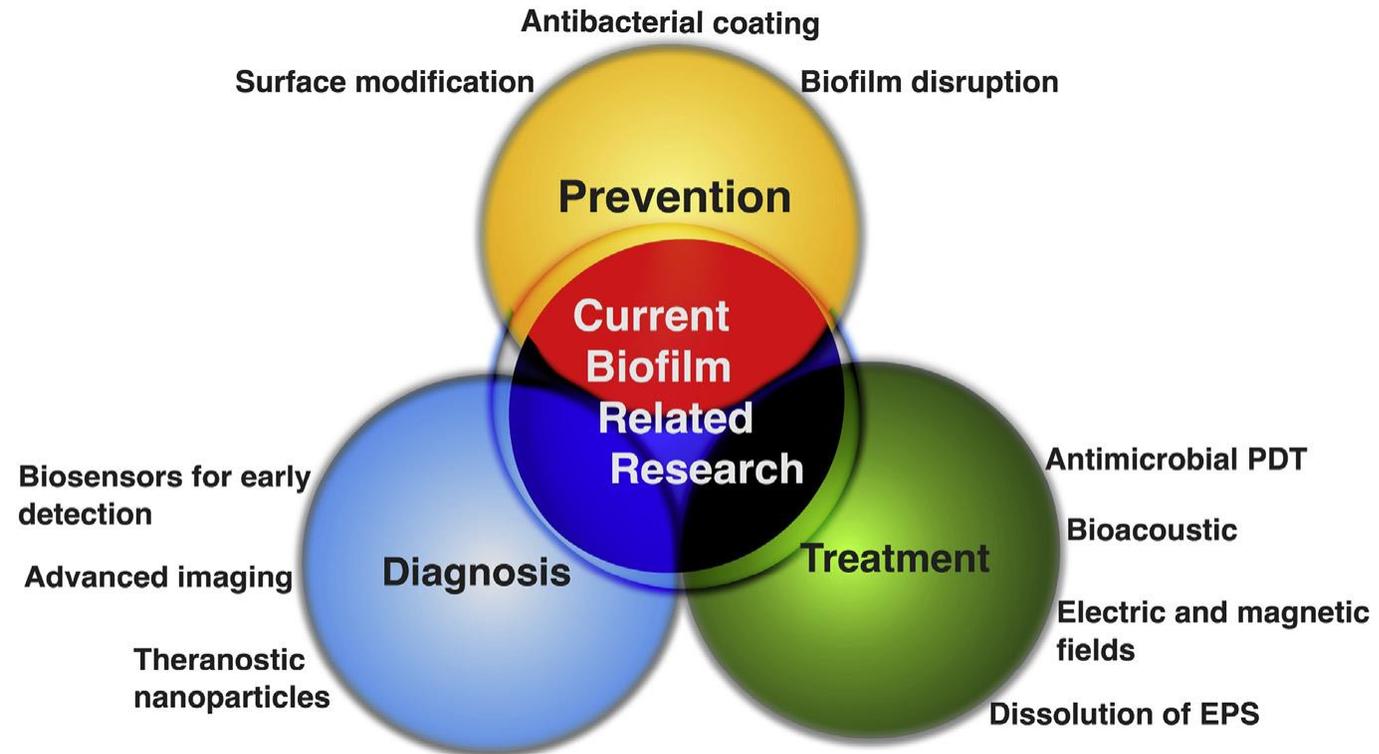
Biofilm treatment strategies

- 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

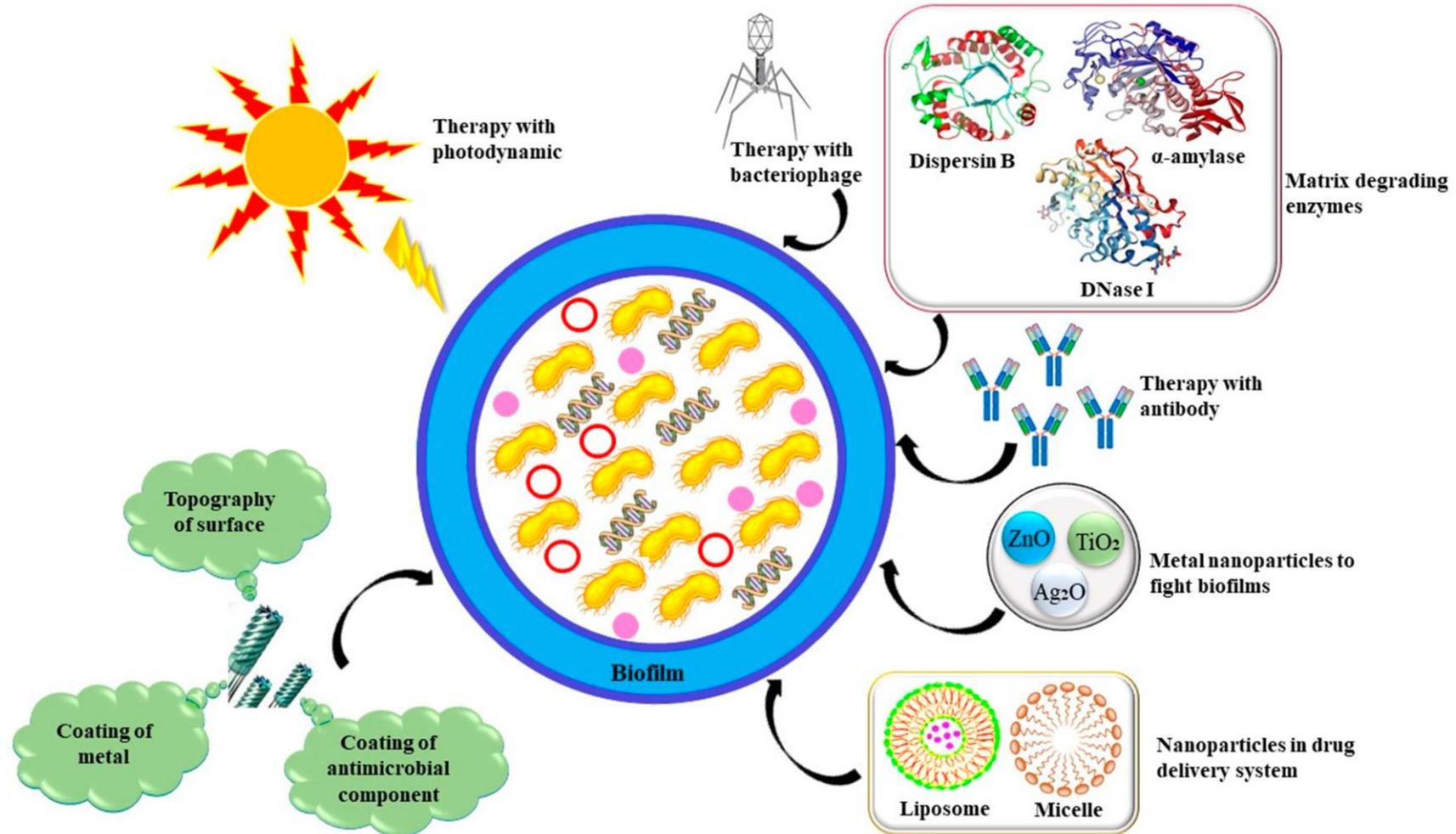
Antibiotic tolerance mechanisms

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

Estrategias de prevención, tratamiento y diagnóstico



Enfoques en la investigación y terapias anti-biofilms para su erradicación



Estrategias

- Métodos físicos:

Métodos eléctricos (electroporación)

Campos magnéticos

Ultrasonido

(Industria, dispositivos médicos, odontología, etc.)

Combinaciones



Estrategias

- Métodos químicos:

Antisépticos

Antibióticos

Enzimas

(proteínasa 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 (lípidos o ácido graso))

Quelantes, anticoagulantes

(EDTA, heparina, etc.)

Terapia fotodinámica

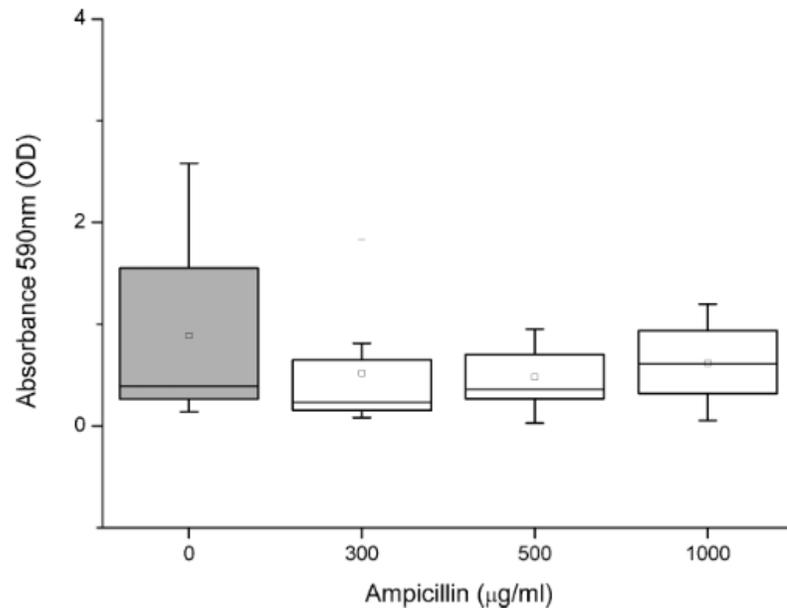
Table 1 Selected biosurfactants reported in literature with antibiofilm/microbial activities

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

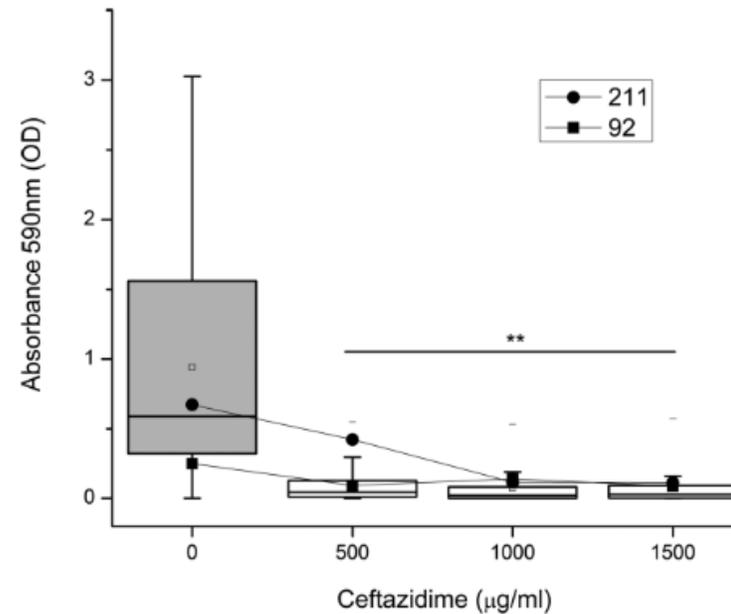
NS not specified

Efecto de diferentes antibióticos sobre biofilms producidos por *Escherichia coli* uropatógena aislada de niños con UTI

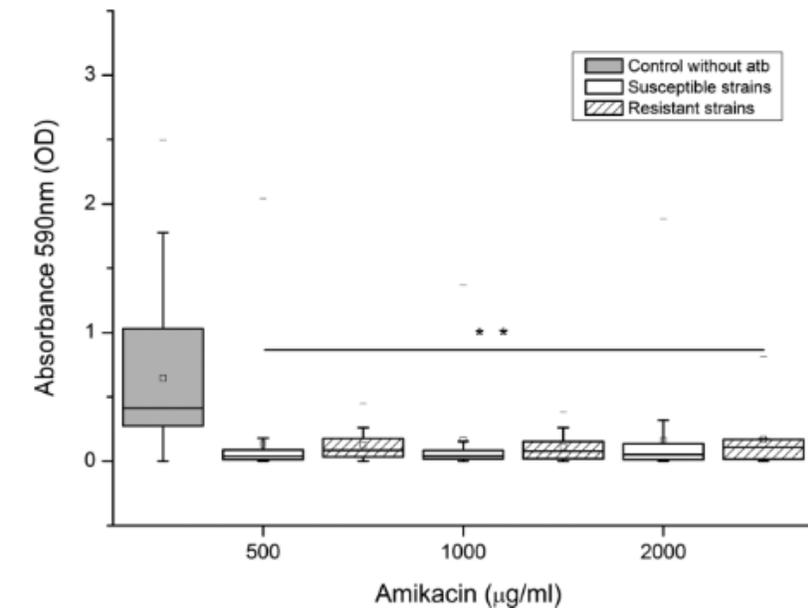
González et al., 2017



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

Enzimas (matriz)

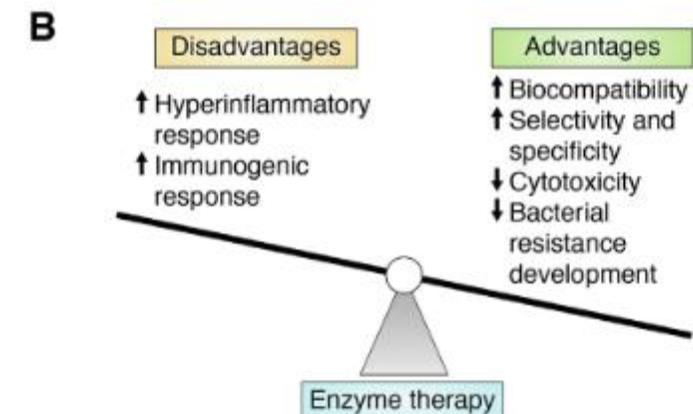
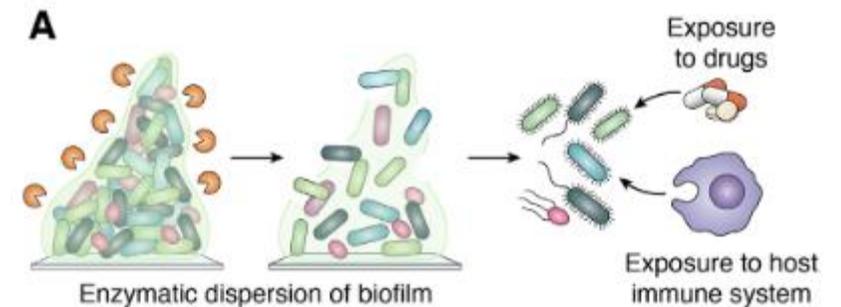
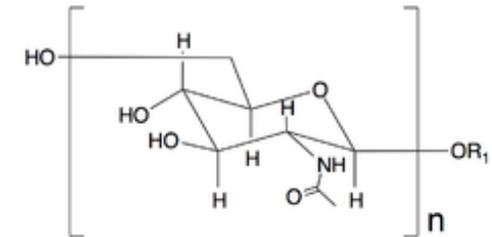
Dispersina B (DspB; glycoside hydrolase
Actinobacillus actinomycetemcomitans, 40 kDa)

Reported glycoside hydrolases for biofilm dispersion

Glycoside hydrolase	Targeting glycosidic linkage	Monosaccharide composition of the polysaccharides
Dispersin B	GlcNAc -(β -1,6)- GlcNAc	<i>N</i> -acetyl-D-glucosamine
α -Amylase	Glc-(α -1,4)-Glc	D-glucose
Cellulase	Glc-(β -1,4)-Glc	D-glucose
PslG	Man α -(β -1,3)- Man α ^a	D-mannose, D-glucose and L-rhamnose
PelA	(1,4) ^b	Partially de- <i>N</i> -acetylated <i>N</i> -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	<i>N</i> -acetyl-D-galactosamine
CcsZ	Glc-(β -1,4)-Glc	D-glucose
PssZ	ManN-(β -1,4)-ManN	D-mannosamine

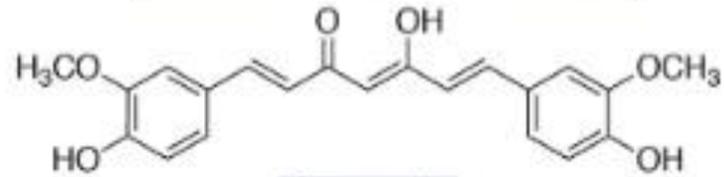
^a The targeting glycosidic linkage of PslG is predicted.

^b PelA enzyme works on Pel, a linear cationic polysaccharide composed of partially de-*N*-acetylated 1,4 linked *N*-acetylgalactosamine.

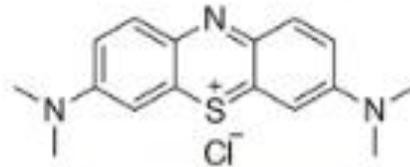


Terapia fotodinámica antimicrobiana

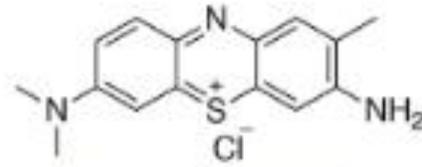
Antimicrobial PDT molecules



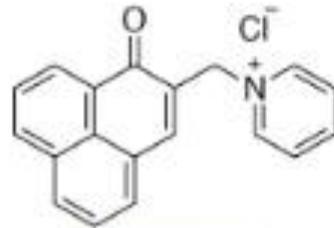
Curcumin



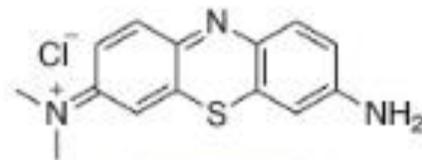
Methylene blue



Toluidine blue



SAPYR



Azure A

Mecanismos de regulación

No existe un mecanismo único responsable de la regulación: especificidades

- Quorum sensing

Principal mecanismo de regulación (no el único!)

Autoinductores (Bacterias Gram – y Gram +)

Modulación adhesión/dispersión:

La mayoría de las especies aumentan los comportamientos asociados a la formación de biofilms a altas concentraciones celulares.

Mutantes afectadas en el QS forman biofilms débiles – función en la producción de ADN extracelular

Comunicación entre microorganismos: QS en bacterias Gram positivas y Gram negativas

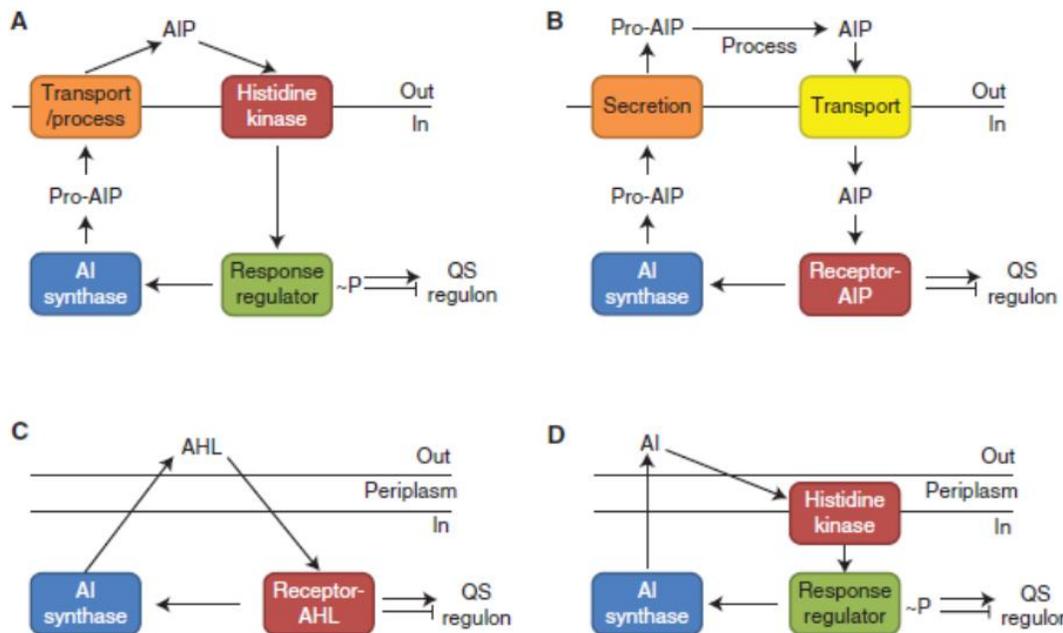
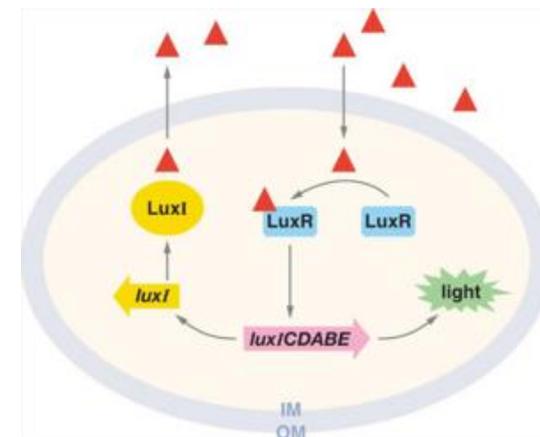


Figure 1. Canonical bacterial quorum-sensing (QS) circuits. Autoinducing peptide (AIP) QS in Gram-positive bacteria by (A) two-component signaling, or (B) an AIP-binding transcription factor. Small molecule QS in Gram-negative bacteria by (C) a LuxI/LuxR-type system, or (D) two-component signaling.



Vibrio fischeri



Euprymna scolopes

Mecanismos generales de QS

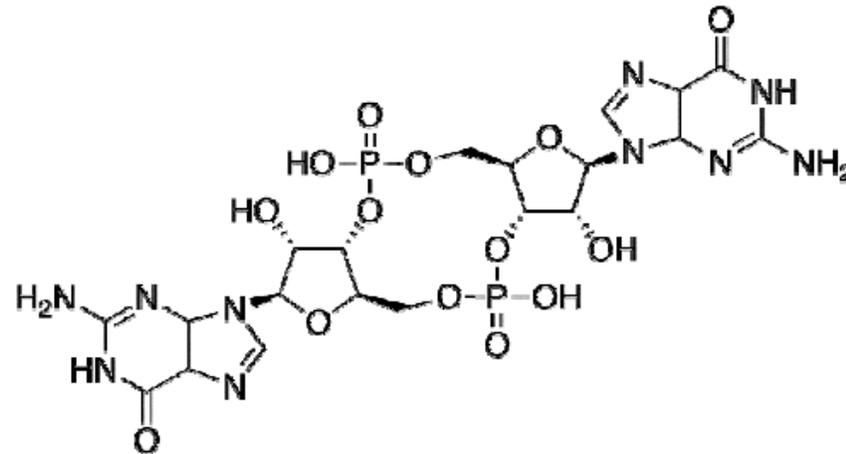
Procesos controlados por Quorum Sensing

- Bioluminiscencia
- Esporulación
- Competencia
- Producción de antibióticos
- Secreción de factores de virulencia
- **Formación de biofilms**

Ejemplos de sistemas de Quorum Sensing y su influencia sobre comportamientos sociales bacterianos

Microrganism	Major Signal Molecules	Regulatory System	Group-Derived Benefits	References
<i>Bacillus subtilis</i>	ComX CSF (PhrC) PhrA,-E, -F, -K, -H	ComP/ComA Rap proteins	Competence, sporulation, biofilm formation, antibiotic production,	[7-10,32]
<i>Myxococcus xanthus</i>	A-signal C-signal	SasSRN	Fruiting body formation or sporulation	[7-10]
<i>Pseudomonas aeruginosa</i>	3O-C12-HSL C4-HSL	LasI/LasR RhlI/RhlR OscR (orphan)	Structured biofilm formation, virulence factors	[7-10, 28-30]
<i>Staphylococcus aureus</i>	AIP-I, AIP-II, AIP-III, AIP-IV	AgrC/AgrA	Biofilm formation, virulence factors	[7-9,31]
<i>Streptococcus mutans</i>	CSP (ComC) XIP (ComS)	ComD/ComE ComR	Bacteriocins, biofilm formation, competence	[33-36]
<i>Streptococcus pneumoniae</i>	CSPs	ComD/ComE	Competence, fratricide, biofilm formation, virulence	[8,32]
<i>Vibrio harveyi</i>	HAI-1, CAI-1 AI-2	LuxLM/LuxN LuxP/LuxQ	Bioluminescence emission, symbiosis	[7-9,11,26]

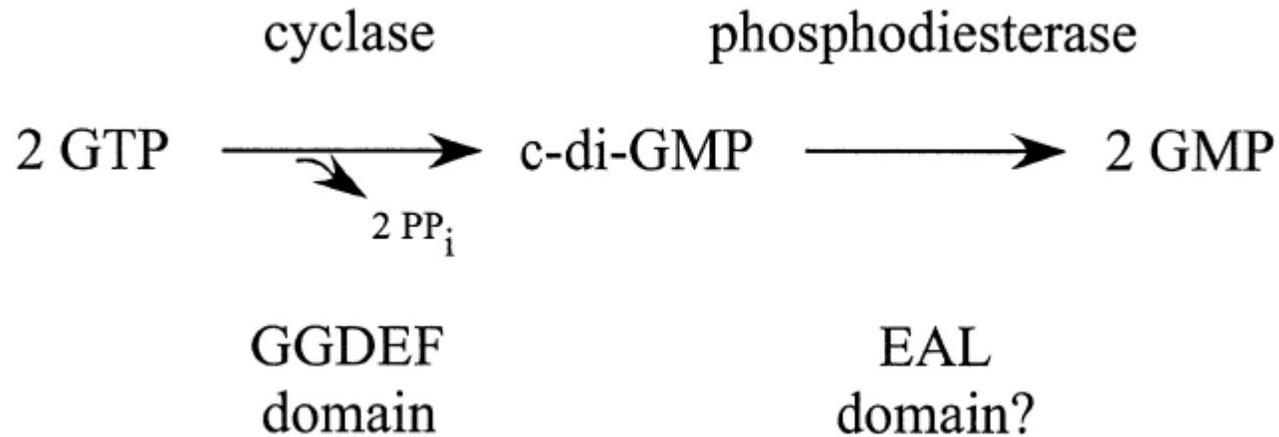
Segundo mensajero: di-GMP (dimeric guanosine monophosphate) cíclico



Caulobacter crescentus, 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).

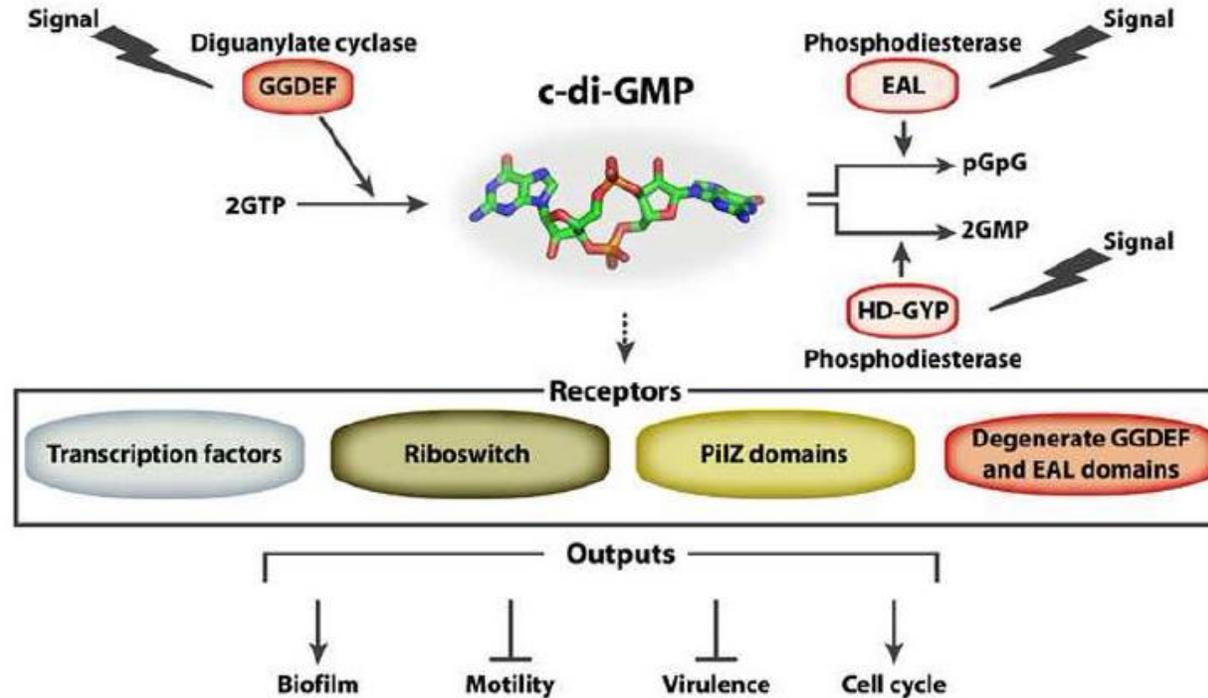
Regulación por c-di-GMP



- Comunicación intercelular
 - Síntesis fimbrial
 - Producción de EPS (matriz)
 - Biofilms
- Motilidad
 - Virulencia
 - Resistencia a metales pesados

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

Regulación por c-di-GMP



Sondermann et al., 2013

Pequeños RNA (sRNA)

La función de sRNAs (25 a 500 nucleótidos, no codificantes) en la regulación de la formación de biofilms se produce a través de dos mecanismos generales, (i) sRNAs actuando por hibridación con otros ARN y (ii) la unión a proteínas.

Ejemplos de bacterias de relevancia clínica que emplean el segundo mensajero c-di-GMP

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

Mecanismos de interferencia con el QS (*Quorum quenching*)

- Inhibidores con efecto en la vía de síntesis de los AI
- Degradación enzimática de los AI
- Análogos de AHL y compuestos con afinidad por receptores
- Análogos de AIP y compuestos con afinidad por receptores
- Acción a nivel de la cascada de transducción de señales

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 Tariku², Fisseha Gebreyesus³, Shibru Zerihun⁴, Ali Mohammed⁵, Nancy Weiland-Bräuer⁶, Ruth A. Schmitz⁶ 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	<i>Aframomum esculentum</i> mature semi-ripe fruit acetone extract	Oleo resin
2	ACFA2	<i>Aframomum esculentum</i> mature unripe fruit acetone extract	Oleo resin
3	ACFA3	<i>Aframomum esculentum</i> mature ripe fruit acetone extract	Oleo resin
4	ACFO	<i>Aframomum esculentum</i> mature unripe fruit oil	Essential oil
5	ACHO	<i>Aframomum esculentum</i> mature ripe fruit husk oil	Essential oil
6	ASRM	<i>Albizia schimperiana</i> root methanol extract	Crude extract
7	CLRA1	<i>Curatma longa</i> finger rhizome acetone extract	Oleo resin
8	CLRO1	<i>Curatma longa</i> finger rhizome oil	Essential oil
9	CLRO2	<i>Curatma longa</i> main rhizome oil	Essential oil
10	CLRA2	<i>Curatma longa</i> main rhizome acetone extract	Oleo resin
11	EBBP	<i>Erythrina brucei</i> stem bark petroleum ether extract	Crude extract
12	JSSP	<i>Justica schimperiana</i> seed petroleum ether extract	Crude extract
13	NSSO	<i>Nigella sativa</i> seed oil	Essential oil
14	NSSP	<i>Nigella sativa</i> seed petroleum ether extract	Crude extract
15	OSLC	<i>Odmum sauve</i> leaf chloroform extract	Crude extract
16	VALC	<i>Vernonia amygdalina</i> leaf chloroform extract	Crude extract
17	VALM	<i>Vernonia amygdalina</i> leaf methanol extract	Crude extract
18	VALP	<i>Vernonia amygdalina</i> leaf petroleum ether extract	Crude extract

Table 4 Quorum Quenching activities of selected medicinal plants of Ethiopia

Extracts	AHL-QQ activity in <i>E. coli</i> based reporter strain AH1-QQ.1	Antimicrobial activity against <i>E. coli</i> 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



Short communication

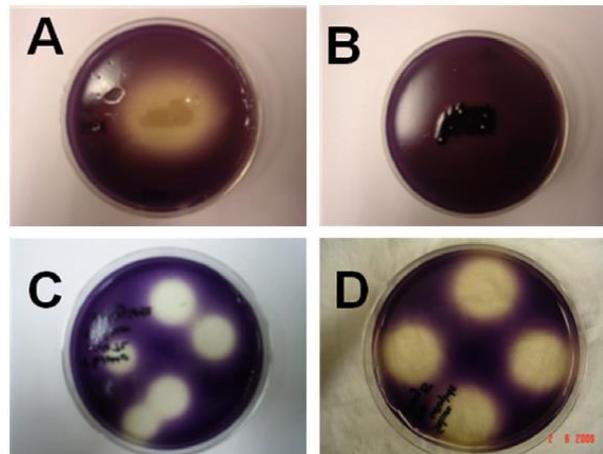
Use of quorum sensing antagonists to deter the formation of crystalline *Proteus mirabilis* biofilms

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

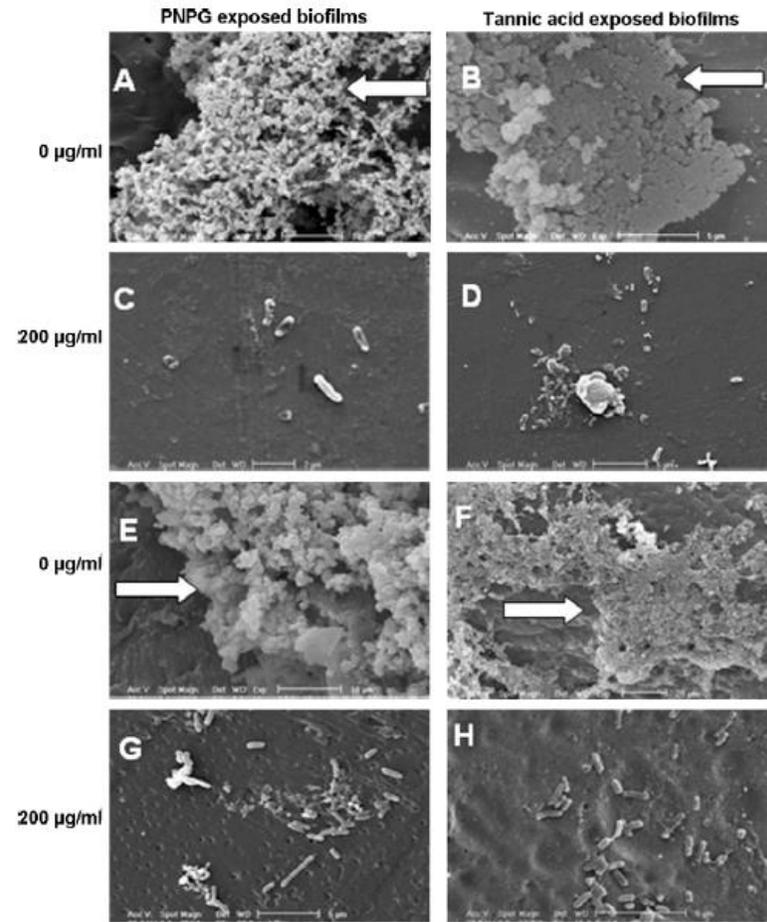
Evaluación del efecto antibiofilm de inhibidores del QS:

- p-nitrofenilglicerol
- taninos

Evaluación: efecto sobre la producción de pigmentos de *Chromobacterium violaceum*



Efecto preventivo y de disrupción del biofilm formado



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 PNPGE 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

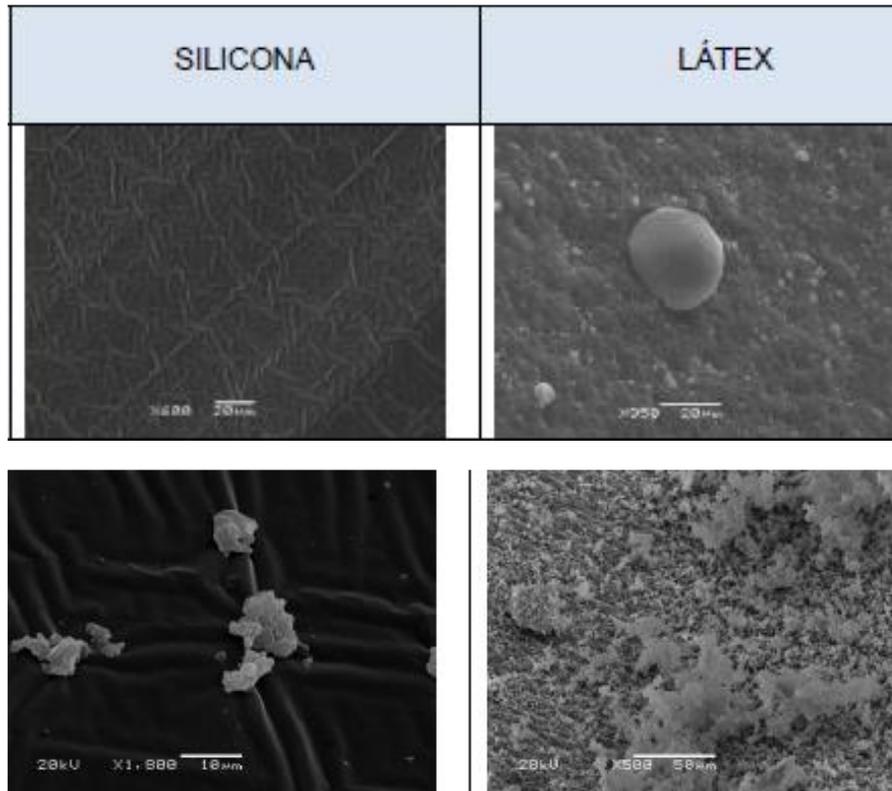
Cerámicas

Nanopatrones

Biosurfactantes (ej. lipopéptido producido por *B. subtilis* ATCC 19659)

Superficies - Materiales

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

Ej. 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.)

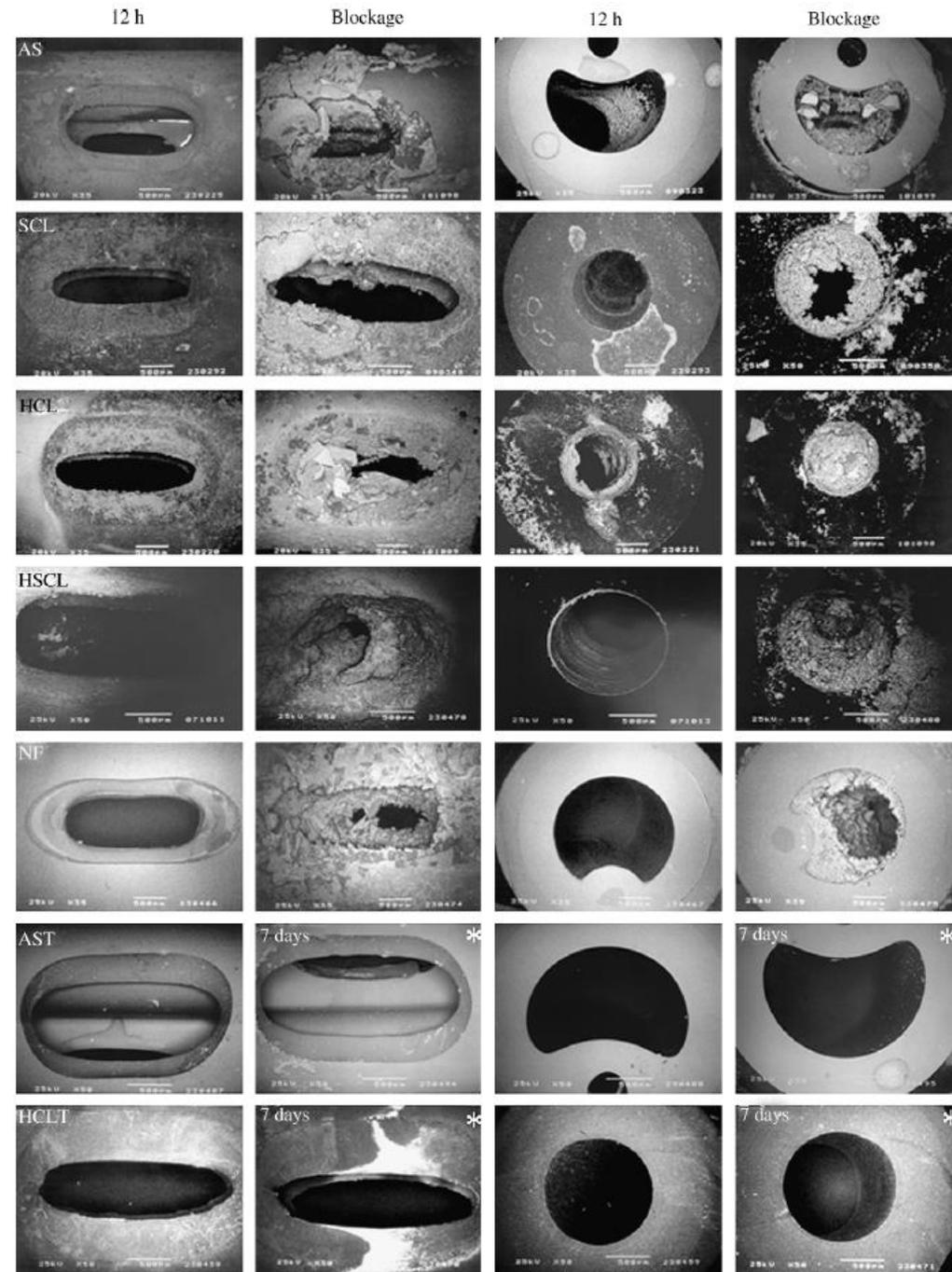
➤ **Coberturas**

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



Estrategias de prevención

- Antimicrobianos (Aslam, 2008)
- Inhibidores del *quorum sensing* (taninos, p-nitrophenyl glycerol - PNPG -, etc.; Jones *et al.*, 2009)
- Jugos y extractos vegetales (arándano, Martino *et al.*, 2005; *Ibicella lutea*, Sosa y Zunino, 2009; cinamaldehído, Amalaradjou *et al.*, 2010)
- EDTA (Percival *et al.*, 2009)
- Bacteriófagos líticos (Carson *et al.*, 2010)
- Interferencia con los mecanismos de captación de Fe (Hancock *et al.*, 2010)
- Uso de bacterias probióticas (ej. *Lactobacillus acidophilus* - Hawthorn & Reid, 1990)
- Impregnación con antisépticos (Hachem *et al.*, 2007)
- Muchos otros...

Nanopartículas

Research Article

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

Victoria Iribarnegaray¹, Nicolás Navarro^{2,3}, Luciana Robino⁴, Pablo Zunino¹, Javier Morales^{1,2,3} & Paola Scavone^{*,†,1}

¹Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, Av. Italia 3318, PC 11600, Montevideo, Uruguay

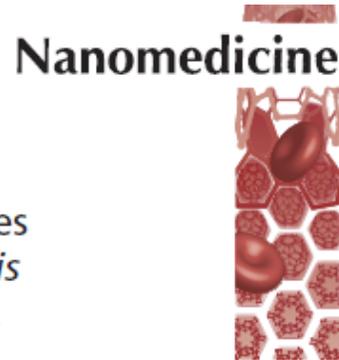
²Departamento de Ciencias y Tecnologías Farmacéuticas, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santos Dumont 964, Independencia, Santiago, Chile

³Advanced Center for Chronic Diseases, Santiago, Chile

⁴Departamento de Bacteriología y Virología, Facultad de Medicina, Universidad de la República, Alfredo Navarro 3051, PC 11600, Montevideo, Uruguay

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†Authors share senior authorship



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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 

Published Online: 31 May 2023 | <https://doi.org/10.2217/nm-2022-0239>

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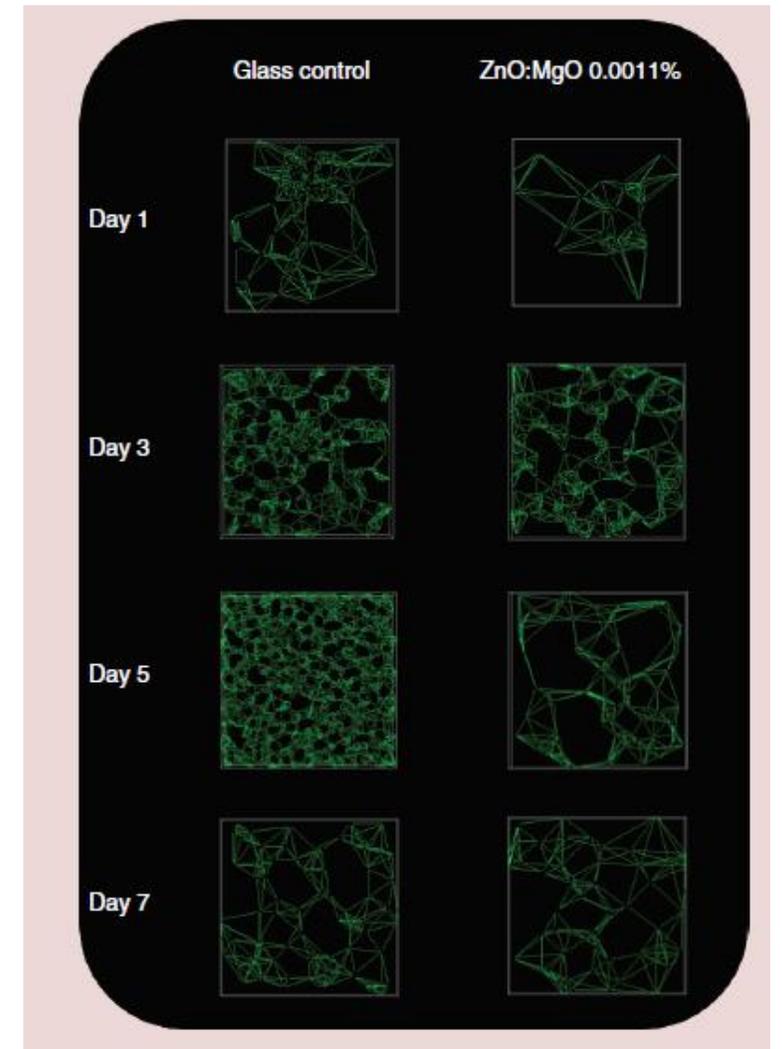
 RELATED

 DETAILS

Info Tab



Vol. 18, No. 10



Ejemplos particulares: catéteres vasculares

Table 1 Summary of the methods that have been attempted or proposed for preventing artificial surface fouling from infectious or thrombotic causes

Infection prevention methods

Antibiotic infusion

Minocycline (16-18)

Rifampin (16-18)

Catheter materials

Polytetrafluoroethylene (21)

Polyurethane (21)

Antiseptic coating

Chlorhexidine (19,20)

Sulfadiazine (19,20)

Other

Silver (11)

Heparin (16)

Thrombosis prevention methods

Hydrophilic polymers

Polyethylene glycol (49)

Zwitterionic materials

Phosphorylcholines (51,52)

Other coatings

Pyrolytic carbon (55-57)

Albumin (62)

Heparin (65)

Direct thrombin inhibitors (71-73)

Sirolimus (74)

SLIPs (77-81)

Micropatterning

Lotus leaf conical cells (85)

Shark skin microgrooves (86)

“Moléculas pequeñas”

Compuesto orgánico de bajo peso molecular (no más de 800 Da), lo que le posibilita la rápida difusión a través de la membrana celular

Acciones biológicas diversas

Table 1. Small molecules that can inhibit biofilm formation.

Agent	Mechanism	Effect	Reference
Anti-virulence compounds	Inhibition of gene expression of virulence factors	Inhibition of biofilm formation by <i>S. aureus</i>	[23]
Anti-biofilm compounds	Unknown	Inhibition of biofilm formation by <i>S. epidermidis</i>	[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 <i>S. aureus</i> and <i>S. epidermidis</i>	[29]
Cis-2-decenoic acid	Unknown	Dispersion of biofilms by <i>E. coli</i> , <i>K. pneumoniae</i> , <i>P. mirabilis</i> , <i>S. pyogenes</i> , <i>B. subtilis</i> , <i>S. aureus</i> , and <i>C. albicans</i>	[30]
D-amino acids	Unknown	Inhibition of biofilm formation by <i>S. aureus</i> and <i>P. aeruginosa</i>	[31]
<i>N</i> -acetylcysteine	Interference with exopolysaccharide formation in biofilms	Inhibition of biofilm formation by <i>S. epidermidis</i>	[32]
Chelators	Interference with metal ion's function in biofilm formation	Inhibition of biofilm formation by <i>S. aureus</i>	[33]

Ejemplos de moléculas pequeñas

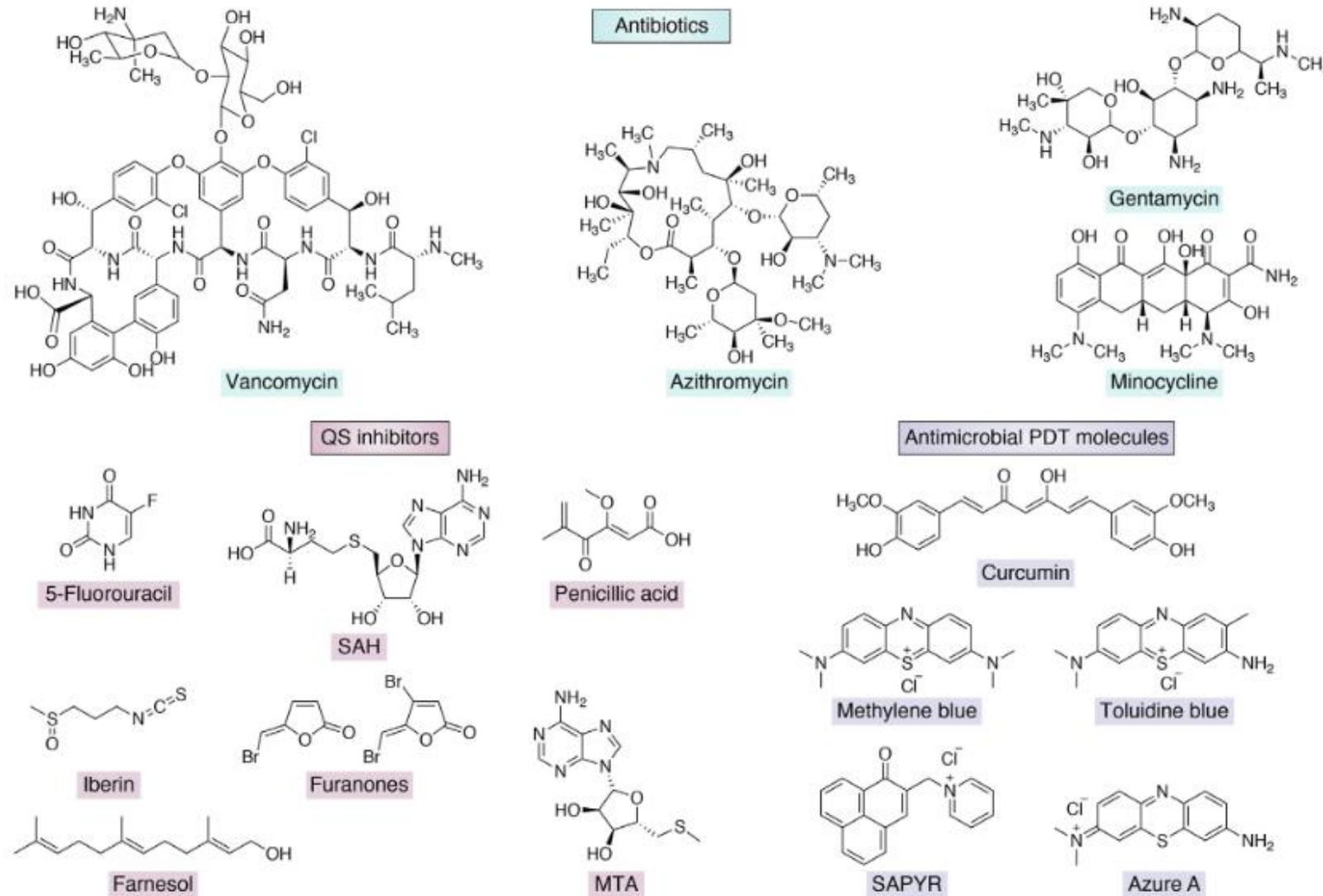
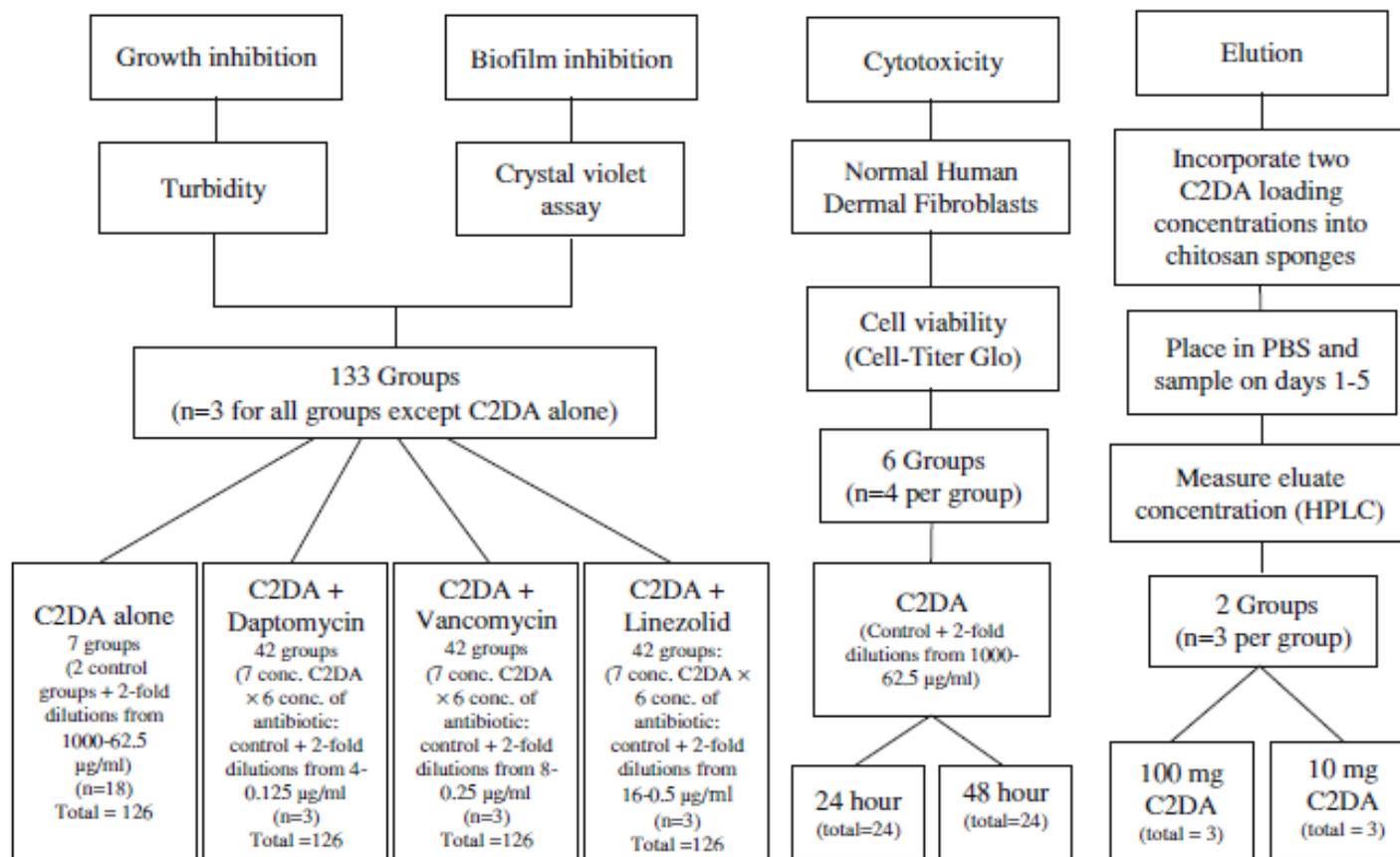


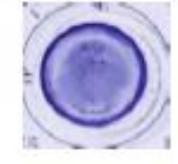
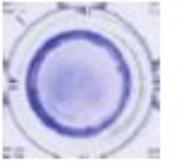
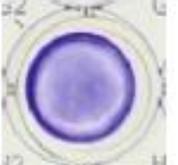
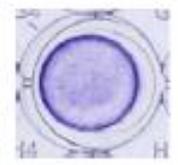
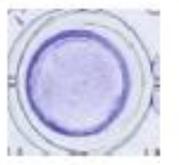
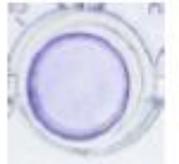
Figure 3. Examples of small molecules used for the treatments of biofilm-associated infections.

SYMPOSIUM: 2011 MUSCULOSKELETAL INFECTION SOCIETY

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

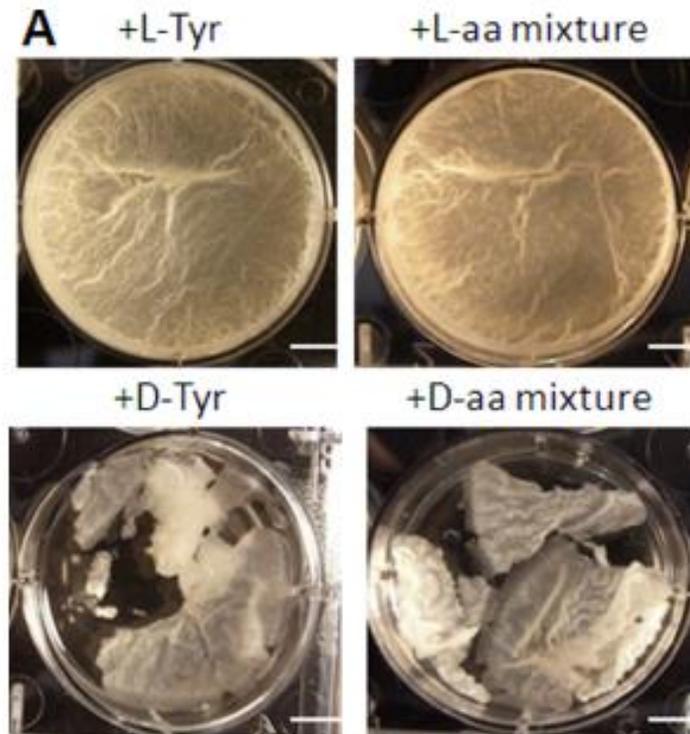
Jessica Amber Jennings PhD, Harry S. Courtney PhD,
Warren O. Haggard PhD



 Blank well (broth with no bacteria)	No antibiotic	1 µg/ml Daptomycin	1 µg/ml Vancomycin	0.5 µg/ml Linezolid
0 µg/ml C2DA				
125 µg/ml C2DA				
500 µg/ml C2DA				
1000 µg/ml C2DA				

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

Disrupción: D-aminoácidos



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

D-Amino Acids Trigger Biofilm Disassembly

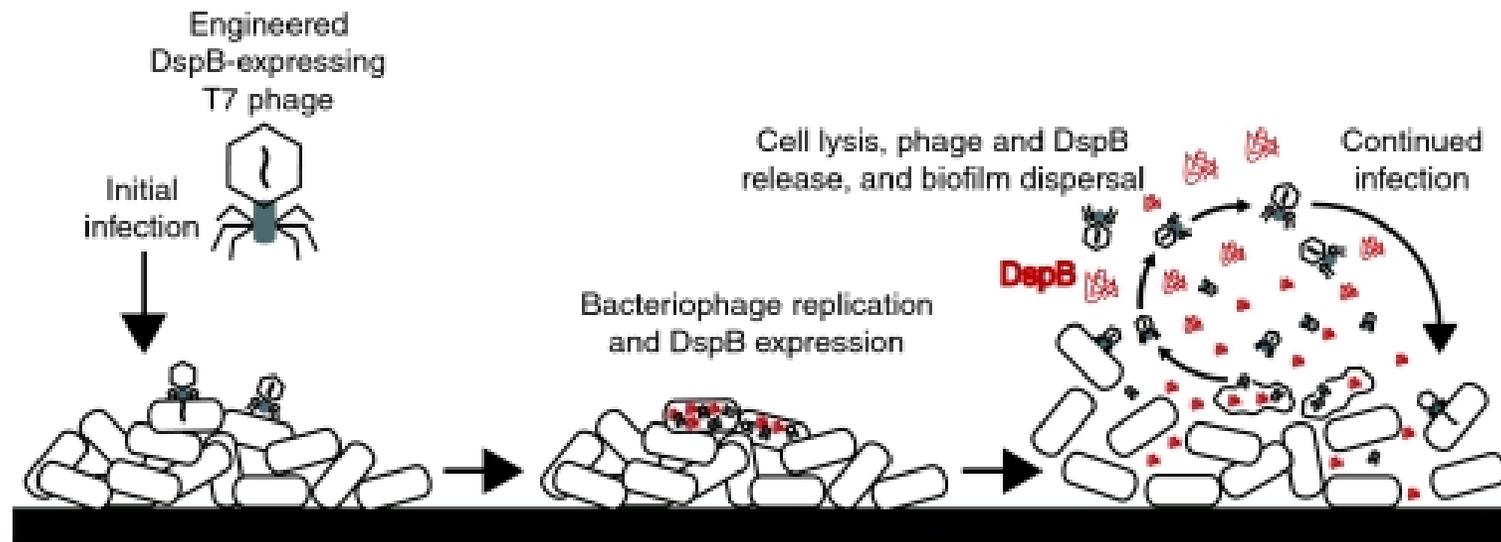
Illana Kolodkin-Gal¹, Diego Romero², Shugeng Cao³, Jon Clardy³, Roberto Kolter², and Richard Losick^{1,2}

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

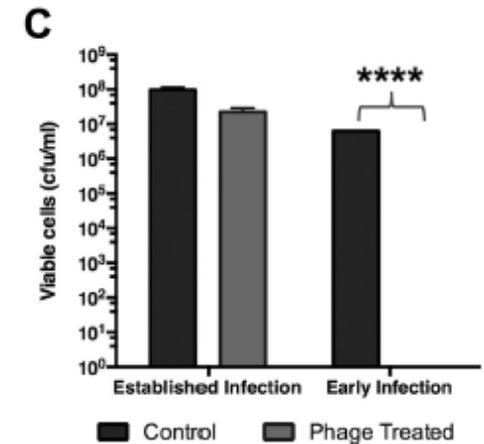
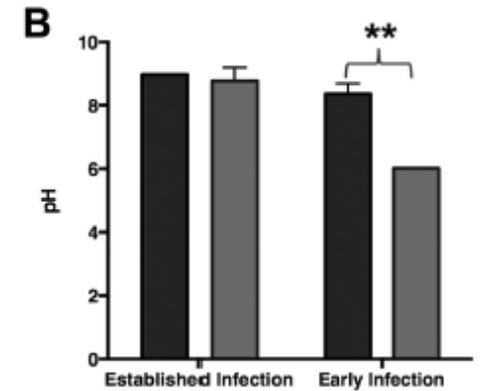
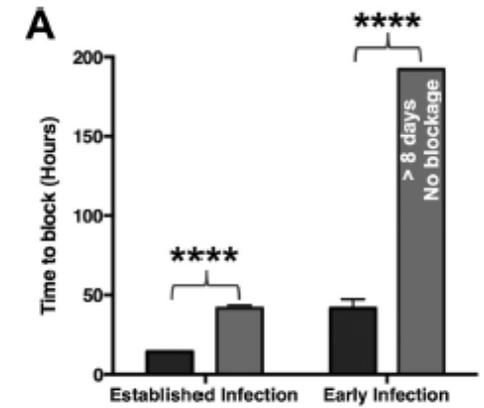
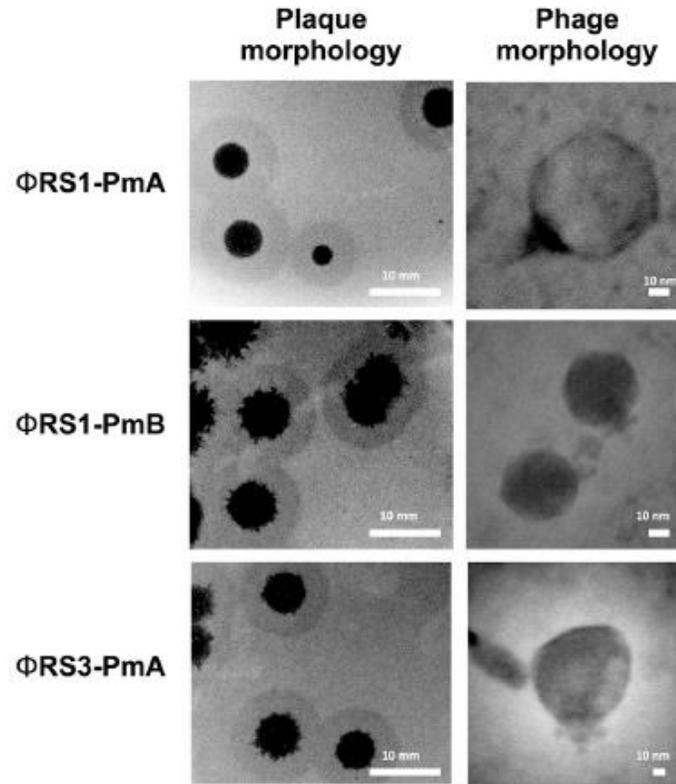
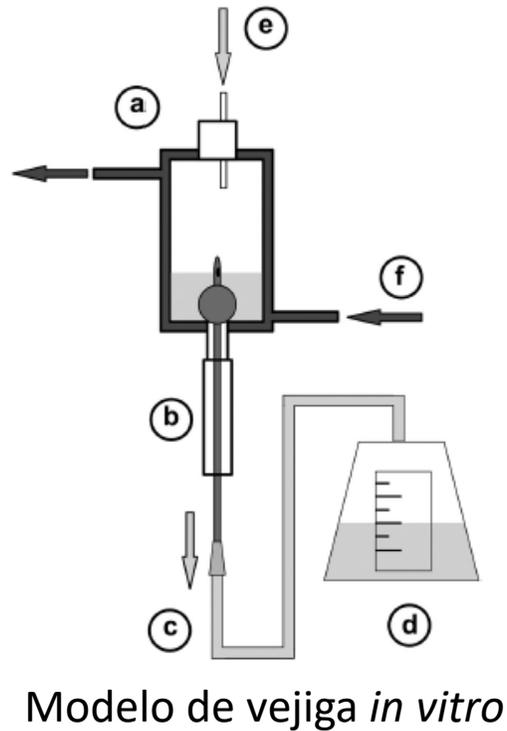
Terapia fágica

- Fagos salvajes
- Fagos modificados



Fagos y biofilms de *P. mirabilis*

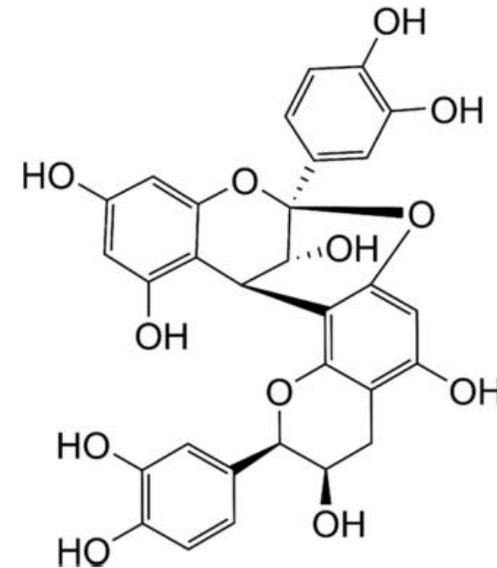
Nzakizwanayo *et al.*, 2016



Productos naturales

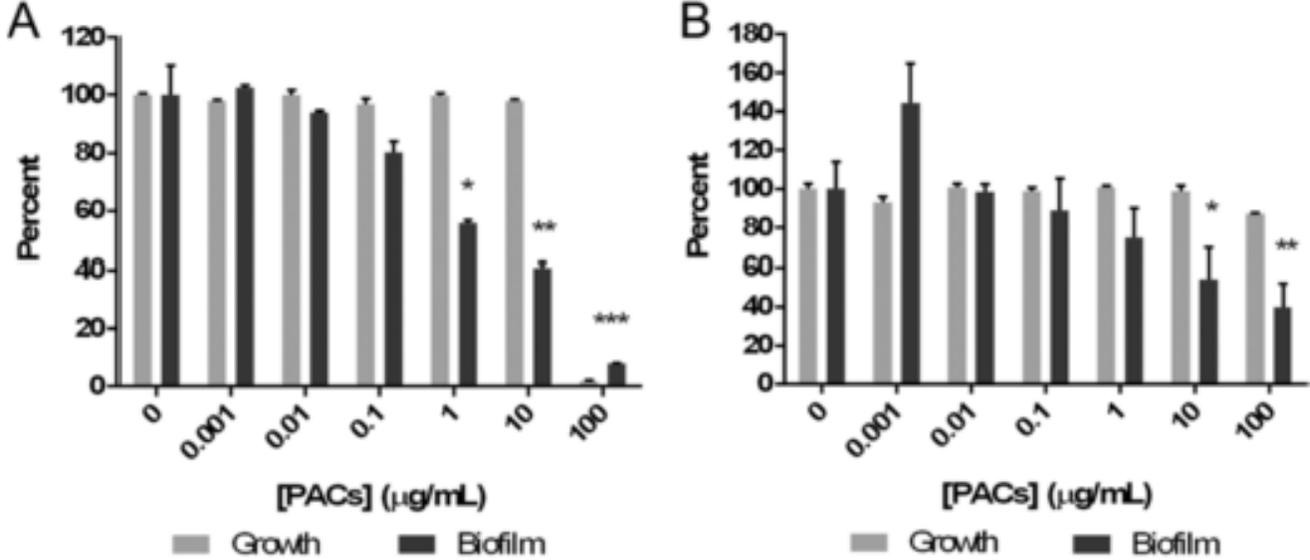
Arándanos

(*Vaccinium macrocarpon*)



Proantocianidina de arándano

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



Prevención

Disrupción

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

Vanessa Sosa and Pablo Zunino

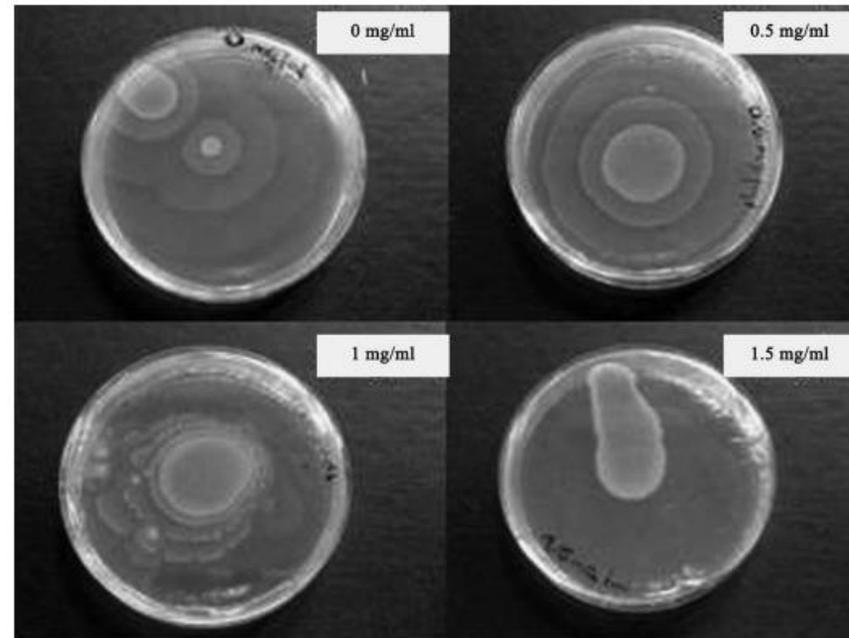
J Infect Dev Ctries 2009; 3(10):762-770.**Table 2.** Effect of *I. lutea* extract on biofilm formation by Pr2921 in polystyrene and glass and on auto-aggregation.

Concentration of <i>I. lutea</i>	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”)

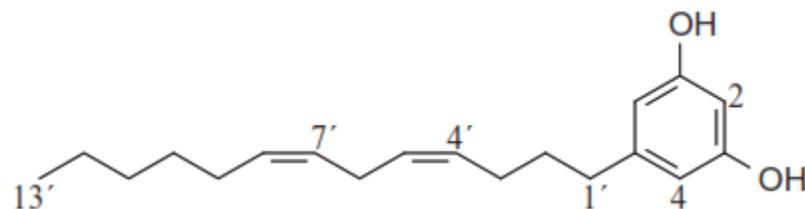


Table 1

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.

Prevención de biofilms en la industria de alimentos

Methodology	Examples	Mechanism of action	Reference
Chemical treatments	Sanitizers (NaOCl, peracetic acid, NaOH, H ₂ O 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; Møretro 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
Steel coatings	DNAses	Coughlan et al., 2016	
	Nanoparticles (Ag ²⁺ , Fe ₃ O 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	Stempel 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
High hydrostatic pressure	H ₂ O	Bactericidal (also endospores)	Evelyn and Silva, 2015; Santos et al., 2017
Non-thermal plasma	UV plus O ₂ , N ₂ , O ₃ , H ₂ O 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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