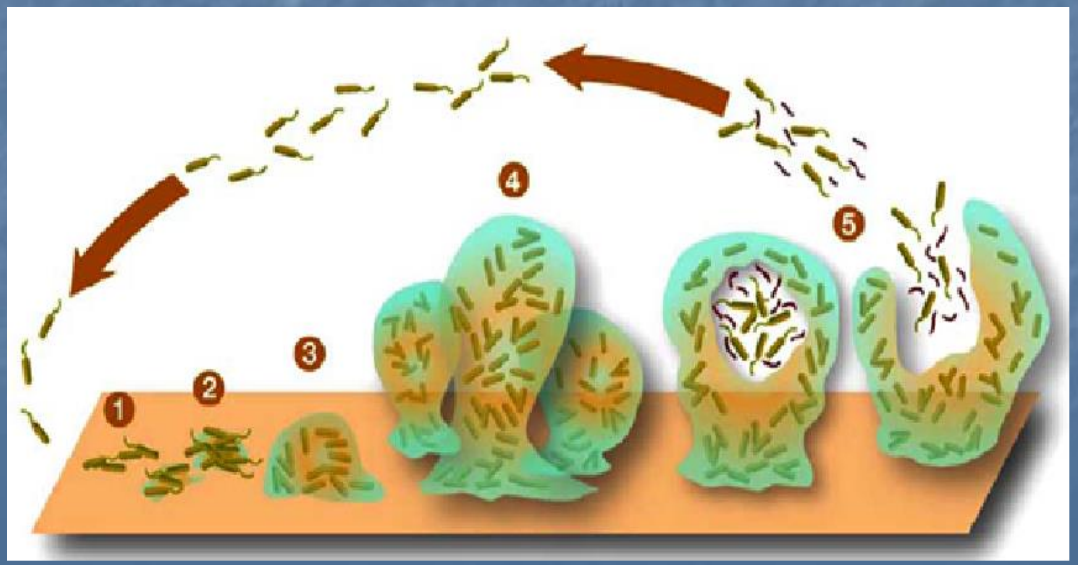
Relevancia de los biofilms microbianos y características genéricas

Pablo Zunino
Departamento de Microbiología, IIBCE

Biofilms bacterianos

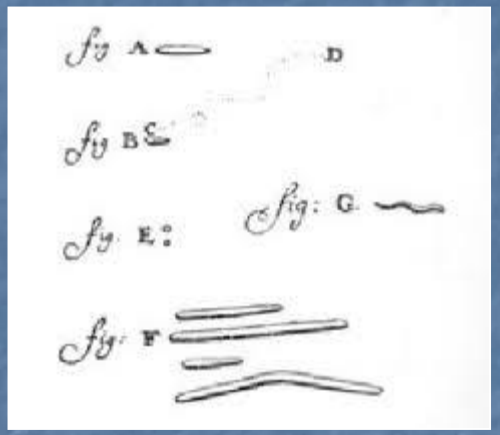
La forma de vida predominante de los microorganismos en cualquier sistema biológico hidratado es una comunidad cooperativa denominada biofilm (Trautner & Darouiche, 2004).

Comunidades formadas por microorganismos adheridos de manera irreversible a un sustrato o interfase, embebidos en una matriz de polímeros extracelulares de producción propia y que exhiben un fenotipo particular en relación a las tasas de crecimiento y expresión de genes (Donlan & Costerton, 2002).



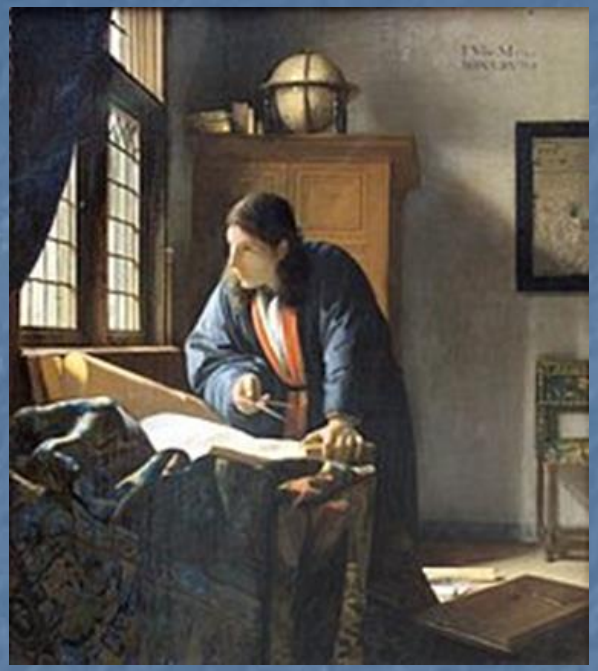
Ciclo de un biofilm

1632

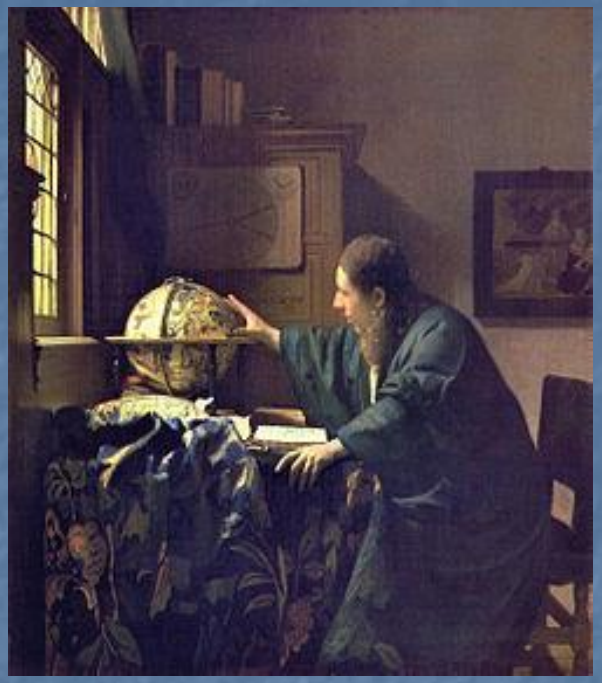


Antonie van Leeuwenhoek (1632-1723)

“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



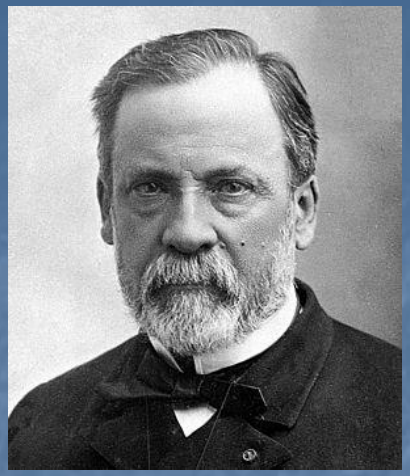
El astrónomo

Johannes Vermeer, baut. 31 de octubre de 1632

Anton van Leeuwenhoek, n. 24 de octubre de 1632

Christiaan Huygens, n. 1629

Roberto Koch (1843 - 1910)



Louis Pasteur (1822 - 1895)

Fanny Hesse (1850 - 1934)

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EDITORIAL

Classic Spotlight: Before They Were Biofilms

George A. O'Toole

Department of Microbiology and Immunology, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire, USA

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.

Address correspondence to georgeo@Dartmouth.edu.

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

W. F. McCoy
Department of Biology, The University of Calgary, Calgary, Alta., Canada T2N 1N4
J. D. Bryers[1]
Department of Chemical and Petroleum Engineering, The University of Calgary, Calgary, Alta., Canada T2N 1N4
J. Robbins
Department of Biology, The University of Calgary, Calgary, Alta., Canada T2N 1N4
AND
J. W. Costerton
Department of Biology, The University of Calgary, Calgary, Alta., Canada T2N 1N4

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

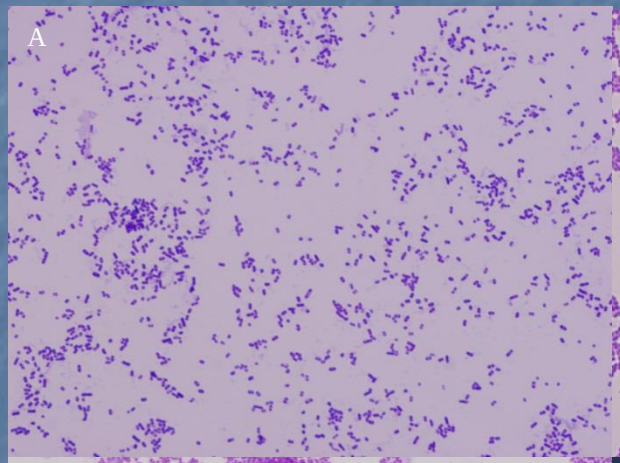
by J. W. Costerton, G. G. Geesey and K.-J. Cheng

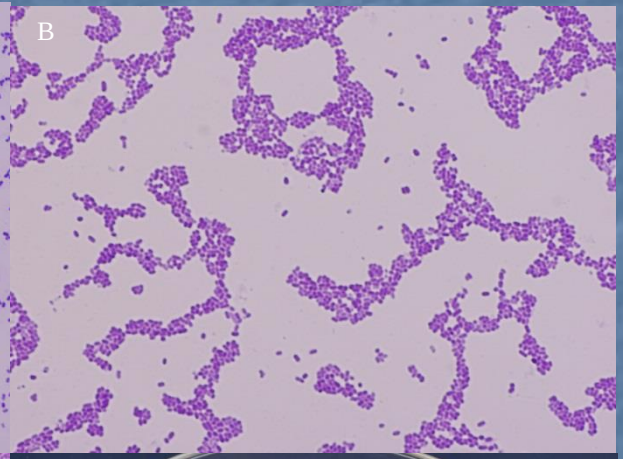
SCIENTIFIC AMERICAN
JANUARY 1978
VOL. 238, NO. 1 PP. 86–95

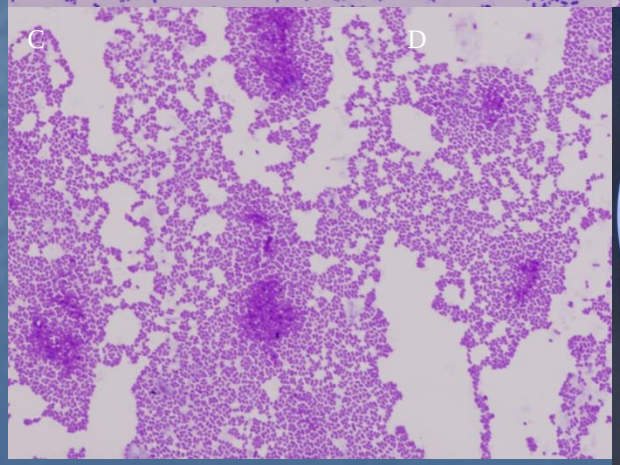
Biofilms

Adhesión entre bacterias? Adhesión a superficies abióticas? Son factores de virulencia?
Las colonias bacterianas son biofilms?

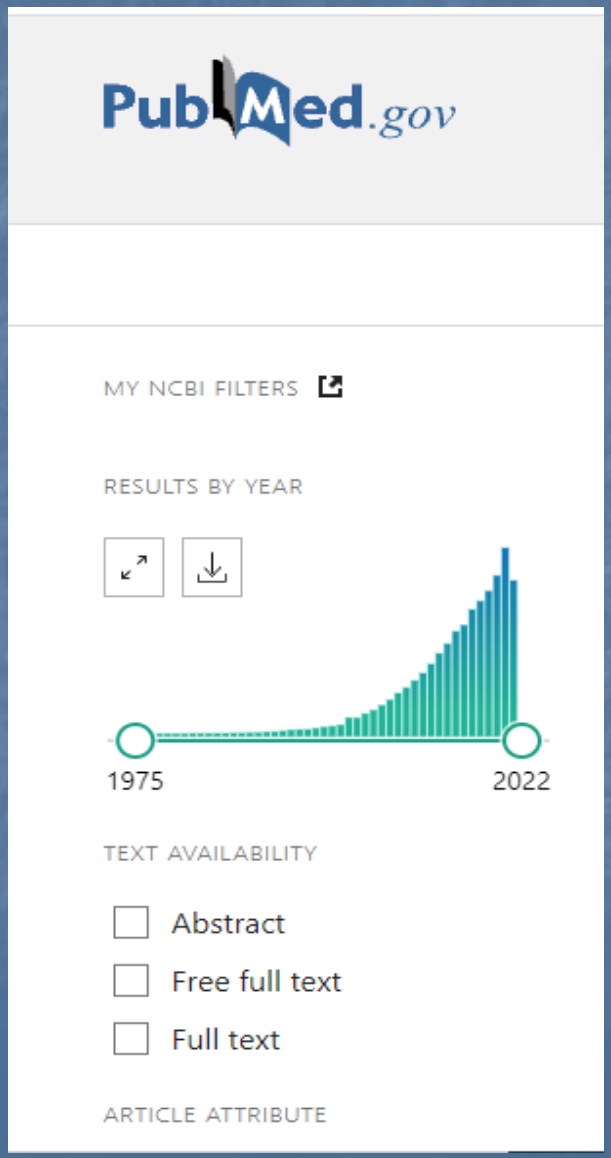
J. William Costerton
(1934 – 2012)







Artículos científicos sobre biofilms (Pubmed - NCBI)



2020: 7.572 artículos

- 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 ferrujinosas (Dodd et al., 2017, Nature)

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

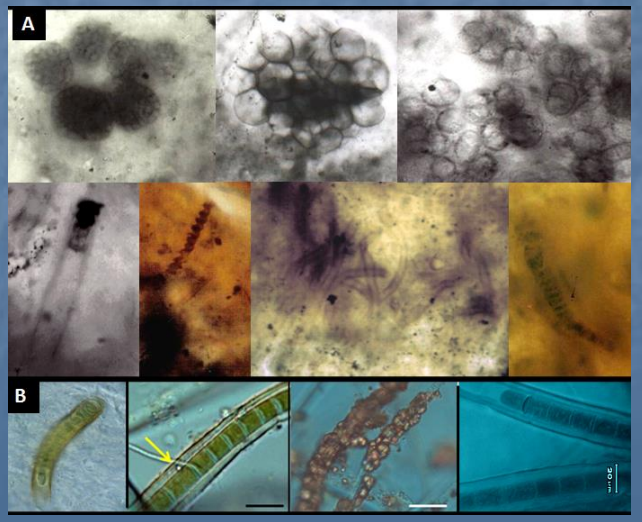
Las bacterias constituyen las primeras formas de vida en la Tierra, teniendo un rol crítico en la generación de condiciones para formas de vida posteriores, fundamentalmente a través del desarrollo de la fotosíntesis y generación de oxígeno en la atmósfera (vida humana aprox. 2,5 Ma)

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)



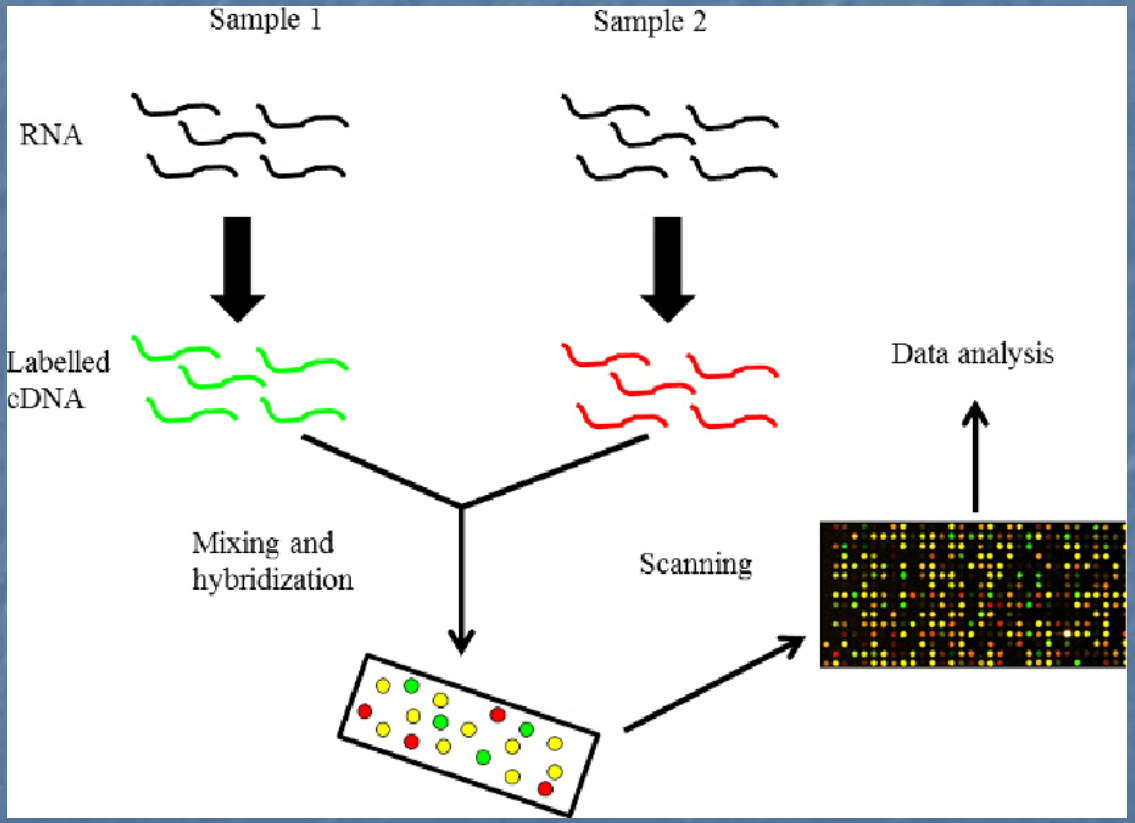
Lester Park, Saratoga Springs, New York (cámbrico)

Fenotipo de las bacterias que forman biofilms, diferente a sus contrapartes planctónicos

- Producción de polímeros extracelulares
- Tasas de crecimiento reducidas
- Mayor adaptación al estrés ambiental
- Resistencia elevada a agentes antimicrobianos (ej. antibióticos, desinfectantes)
- Regulación de genes específicos

Estudios basados en *microarrays* sugieren que las bacterias en biofilms tienen al menos un 10% de expresión génica diferencial con respecto a las formas planctónicas (*P. aeruginosa, E. coli, S. aureus*, etc.)

Análisis global de expresión génica en biofilms: *microarrays*



Afzal et al, 2015

Expresión génica de *E. coli* en biofilms (*microarrays*)

		Number of genes[a]			
		Biofilm versus exponential		Biofilm versus stationary	
Functional group	Total	Higher in biofilm	Lower in biofilm	Higher in biofilm	Lower in biofilm
Whole genome	4290	206	27	389	192
Amino acid biosynthesis	97	0	0	45	0
Biosynthesis of cofactors, prosthetic groups and carriers	106	0	0	20	1
Carbon compound catabolism	124	7	0	5	6
Cell processes	170	12	2	15	11
Cell structure	85	5	1	9	0
Central intermediary metabolism	149	5	5	32	4
DNA replication, repair, restriction/modification	105	0	0	6	2
Energy metabolism	136	24	1	28	4
Fatty acid and phospholipids metabolism	41	1	0	6	1
Hypothetical, unclassified, unknown	1428	74	2	46	94
Nucleotide biosynthesis and metabolism	66	2	1	22	3
Phage, transposon or plasmid	91	2	0	2	2
Putative cell structure	438	1	0	0	2
Putative enzymes	453	25	2	28	21
Putative factors	67	3	0	1	4
Putative membrane proteins	54	1	0	3	3
Putative regulatory proteins	167	8	0	3	4
Putative transport proteins	291	18	3	14	11
Regulatory function	208	5	4	13	7
Transcription, RNA processing and degradation	28	0	0	3	0
Translation and post-translational modification	128	1	2	53	2
Transport and binding proteins	254	12	4	35	10

a. Number of genes showing significant ($P < 0.05$, *t*-test; $P < 0.03$, Mann–Whitney test) expression fold ratios ($\geq \pm 2.5$).

Schembri, Kjærgaard and Klemm; 2003

Expresión génica de *S. aureus* en biofilms

TABLE 3. Genes differentially expressed in a biofilm versus exponential growth phase

N315 ORF[a]	Common name[a]	Product[a,b]	ER[c]
Cell envelope and cellular processes			
N315-SA1960	*mtlF*	PTS system, mannitol-specific IIBC component	5.46
N315-SA1882	*kdpD*	Sensor protein KdpD	5.13
N315-SA2311		Similar to NAD(P)H-flavin oxidoreductase	2.71
N315-SA1156		ABC transporter (ATP-binding protein) homolog	2.68
N315-SA0724		Similar to cell division inhibitor	0.496
N315-SA2253	*opp-1C*	Oligopeptide transporter putative membrane permease domain	0.490
N315-SA0567		Similar to iron(III) ABC transporter permease protein	0.474
N315-SA2216		Similar to ABC transporter, ATP-binding protein	0.471
N315-SA0980		Similar to ferrichrome ABC transporter	0.471
N315-SA0981		Similar to ferrichrome ABC transporter	0.468
N315-SA0592	*tagA*	Teichoic acid biosynthesis protein	0.462
N315-SA1935	*hmrA*	Similar to amidase	0.442
N315-SA1169		γ-Aminobutyrate permease	0.425
N315-SA0243	*tagB*	Similar to teichoic acid biosynthesis protein B	0.423
N315-SA0110	*sirB*	Lipoprotein	0.399
N315-SA2100		Similar to autolysin E	0.394
N315-SA1458	*lytH*	*N*-Acetylmuramoyl-L-alanine amidase	0.393
N315-SA0109	*sirC*	Lipoprotein	0.378
N315-SA0106	*lctP*	L-Lactate permease homolog	0.376
N315-SA0682		Similar to ditripeptide ABC transporter	0.370
N315-SA2053		Glucose uptake protein homolog	0.331
N315-SA0479	*nupC*	Pyrimidine nucleoside transport protein	0.315
N315-SA0111	*sirA*	Lipoprotein	0.312
N315-SA2339		Similar to antibiotic transport-associated protein	0.291
N315-SA0566		Similar to iron-binding protein	0.287
N315-SA2233		Similar to integral membrane efflux protein	0.281
N315-SA0325	*glpT*	Glycerol-3-phosphate transporter	0.281
N315-SA2112		Similar to sodium-dependent transporter	0.278
N315-SA1025	*mraY*	Phospho-*N*-muramic acid-pentapeptide translocase	0.265
N315-SA0600		Similar to pyrimidine nucleoside transporter	0.255
N315-SA1978		Similar to ferrichrome ABC transporter (permease)	0.203
N315-SA0010	*azlC*	Similar to amino acid permease	0.161
N315-SA2300		Similar to glucarate transporter	0.141
N315-SA0691	*sstD*	Lipoprotein, similar to ferrichrome ABC transporter	0.126
N315-SA0374	*pbuX*	Xanthine permease	0.089
N315-SA0579		Similar to Na^+/H^+ antiporter	0.080
N315-SA0411	*ndhF*	NADH dehydrogenase subunit 5	0.065
N315-SA2302	*sipC*	Similar to ABC transporter	0.046
N315-SA2303	*smpC*	Similar to membrane-spanning protein	0.041
Information pathways			
N315-SA1883	*kdpE*	KDP operon transcriptional regulatory protein	5.42
N315-SA2429	*ArgR*	Similar to arginine repressor	3.92
N315-SA2296		Similar to transcriptional regulator, MerR family	3.72
N315-SA2418		Similar to two-component response regulator	2.13
N315-SA0460	*pth*	Peptidyl-tRNA hydrolase	0.490
N315-SA0652		Similar to transcription regulation protein	0.452
N315-SA1853		Similar to DNA mismatch repair protein MutS	0.445
N315-SA1287	*asnS*	Asparaginyl-tRNA synthetase	0.441
N315-SA0348		Similar to transcription terminator	0.440
N315-SA2358		Similar to transcriptional regulator (TetR/AcrR family)	0.382
N315-SA1697		Similar to protein-tyrosine phosphatase	0.364
N315-SA1120		Similar to transcription regulator GntR family	0.354
N315-SA0298	*pfoR*	Similar to regulatory protein PfoR	0.333
N315-SA1550	*tyrS*	Tyrosyl-tRNA synthetase	0.325
N315-SA2482	*pcp*	Pyrrolidone-carboxylate peptidase	0.297
N315-SA1583	*rot*	Repressor of toxins (Rot)	0.295
N315-SA0653	*fruR*	Similar to transcription repressor of fructose operon	0.229
N315-SA0904		Probable ATL autolysin transcription regulator	0.191
N315-SA1725	*sspB*	Staphopain, cysteine proteinase	0.074
Intermediary metabolism			
N315-SA0328		Similar to NADH-dependent FMN reductase	7.25
N315-SA0122	*butA*	Acetoin (diacetyl)reductase	5.04
N315-SA2297		Similar to GTP-pyrophosphokinase	3.25
N315-SA1142	*glpD*	Aerobic glycerol-3-phosphate dehydrogenase	2.97
N315-SA0016	*purA*	Adenylosuccinate synthase	2.37
N315-SA2397		Similar to pyridoxal-phosphate-dependent aminotransferase	2.04
N315-SA2001		Similar to oxidoreductase, aldo/keto reductase family	2.01
N315-SA1201	*trpD*	Anthranilate phosphoribosyltransferase	0.495
N315-SA1685	*mutY*	Similar to A/G-specific adenine glycosylase	0.481

Continued on following page

Beenken et al., 2004

Expresión génica de *A. baumaniii* en biofilms: análisis de transcriptomas

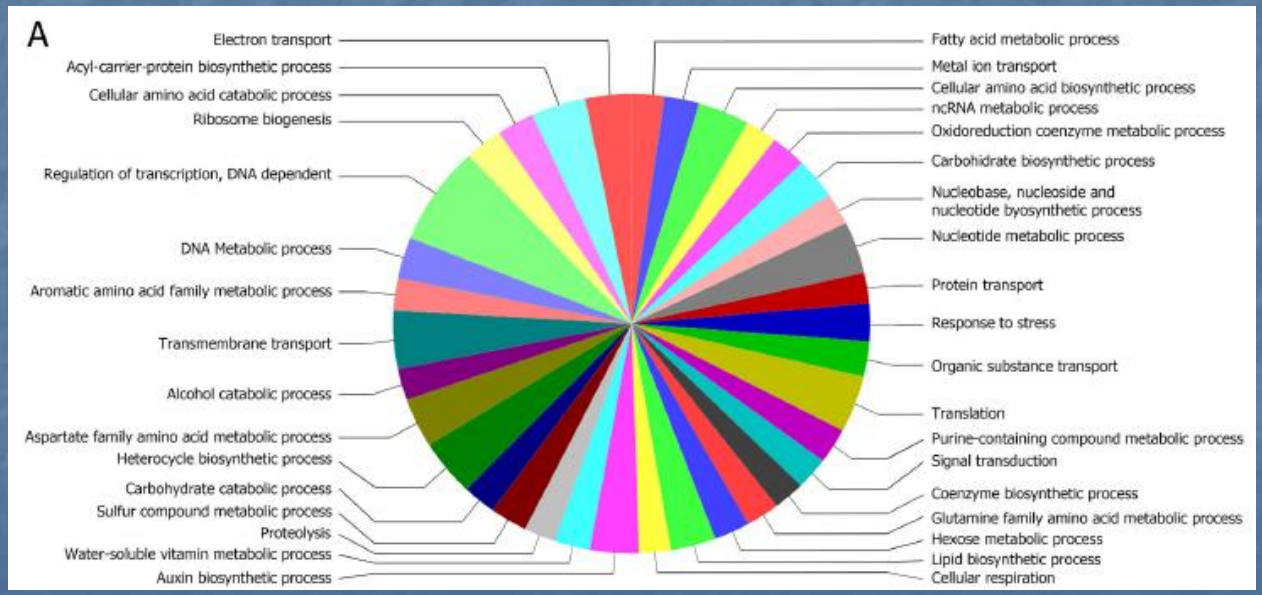
OPEN ACCESS Freely available online

PLOS ONE

Whole Transcriptome Analysis of *Acinetobacter baumannii* Assessed by RNA-Sequencing Reveals Different mRNA Expression Profiles in Biofilm Compared to Planktonic Cells

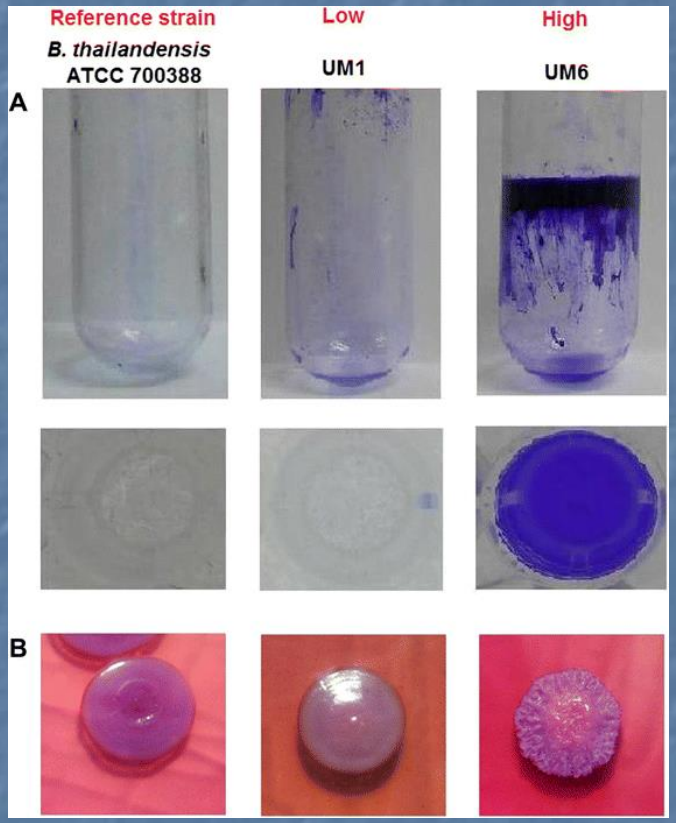
Soraya Rumbo-Feal[1☯], Manuel J. Gómez[2☯], Carmen Gayoso[1☯], Laura Álvarez-Fraga[1], María P. Cabral[1], Ana M. Aransay[3], Naiara Rodríguez-Ezpeleta[3,4], Ane Fullaondo[3], Jaione Valle[5], María Tomás[1], Germán Bou[1*], Margarita Poza[1*]

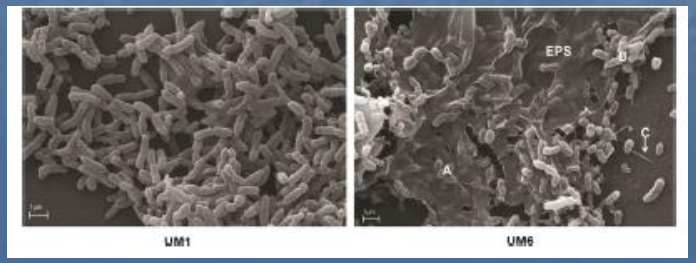
1 Department of Microbiology, Biomedical Research Institute, University Hospital, A Coruña, Spain, **2** Department of Molecular Evolution, Center for Astrobiology, INTA-CSIC, Madrid, Spain, **3** Genome Analysis Platform, CIC bioGUNE & CIBERehd, Derio, Spain, **4** Marine Research Division, AZTI, Tecnalia, Sukarrieta, Spain, **5** Department of Microbial biofilms, Agrobiotechnology Institute, Navarra, Spain

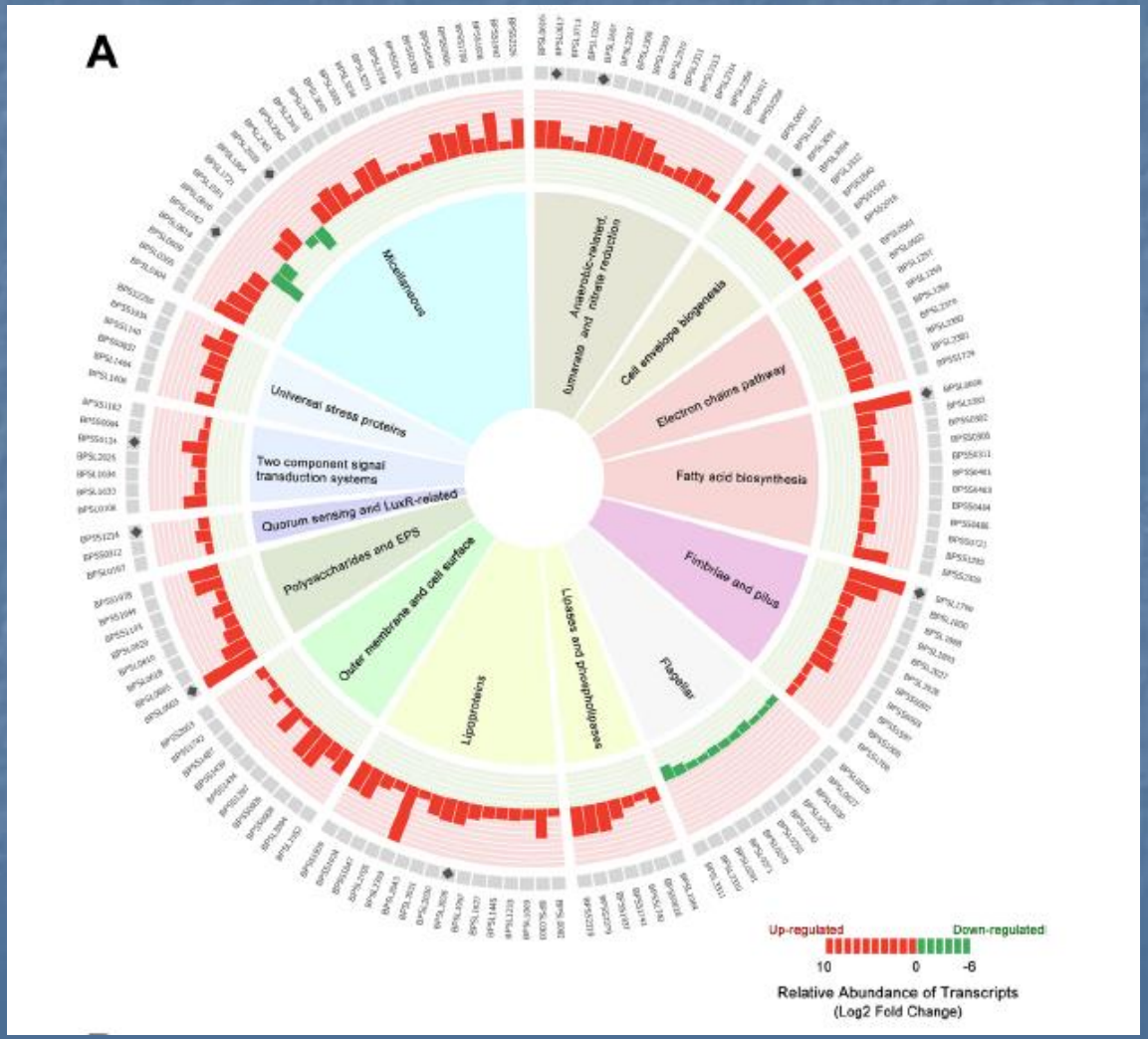


Distribución de secuencias de los 1621 genes identificados "up-regulated"en bacterias formando biofilms o en fase estacionaria. Genes involucrados en: A) procesos biológicos, B) componentes cleulares, C) funciones moleculares

Transcriptoma (RNA-Seq) de *Burkholderia pseudomallei* (Chin et al., 2015)







Expresión génica en biofilms bacterianos en distintas condiciones

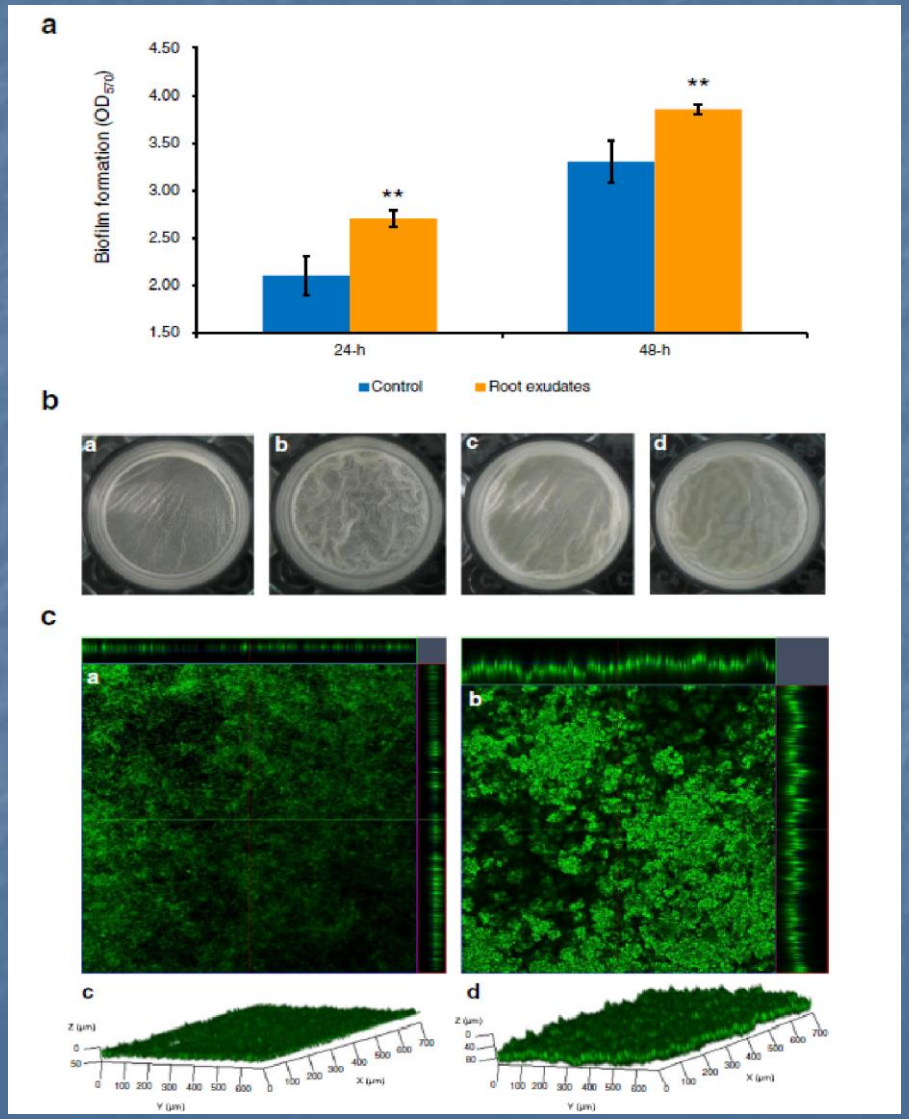
Zhang et al. BMC Genomics (2015) 16:685
DOI 10.1186/s12864-015-1825-5

BMC Genomics

RESEARCH ARTICLE Open Access

Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates

Nan Zhang[1†], Dongqing Yang[1†], Dandan Wang[1], Youzhi Miao[1], Jiahui Shao[1], Xuan Zhou[1], Zhihui Xu[1], Qing Li[1], Haichao Feng[1], Shuqing Li[1], Qirong Shen[1,3*] and Ruifu Zhang[1,2,3*]



Effects of concentrated maize root exudates on biofilm formation of SQR9.
a) Effects of maize root exudates on the biomass of biofilm formed by SQR9. b) Effects of root exudates on the appearance of biofilm formed by SQR9: a, Control, 24 h post-inoculation; b, Treatment with maize exudates, 24 h post-inoculation; c, Control, 48 h post-inoculation; d, Treatment with maize exudates, 48 h post-inoculation). c) Effects of root exudates on the three-dimensional structure of a biofilm formed by SQR9 visualized by confocal laser scanning microscopy (CLSM) 24 h post-inoculation. a, c. Control; b, d. Treatment with maize root exudates

Diversos genes varían su expresión en biofilms bajo diferentes condiciones

Table 4 Functional categories of SQR9 genes that were significantly regulated by the maize root exudates

Functional class	24-h	48-h
1 Cell envelope and cellular processes		
1.1 Cell wall	14	39
1.2 Transport/binding proteins and lipoproteins	80	75
1.3 Sensors (signal transduction)	4	14
1.4 Membrane bioenergetics (electron transport chain and ATP synthase)	13	23
1.5 Mobility and chemotaxis	18	49
1.6 Protein secretion	1	9
1.7 Cell division	2	19
1.8 Sporulation	47	44
1.9 Germination	6	2
1.10 Transformation/competence		1
2 Intermediary metabolism		
2.1 Metabolism of carbohydrates and related molecules		
2.1.1 Specific pathway	52	41
2.1.2 Main glycolytic pathways	2	7
2.1.3 TCA cycle	1	8
2.2 Metabolism of amino acids and related molecules	43	41
2.3 Metabolism of nucleotides and nucleic acids	17	30
2.4 Metabolism of lipids	20	28
2.5 Metabolism of coenzymes and prosthetic groups	24	51
2.6 Metabolism of phosphate	2	1
2.7 Metabolism of sulfur	2	2

Table 3 Numbers of significantly differentially expressed genes in the presence and absence of root exudates

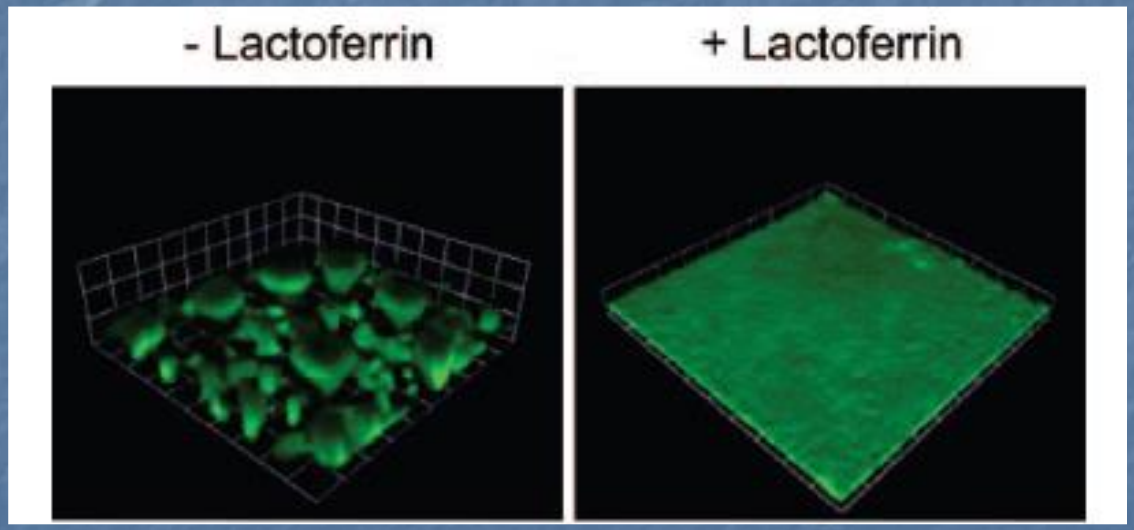
Items	Up-regulated	Down-regulated
RE/Control_24h	382 (9.4 %)	261 (6.4 %)
RE/Control_48h	260 (6.4 %)	764 (18.7 %)

The percentages in parentheses represent the ratios of differentially expressed gene numbers to those of the whole genome (4,078 coding sequences)

3 Information pathways		
3.1 DNA replication	1	12
3.2 DNA restriction/modification and repair	2	19
3.3 DNA recombination		8
3.4 DNA packaging and segregation		4
3.5 RNA synthesis	25	66
3.6 RNA modification		18
3.7 Protein synthesis	2	51
3.8 Protein modification	2	15
3.9 Protein folding	2	1
4 Other functions		
4.1 Adaptation to atypical conditions	18	25
4.2 Detoxification	18	25
4.3 Antibiotic production	6	10
4.4 Phage-related functions	15	15
4.6 Miscellaneous	4	5
Total (with known function)	443	758

Condiciones de desarrollo ejercen fuerte influencia en formación y estructura tridimensional del biofilm: composición del medio, tipo de superficie, osmolaridad, pH, temperatura, disposición de hierro, flujo del medio, etc.

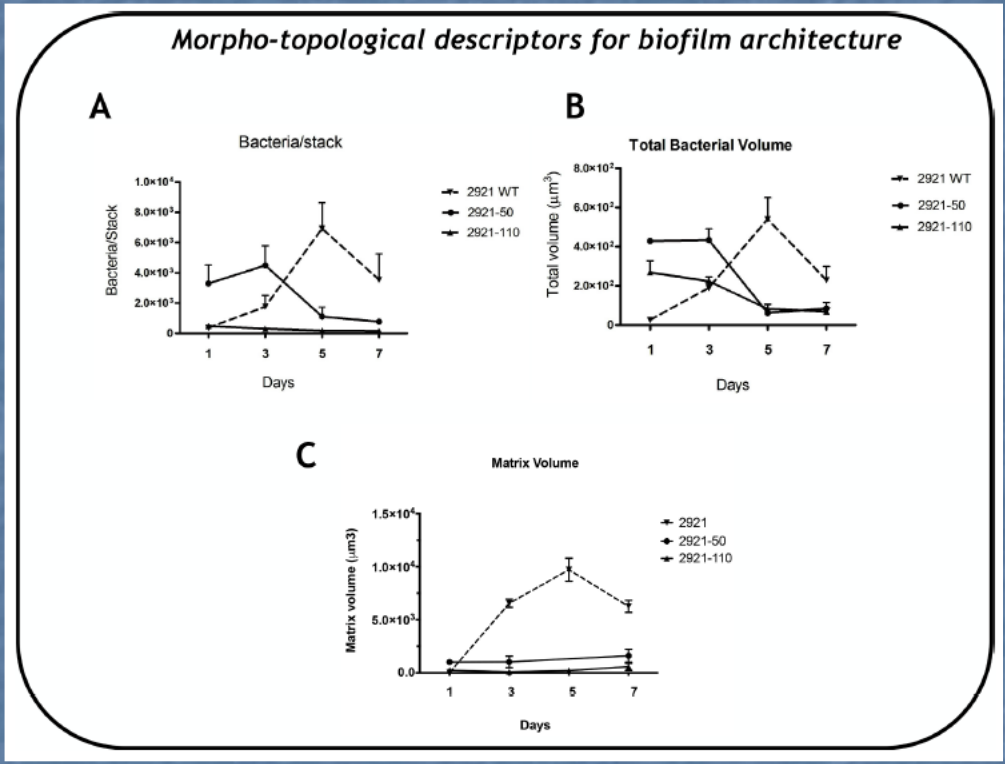
Biofilms de *P. aeruginosa* bajo distintas condiciones ambientales

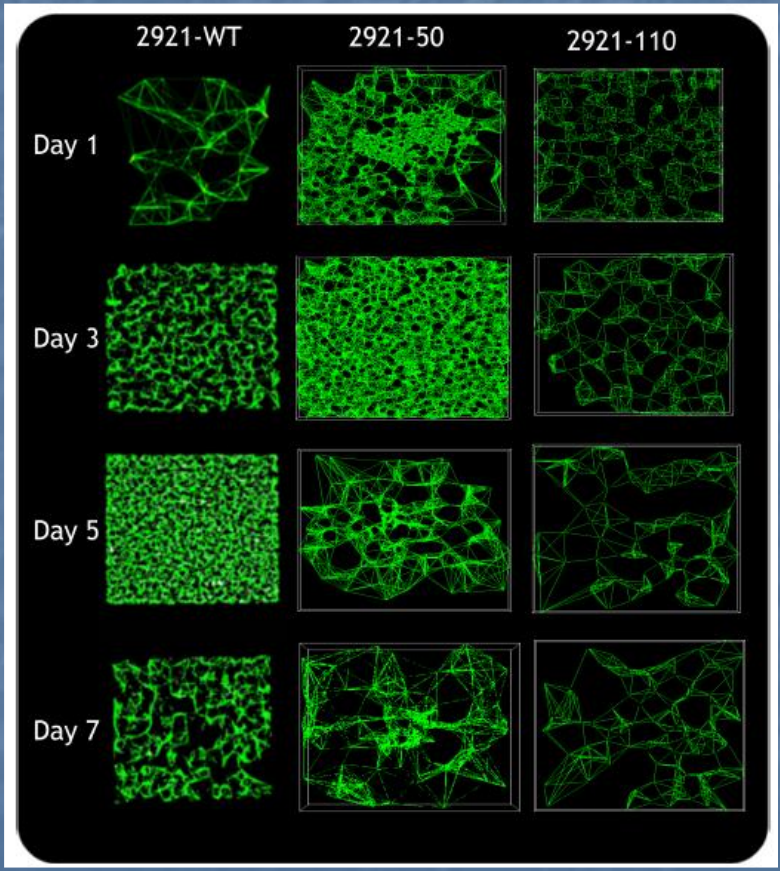


Condiciones de restricción de Fe

Banin et al., 2005

Mutantes de *P. mirabilis* en sistemas relacionados con captación de Fe y biofilms



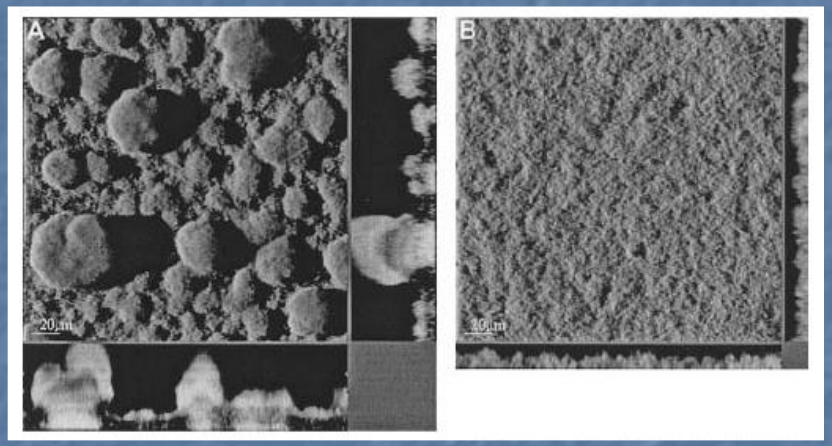


Infectividad atenuada en un modelo de UTI ascendente en ratón

Iribarnegaray et al. 2021

Biofilms bajo distintas condiciones ambientales: fuentes de carbono

P. aeruginosa

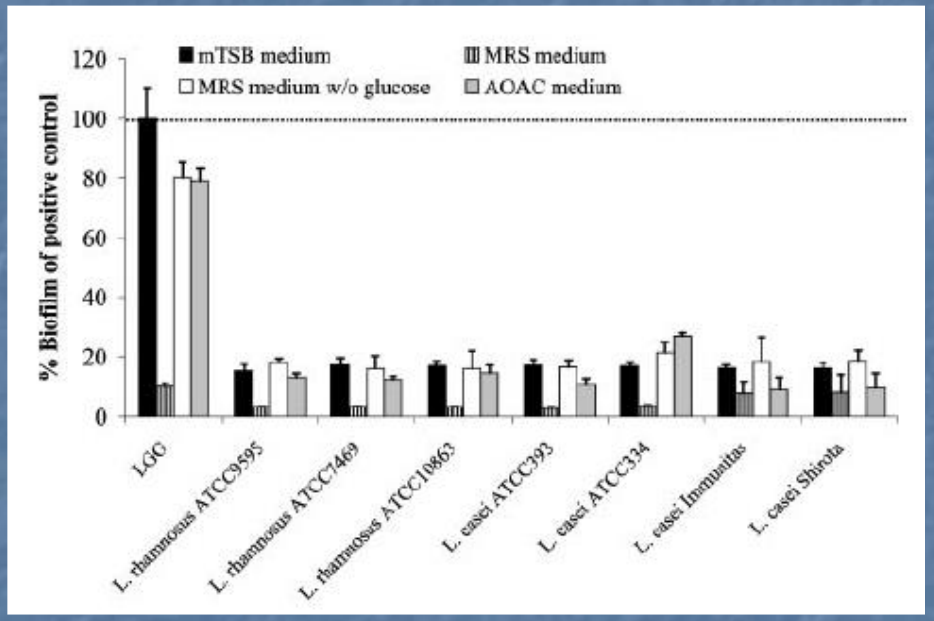


A- Medio mínimo-glucosa

B- Medio mínimo-citrato

Klausen et al., 2003

Lactobacillus spp.



Lebeer et al., 2007

Hallazgos similares en nuestro laboratorio
(Fernández et al., 2018)

Unidad estructural básica: microcolonias

Agregados celulares sumergidos en una matriz extracelular de producción propia (EPS, *extracellular polymeric substances*) entre las que discurren canales acuosos

EPS: Característica distintiva del biofilm

Polisacáridos (rol de cationes bivalentes)

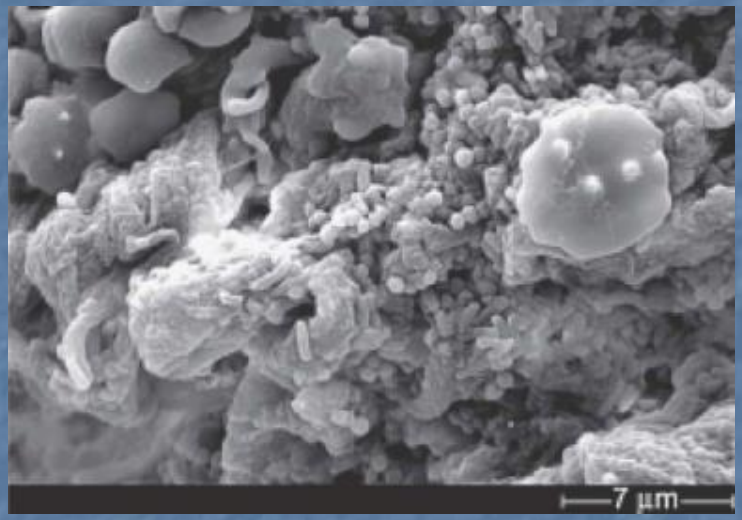
eADN (similar al ADN cromosomal)

Proteínas (PME, 30% en *P.aeruginosa*, prots. secretadas, lisis)

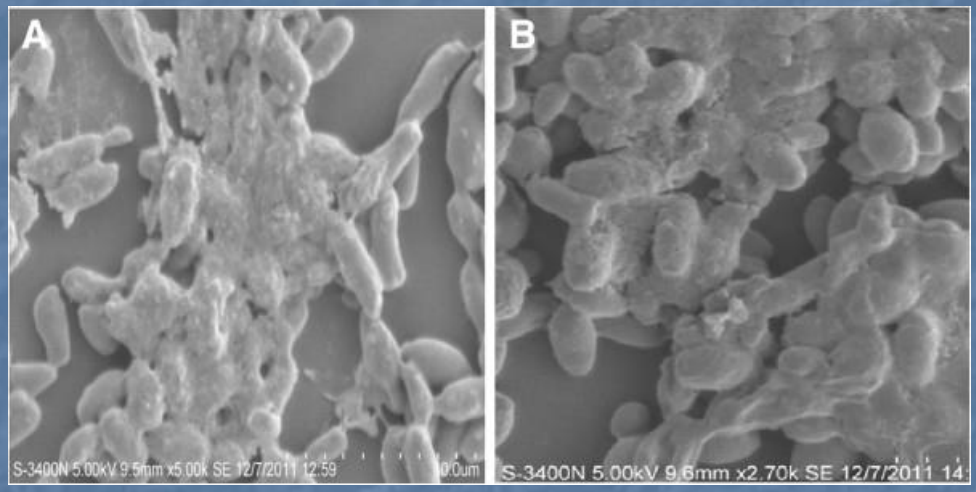
Lípidos y surfactantes (LPS, *P. aeruginosa*)

Agua

EPS, extracellular polymeric substances



Biofilm polimicrobiano en un herida, James et al., 2008



Biofilm de *Candida* sp., Basak et al., 2014

Papel del QS

Polisacáridos (intracelulares y extracelulares)

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

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

Limoli et al, 2014

Otros…

- Manosa
- Galactosa
- Glucosa
- *N*-acetyl-glucosamina,
- Ácido galacturónico,
- arabinosa, ramnosa, xilosa

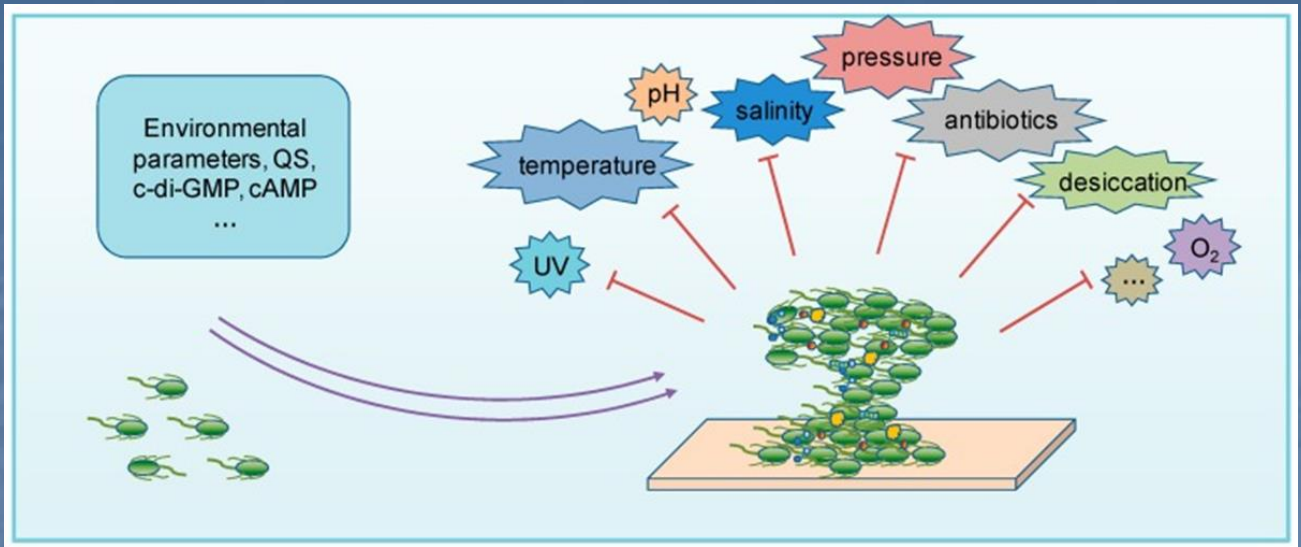
Proteínas

- Enzimas
- Rugosity and BF modulator (RbmA , *Vibrio* spp.)
- Biofilms associated protein (Bap, *S. aureus*)
- Glucanbinding proteins (Gbps, *S. mutans)*
- Amiloides *(*Fap en *P. aeruginosa;* TasA en *B. Subtilis)*
- PME
- Fimbrias
- Flagelos
- Vesículas, entre otras…

Table 2 | **Biofilm enzymes in natural and man-made aquatic environments***

Enzyme	Type of biofilm
Protein-degrading enzymes	
Protease	River biofilms and activated sludge
Peptidase	Drinking-water biofilms, river biofilms, waste-water biofilms, sewer biofilms, marine aggregates and activated sludge
Polysaccharide or oligosaccharide-degrading enzymes	
Endocellulase	River biofilms
Chitinase	River biofilms and estuarine-sediment biofilms
α-glucosidase	River biofilms, sewer biofilms, stream sediment biofilms, lake sediment biofilms, waste-water biofilms, marine aggregates and activated sludge
β-glucosidase	River biofilms, biofilms from trickling biofilters, sewer biofilms, stream sediment biofilms, lake sediment biofilms, marine aggregates and activated sludge
β-xylosidase	River biofilms and lake sediment biofilms
N-acetyl-β-D-glucosaminidase	River biofilms, marine aggregates and activated sludge
Chitobiosidase	Marine aggregates
β-glucuronidase	Activated sludge
Lipid-degrading enzymes	
Lipase	Marine aggregates and activated sludge
Esterase	River biofilms, lake sediment biofilms, drinking-water biofilms, sewer biofilms, stream sediment biofilms and activated sludge
Phosphomonoesterases	
Phosphatase	River biofilms, sewer biofilms, stream biofilms, marine aggregates and activated sludge
Oxidoreductases	
Phenol oxidase	River biofilms
Peroxidase	River biofilms
Extracellular redox activity	Activated sludge

Flemming and Wingender, 2010



Yin et al., 2019

Funciones de la EPS

- Adhesión a superficies
- Soporte estructural
- Prevención de la desecación (fuertemente hidratada)
- Protección frente a agentes externos (compuestos antimicrobianos, organismos predadores, células del sistema inmune)
- Generación de “micronichos” (ej. pH; sitios de adhesión)

Table 1 | Functions of extracellular polymeric substances in bacterial biofilms

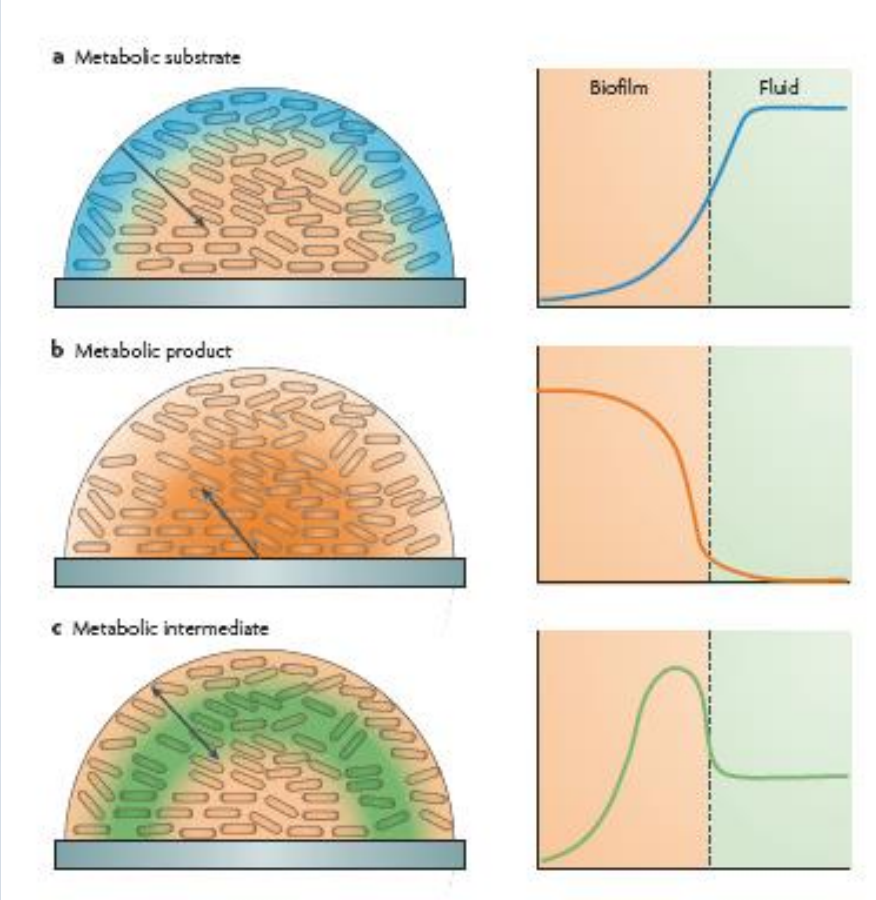
Function	Relevance for biofilms	EPS components involved
Adhesion	Allows the initial steps in the colonization of abiotic and biotic surfaces by planktonic cells, and the long-term attachment of whole biofilms to surfaces	Polysaccharides, proteins, DNA and amphiphilic molecules
Aggregation of bacterial cells	Enables bridging between cells, the temporary immobilization of bacterial populations, the development of high cell densities and cell–cell recognition	Polysaccharides, proteins and DNA
Cohesion of biofilms	Forms a hydrated polymer network (the biofilm matrix), mediating the mechanical stability of biofilms (often in conjunction with multivalent cations) and, through the EPS structure (capsule, slime or sheath), determining biofilm architecture, as well as allowing cell–cell communication	Neutral and charged polysaccharides, proteins (such as amyloids and lectins), and DNA
Retention of water	Maintains a highly hydrated microenvironment around biofilm organisms, leading to their tolerance of dessication in water-deficient environments	Hydrophilic polysaccharides and, possibly, proteins
Protective barrier	Confers resistance to nonspecific and specific host defences during infection, and confers tolerance to various antimicrobial agents (for example, disinfectants and antibiotics), as well as protecting cyanobacterial nitrogenase from the harmful effects of oxygen and protecting against some grazing protoza	Polysaccharides and proteins
Sorption of organic compounds	Allows the accumulation of nutrients from the environment and the sorption of xenobiotics (thus contributing to environmental detoxification)	Charged or hydrophobic polysaccharides and proteins
Sorption of inorganic ions	Promotes polysaccharide gel formation, ion exchange, mineral formation and the accumulation of toxic metal ions (thus contributing to environmental detoxification)	Charged polysaccharides and proteins, including inorganic substituents such as phosphate and sulphate
Enzymatic activity	Enables the digestion of exogenous macromolecules for nutrient acquisition and the degradation of structural EPS, allowing the release of cells from biofilms	Proteins
Nutrient source	Provides a source of carbon-, nitrogen- and phosphorus-containing compounds for utilization by the biofilm community	Potentially all EPS components
Exchange of genetic information	Faciliates horizontal gene transfer between biofilm cells	DNA
Electron donor or acceptor	Permits redox activity in the biofilm matrix	Proteins (for example, those forming pili and nanowires) and, possibly, humic substances
Export of cell components	Releases cellular material as a result of metabolic turnover	Membrane vesicles containing nucleic acids, enzymes, lipopolysaccharides and phospholipids
Sink for excess energy	Stores excess carbon under unbalanced carbon to nitrogen ratios	Polysaccharides
Binding of enzymes	Results in the accumulation, retention and stabilization of enzymes through their interaction with polysaccharides	Polysaccharides and enzymes

EPS, extracellular polymeric substances.

Flemming and Wingender, 2010

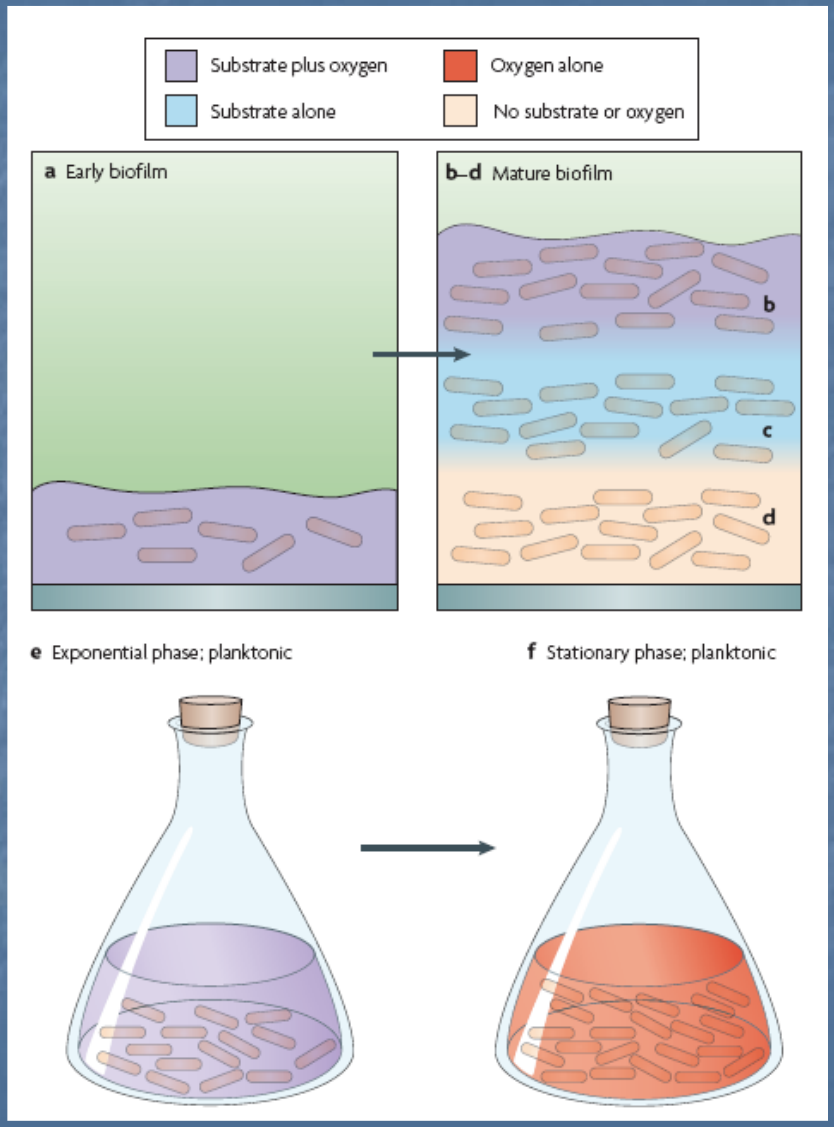
Biofilms: comunidades heterogéneas

Heterogeneidad química de los biofilms: gradientes



Stewart and Franklin, 2008

Heterogeneidad fisiológica de los biofilms



Stewart and Franklin, 2008

Etapas de formación de los biofilms

(particularidades de las distintas especies bacterianas)

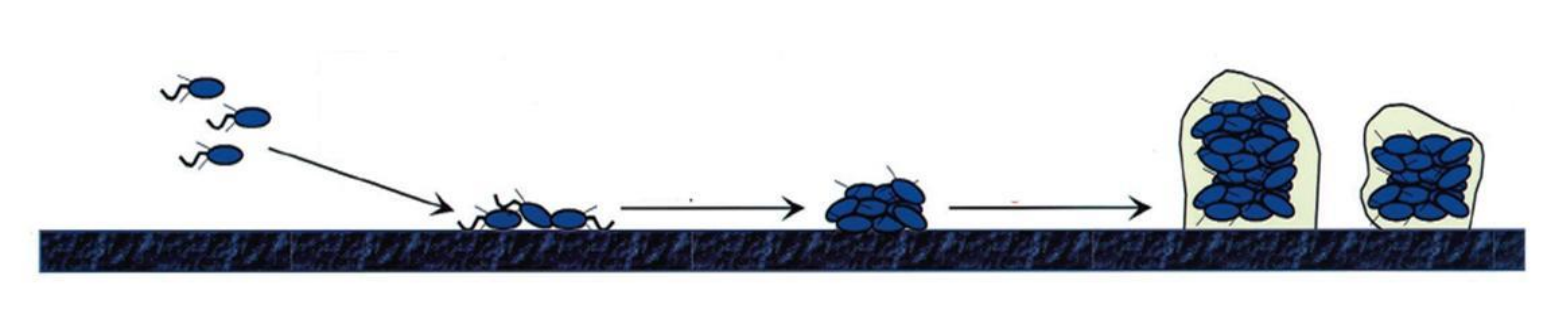
(i) aproximación y adhesión reversible de las bacterias a la superficie (adhesión inespecífica o específica)

(ii) adhesión irreversible y comienzo de producción de EPS

(iii) formación de microcolonias

(iv) maduración del biofilm con gran aumento de EPS

(v) dispersión celular (pasiva o activa)



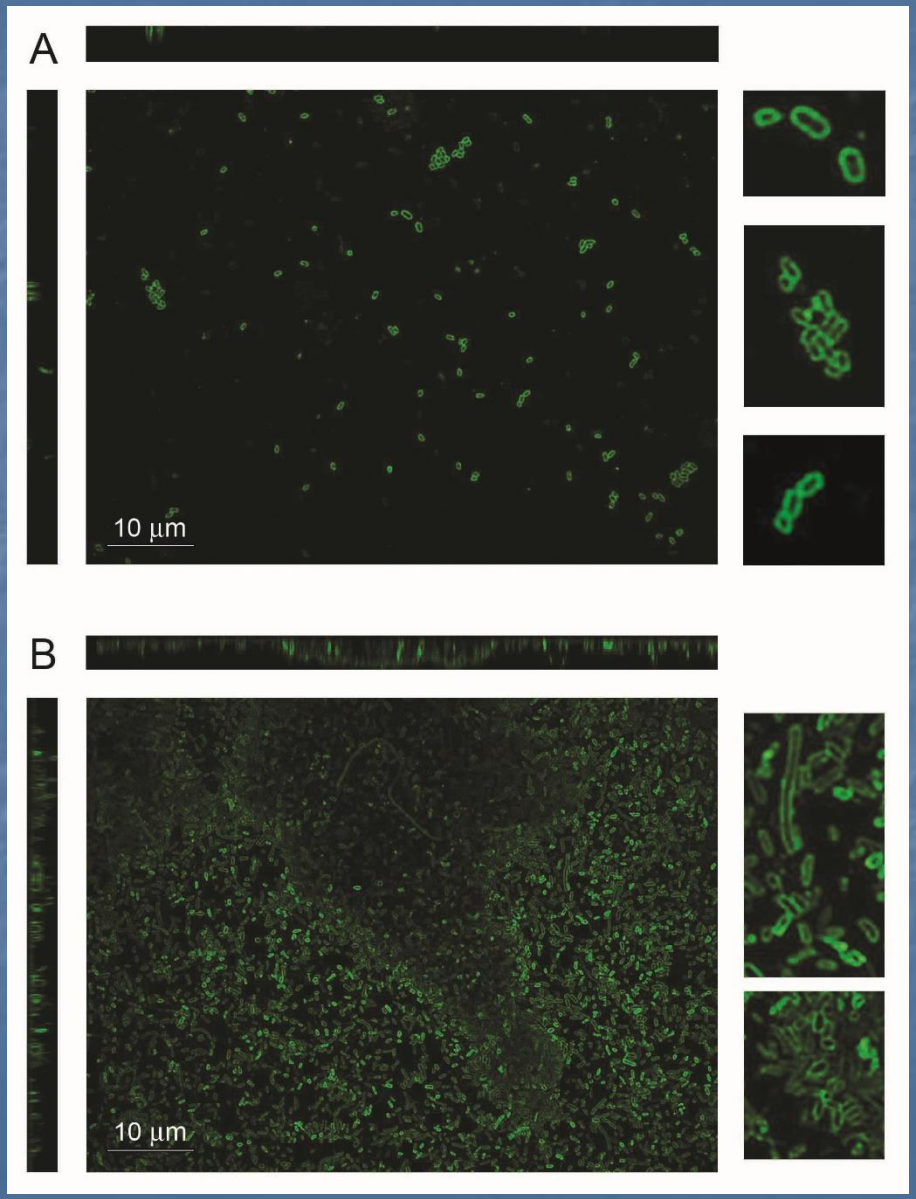
Davey y O'Toole, 2000

Etapas: microscopía laser confocal - *Proteus mirabilis*

Obtención de *stacks*

A - 1 día de incubación

B - 5 días de incubación



Descriptores matemáticos morfo-topológicos y reconstrucción de modelos 2D y 3D

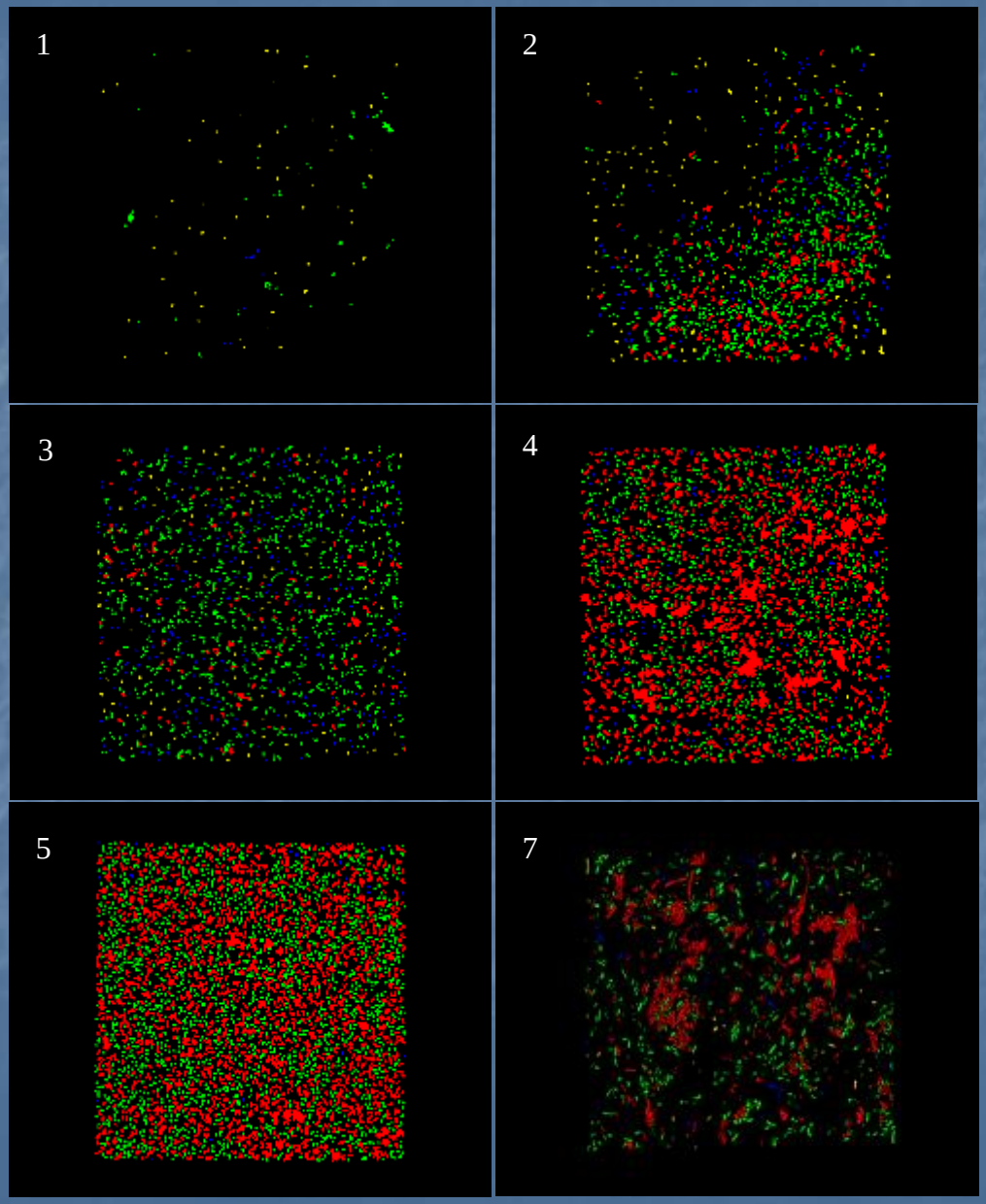
Parámetros relacionados con la formación de biofilms:

- distancias entre bacterias
- complejidad y compactación entre bacterias
- cantidad y volumen de bacterias
- elongación bacteriana: rol de células *swarmer*
- vecindad bacteriana

Modelo de distancias (parámetro 2D)

4 rangos de distancias:

<3 pixeles (rojo)
3-12 pixeles (verde)
12-20 pixeles (azul)
>20 pixeles (amarillo)



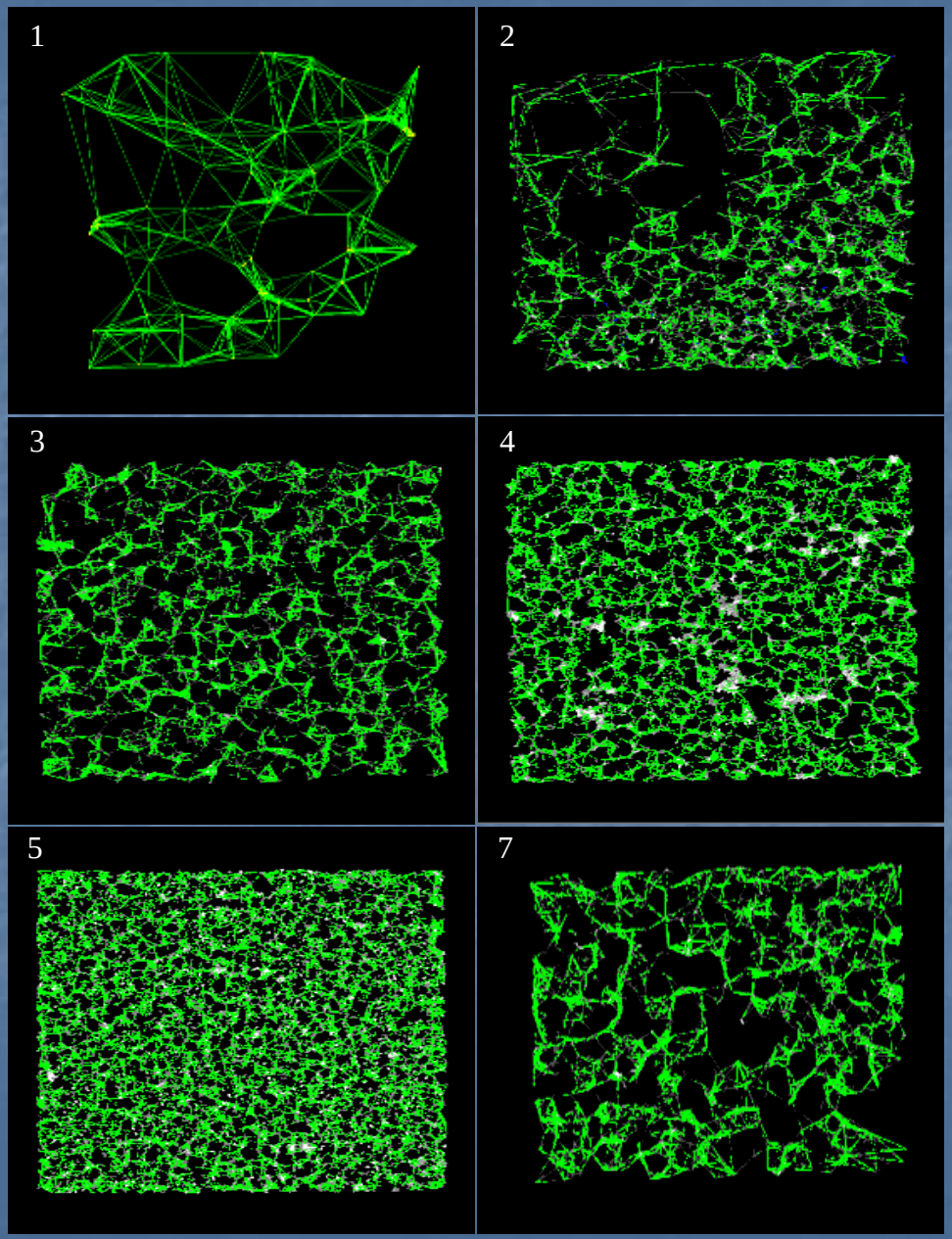
Schlapp. G., Scavone, P., Zunino, P., Hartell, S. Development of 3D architecture of uropathogenic *Proteus mirabilis* batch culture biofilms: a quantitative confocal microscopy approach. Journal of Microbiological Methods 2011. 87:234-240.

Complejidad y compactación (parámetro 2D)

Modelo de *lattice* hexagonal

Líneas verdes indican la conexión entre las seis bacterias vecinas más cercanas a cada una

- Compactación
- Modelo hexagonal

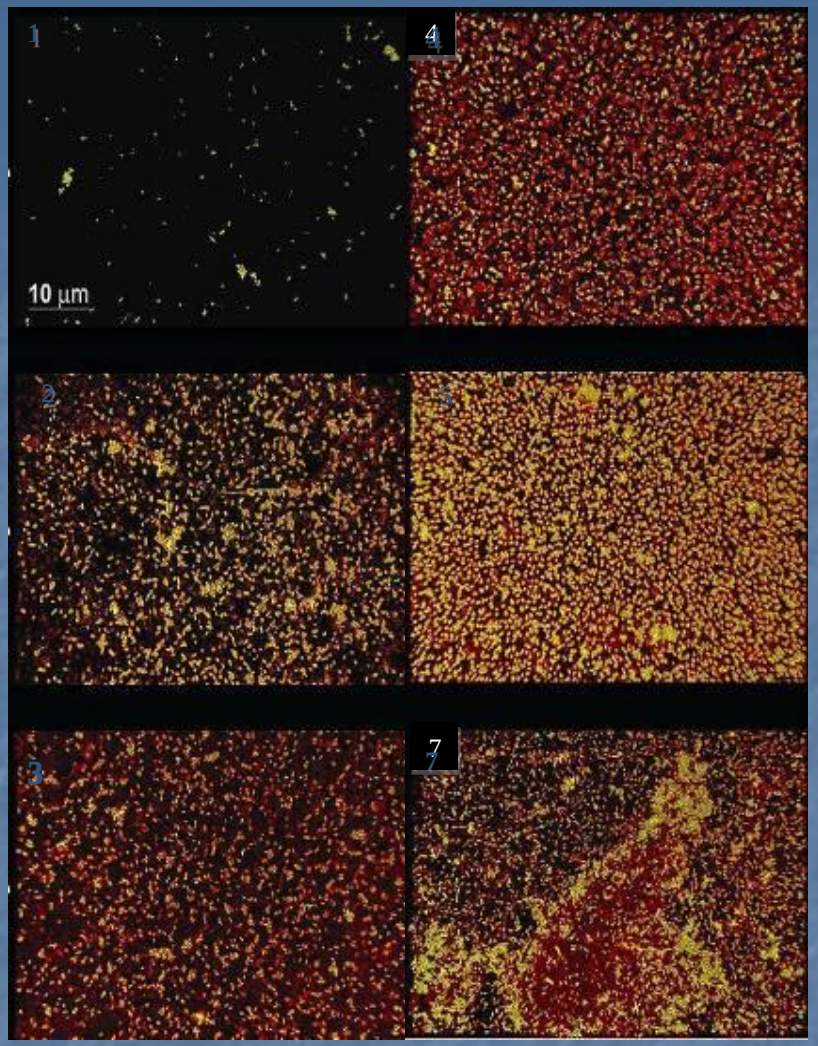


Etapas en la formación del biofilm de *P. mirabilis* Pr2921

Superposición modelos 3D

Volumen total bacteriano (amarillo)

Volumen de biomasa (rojo)



(1) Adhesión reversible de las bacterias a la superficie – día 1
(2) Adhesión irreversible de las bacterias a la superficie y producción de polímeros extracelulares - día 2
(3) Desarrollo inicial de la arquitectura del biofilm – día 3
(4) Formación de microcolonias y maduración del biofilm días 4 y 5
(5) Dispersión de las bacterias – día 7.

Fenotipos celulares en la fase de dispersión

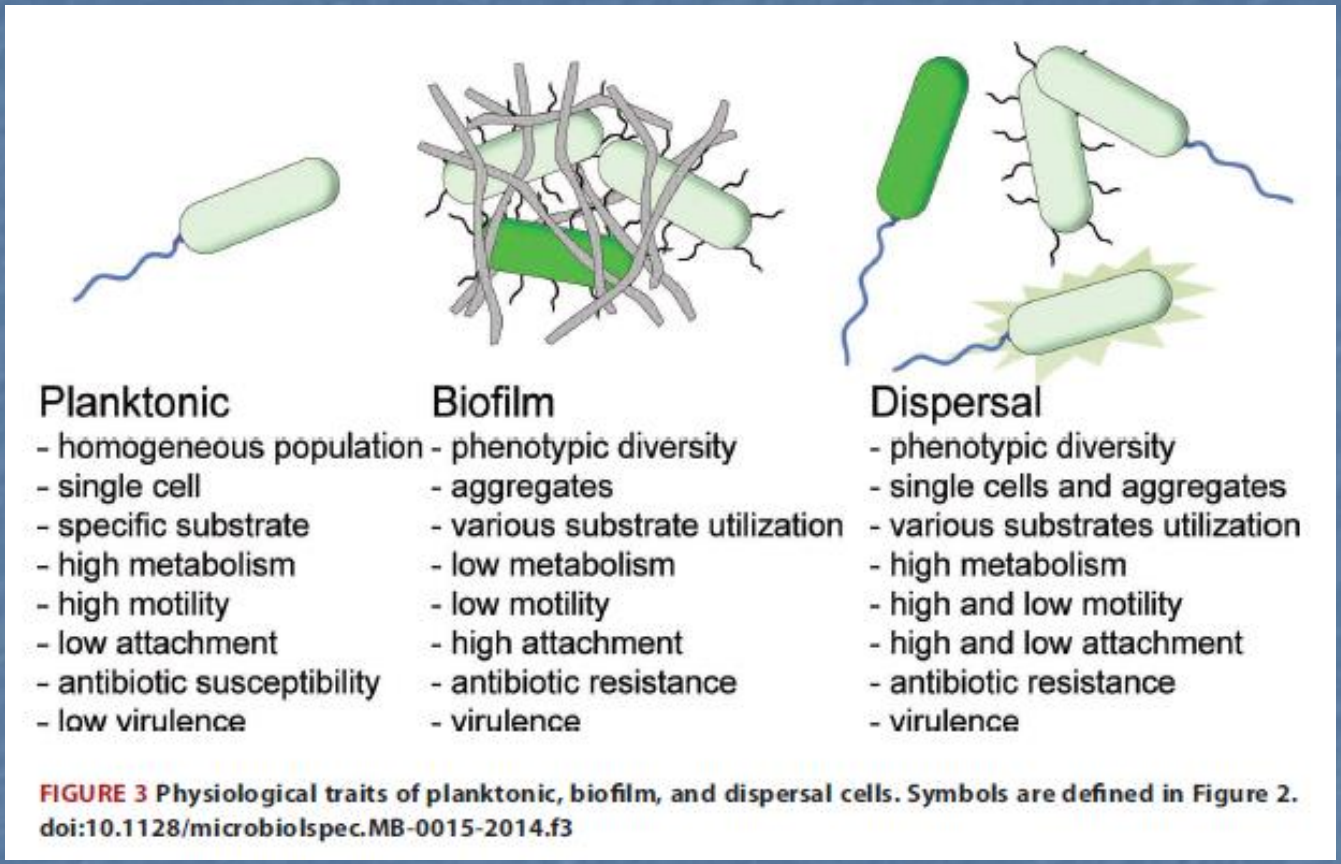


FIGURE 3 Physiological traits of planktonic, biofilm, and dispersal cells. Symbols are defined in Figure 2.
doi:10.1128/microbiolspec.MB-0015-2014.f3

Barraud et al., 2015

DspB protein is responsible for the surface detachment of *Actinobacillus pleuropneumoniae* biofilms (dispersina B)

Factores bacterianos implicados en la etapa de adhesión

- Interacciones no específicas (iniciales)

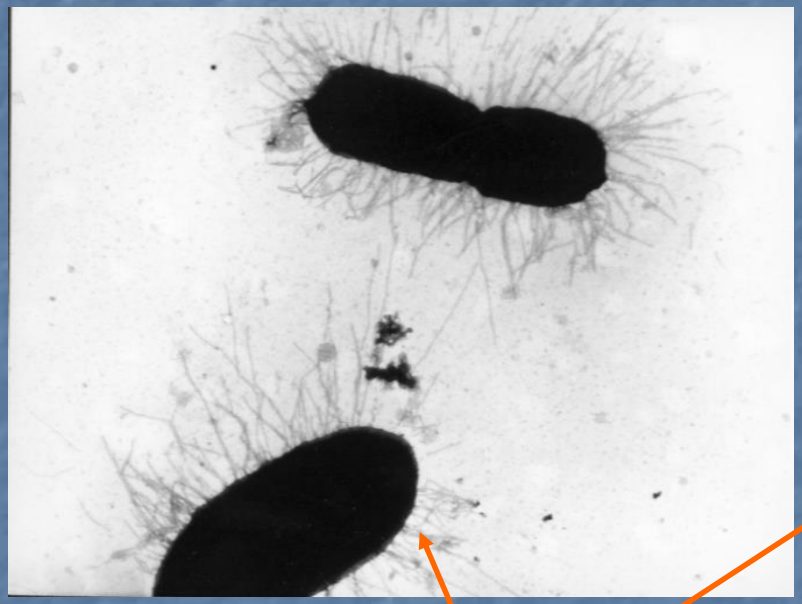
- Hidrofobicidad
- Fuerzas electrostáticas
- Tensión superficial (Darouiche, 2001)

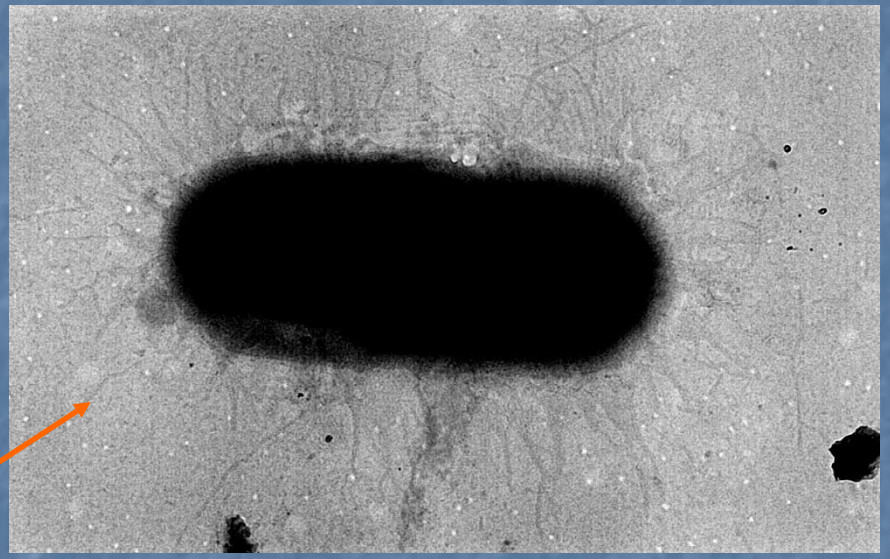
- Factores bacterianos específicos (interacciones más estables)

- Adhesinas fimbriales (fimbria tipo I de *E. coli*, fimbrias tipo IV de *P. aeruginosa*, etc.)

- Proteínas MSCRAMMs (microbial surface components recognizing adhesive matrix molecules; *S. aureus*): pueden unirse a moléculas como el colágeno (a través de Cna), fibronectina (a través de FnbAB), fibrinógeno (ClfAB y Fib), etc.

Fimbrias de *P. mirabilis*





Fimbrias de *P. mirabilis* 2921
(Dep. de Microbiología -IIBCE)

JOURNAL OF BACTERIOLOGY, June 2008, p. 4027–4037 Vol. 190, No. 11
0021-9193/08/$08.00+0 doi:10.1128/JB.01981-07

Complete Genome Sequence of Uropathogenic *Proteus mirabilis*, a Master of both Adherence and Motility[▽]†

Melanie M. Pearson,[1] Mohammed Sebaihia,[2] Carol Churcher,[2] Michael A. Quail,[2] Aswin S. Seshasayee,[3] Nicholas M. Luscombe,[3] Zahra Abdellah,[2] Claire Arrosmith,[2] Becky Atkin,[2] Tracey Chillingworth,[2] Heidi Hauser,[2] Kay Jagels,[2] Sharon Moule,[2] Karen Mungall,[2] Halina Norbertczak,[2] Ester Rabbinowitsch,[2] Danielle Walker,[2] Sally Whithead,[2] Nicholas R. Thomson,[2] Philip N. Rather,[4] Julian Parkhill,[2] and Harry L. T. Mobley[1]*

Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan[1]; Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom[2]; EMBL-European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom[3]; and Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia[4]

Received 20 December 2007/Accepted 16 March 2008

- 17 operones fimbriales y 13 posibles genes fimbriales “huérfanos” (no incluidos en operones)
- genes relacionados con fimbrias tipo IV
- adhesinas no fimbriales (ej. genes similares a *ail* de *Y. enterocolitica* – adhesión e invasión).
- Los genes vinculados a flagelos y movilidad se ubican en un locus de 53,3 kb
- Presenta un sistema de secreción tipo III y al menos 8 loci vinculados a sistemas de captación de hierro

- Polysaccharide intercellular adhesin (PIA) (*icaADBC* operon) polímeros de *N*-acetilglucosamina. Variación ON/OFF (*S. epidermidis* y *S. aureus*)

- Biofilm-associated protein (Bap) (Gram +)

- Adhesinas no fimbriales (PME autotransportadora Ag43 de *E. coli,* Danese et al., 2000) Variación ON/OFF

- Flagelos

- LPS

- Polisacáridos capsulares (ej. *S. epidermidis* y *S. aureus*)

- Factores del huésped (fibrinógeno, colágeno, etc.)

OXFORD FEMS JOURNALS investing in science

Pathogens and Disease, 74, 2016, ftw033

doi: 10.1093/femspd/ftw033
Advance Access Publication Date: 17 April 2016
Research Article

RESEARCH ARTICLE

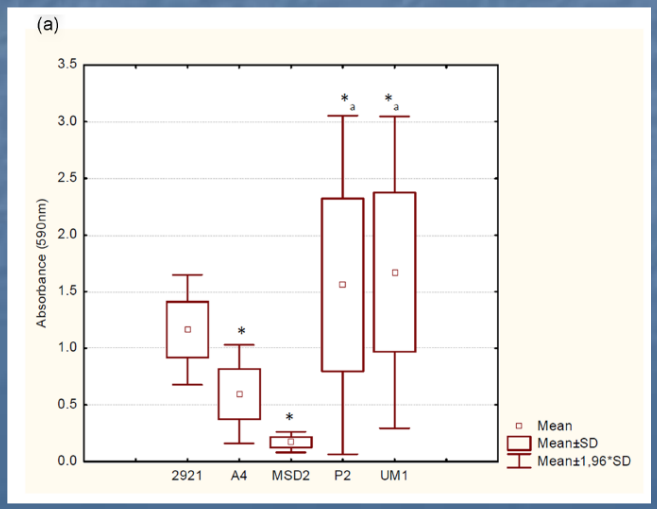
Fimbriae have distinguishable roles in *Proteus mirabilis* biofilm formation

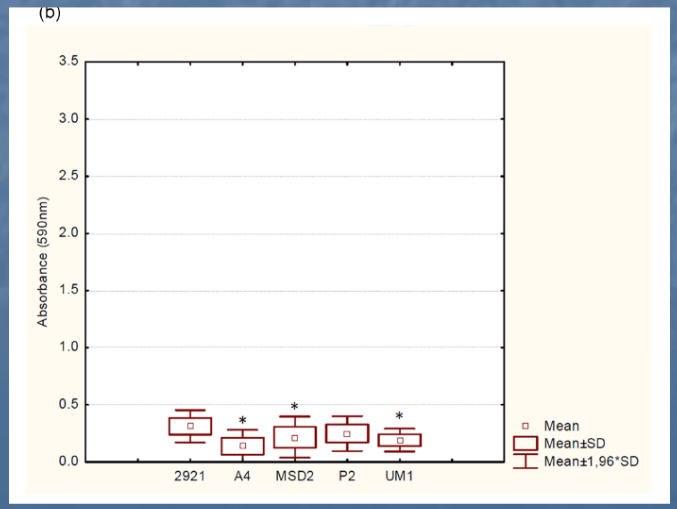
Paola Scavone[1], Victoria Iribarnegaray[1], Ana Laura Caetano[1], Geraldine Schlapp[1], Steffen Härtel[2] and Pablo Zunino[1,*]

Table 2. Bacterial ability to migrate across urinary catheter sections, and swimming and swarming motility.

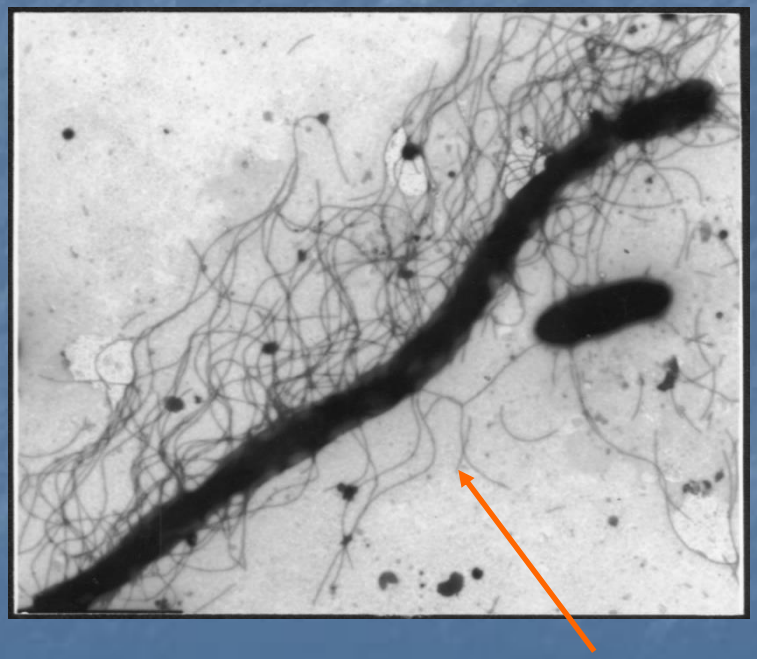
Strain	Latex (%, P)	Silicone (%, P)	Swimming (cm^2) (P)[a]	Swarming (cm^2) (P)[a]
Pr2921	15/15 (100%)	15/15 (100%)	49.23 ± 7.79	50.27 ± 0.10
MSD2	11/15 (73%, 0.03)	8/15 (53%, 0.0025)	30.27 ± 16.09 (0.28)	31.37 ± 19.29 (0.14)
P2	5/15 (33%, 0.0001)	9/15 (60%, 0.0062)	42.73 ± 12.83 (0.34)	43.01 ± 12.70 (0.46)
UM1	11/15 (73%, 0.03)	9/15 (60%, 0.0062)	37.99 ± 18.42 (0.18)	28.27 ± 0.10 (0.06)
A4	2/15 (13%, <0.0001)	10/15 (66%, 0.014)	39.81 ± 14.55 (0.31)	34.21 ± 12.70 (0.17)

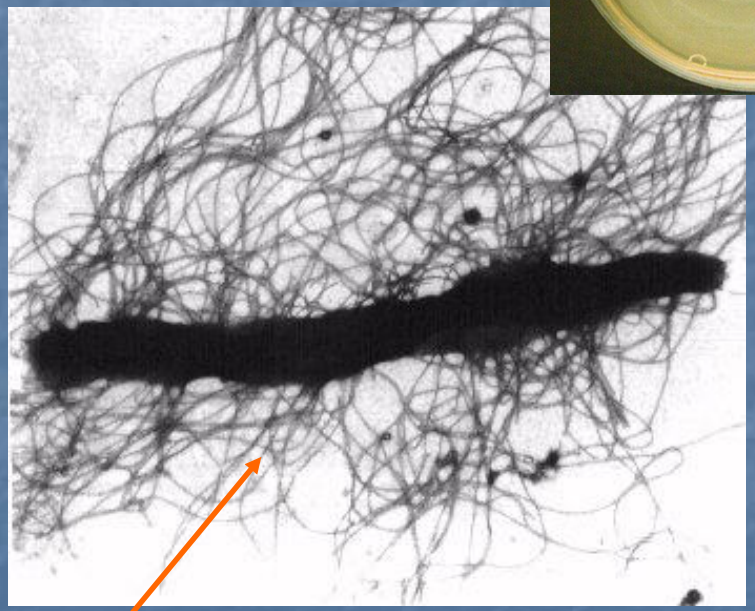
Strains used in this assay were Pr2921 wild type strain and UCA, PMF, ATF and MR/P fimbrial mutants (UM1, P2, A4 and MSD2, respectively).
[a]P values were calculated comparing motility areas of the wild type and each mutant.





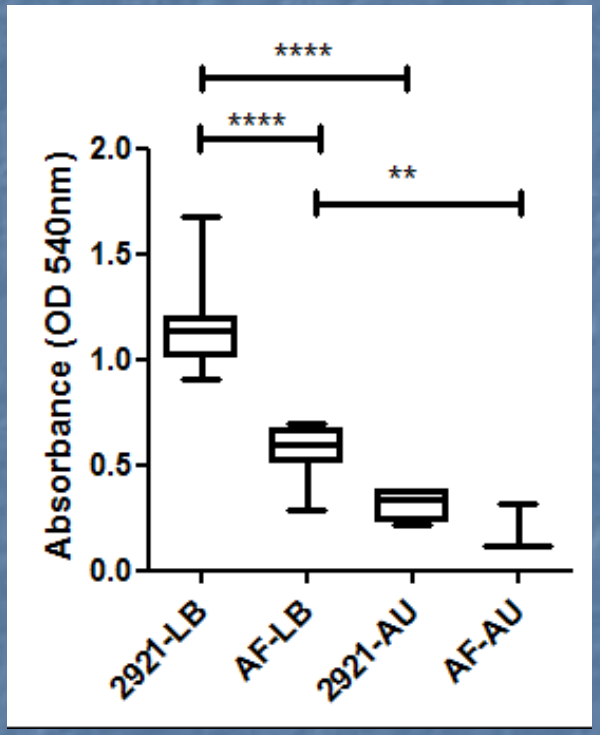
Flagelos de *P. mirabillis*

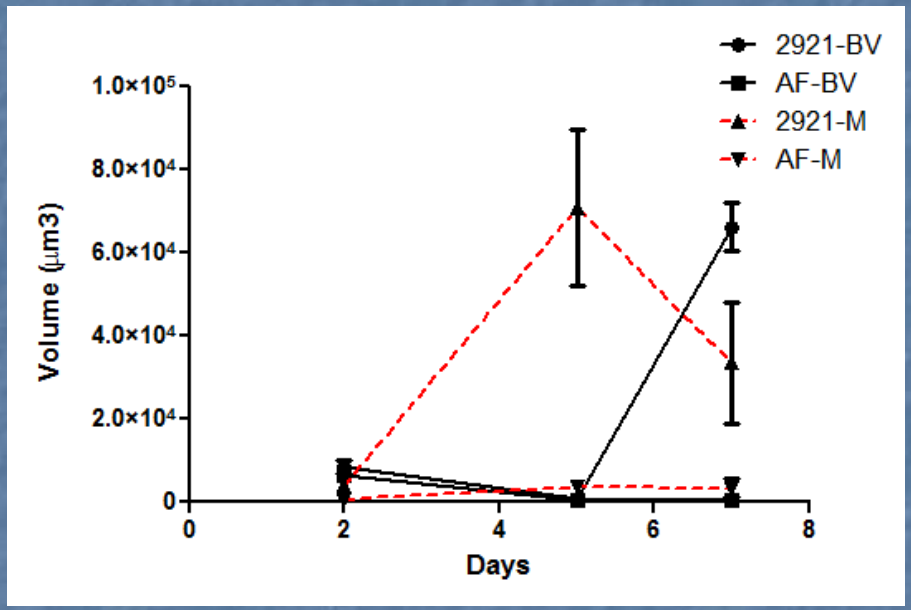




Flagelos de la cepa clínica de *P. mirabilis* Pr2921 (Zunino *et al*., 1994).

Mutante aflagelada y BF





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 produción de ADN extracelular

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

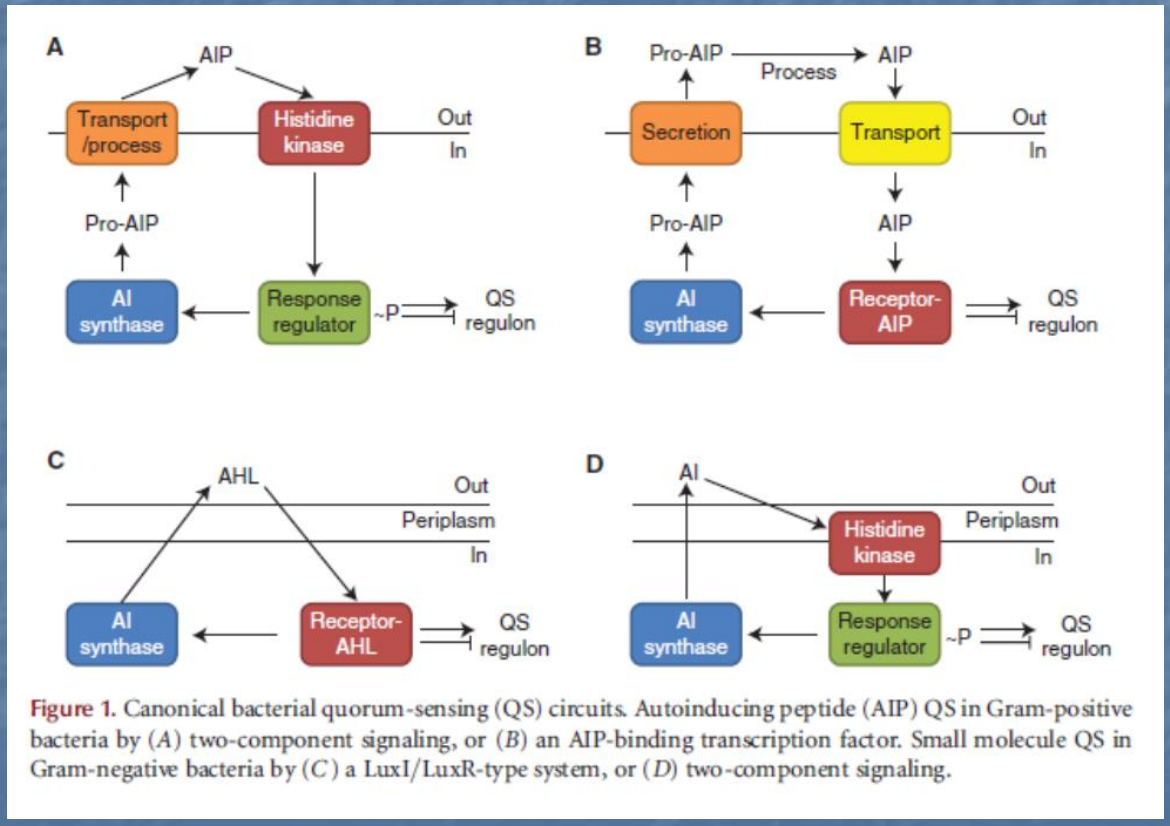
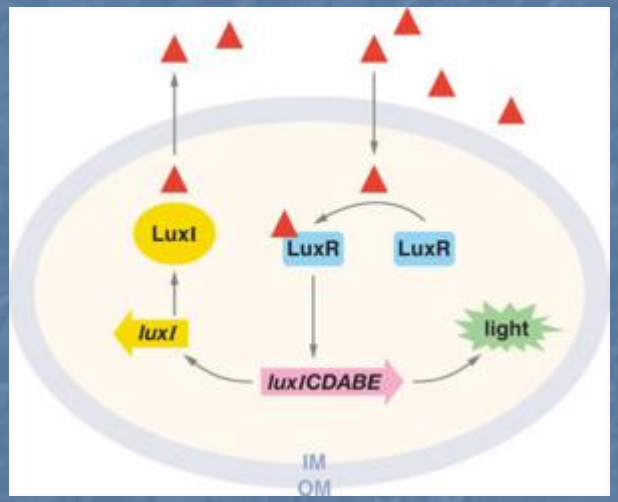


Figure 1. Canonical bacterial quorum-sensing (QS) circuits. Autoinducing peptide (AIP) QS in Gram-positive bacteria by (*A*) two-component signaling, or (*B*) an AIP-binding transcription factor. Small molecule QS in Gram-negative bacteria by (*C*) a LuxI/LuxR-type system, or (*D*) two-component signaling.

Mecanismos generales de QS



Vibrio fischeri

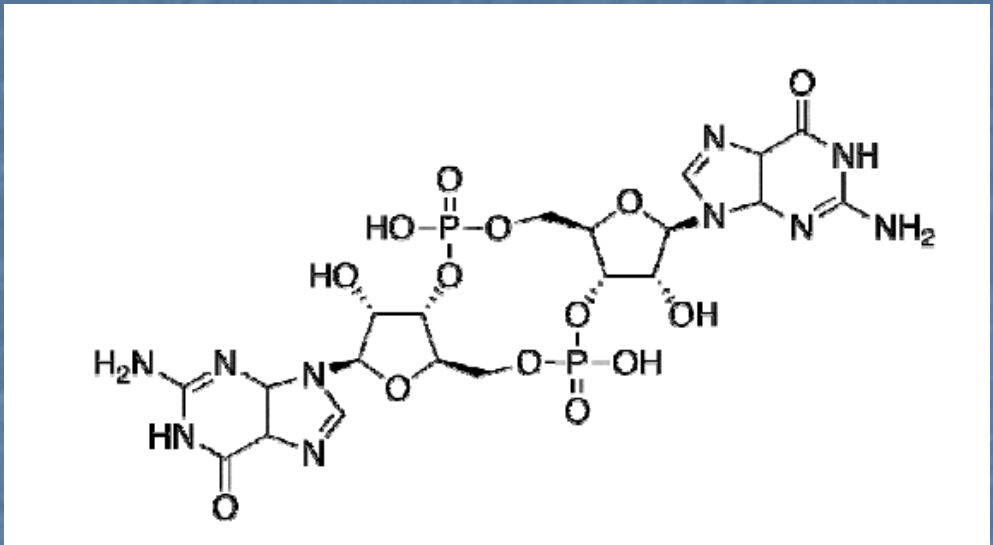
Procesos controlados por Quorum Sensing:

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

Ejemplos de sistemas de Quorum Sensing y su influencia sobre comportamientos sociales bacterianos (Yung & Xialin, 2012)

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

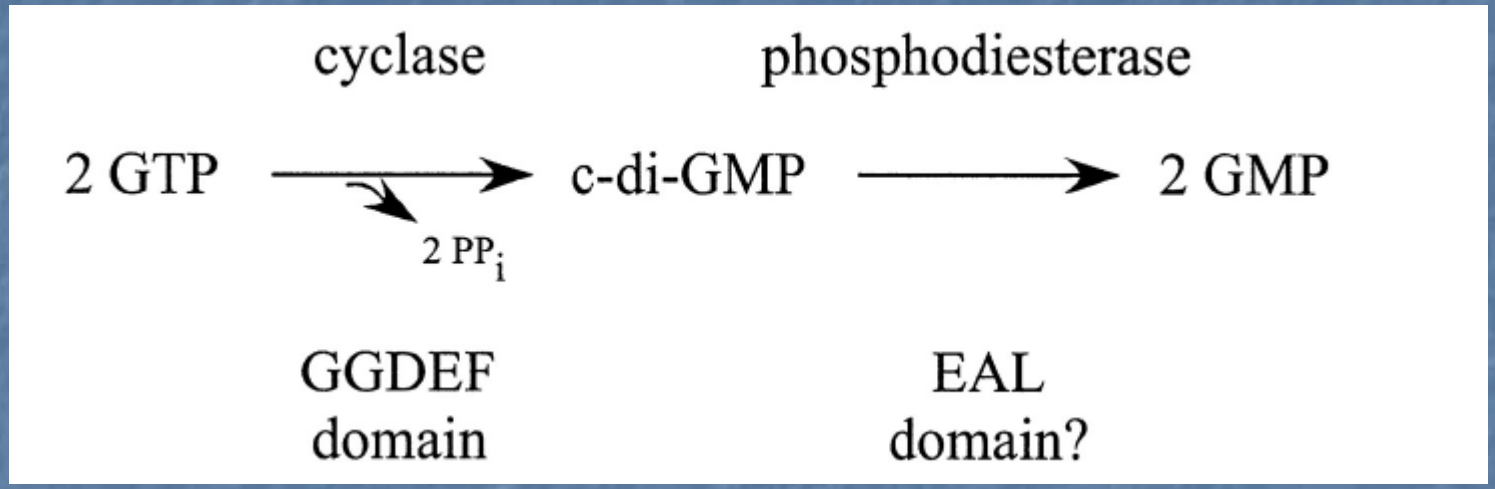
Segundo mensajero: di-GMP (dimeric guanosine monophosphate) cíclico



Caulobacter 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).

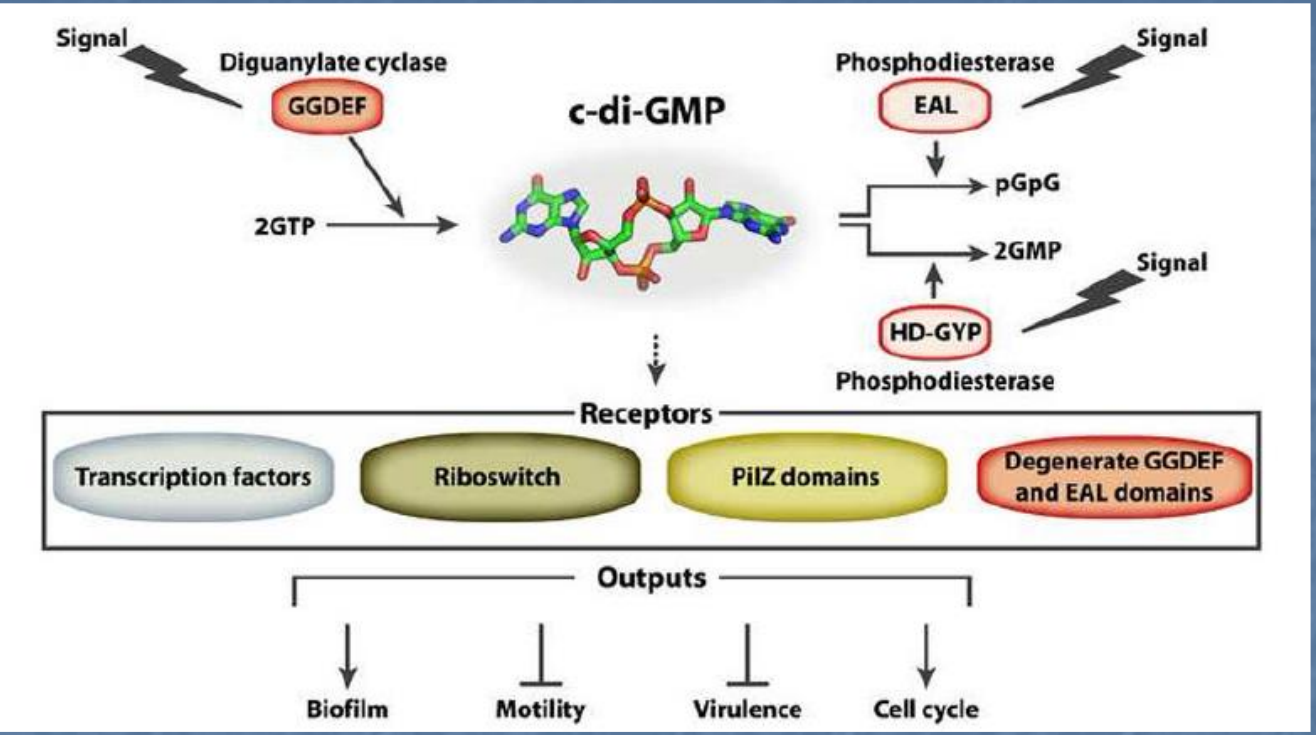
Regulación por c-di-GMP



- 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*

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
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

Biofilms y patogenicidad bacteriana

Los porcentaje de infecciones bacterianas que involucran biofilms se estiman entre un 65% (CDC) y un 80% (NIH)

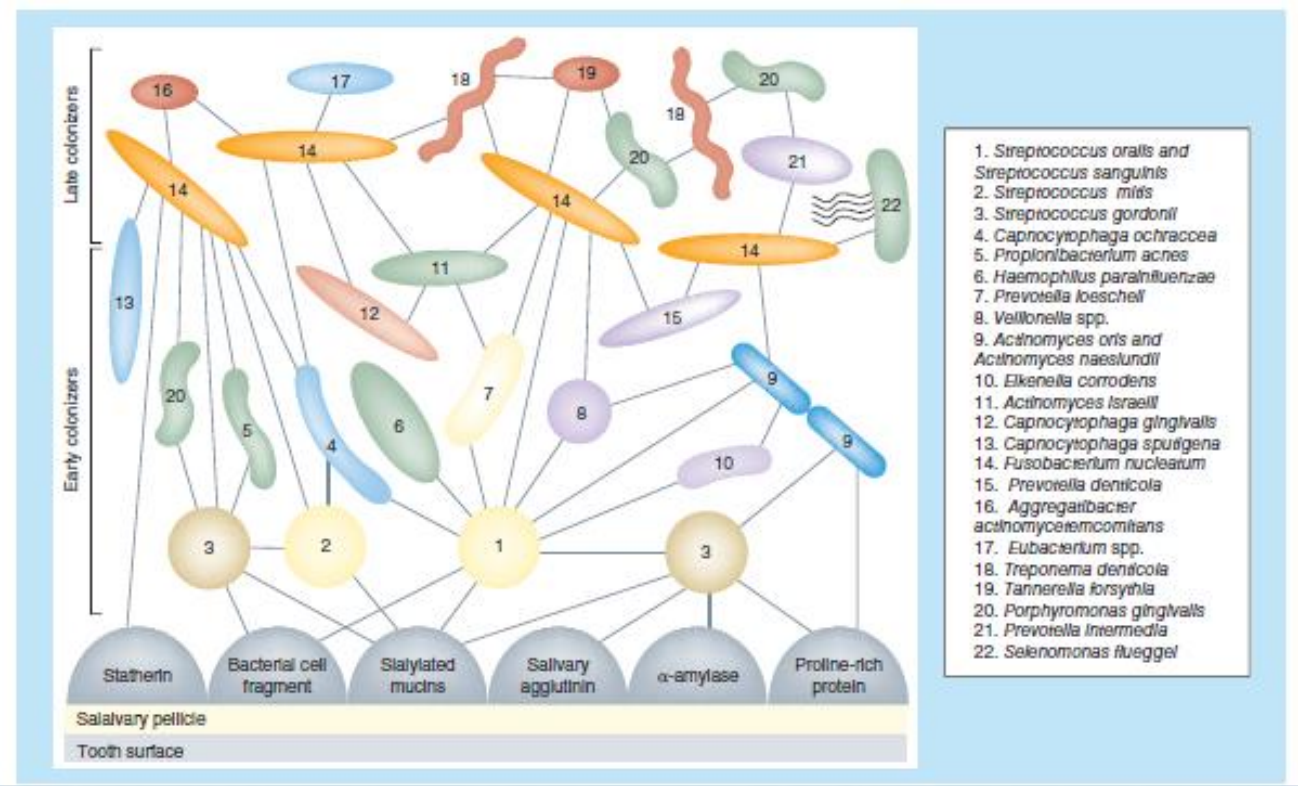
Factores en la formación del biofilm

Factores de virulencia

Estrategias de prevención y eliminación

Enfermedades relacionadas con biofilms

Placa dental



Rabin et al. 2021

Biofilms polimicrobianos
Más de 700 especies de bacterias y arqueas reportadas

- Fibrosis quística

80% de los casos asociados a infecciones por *P. aeruginosa*

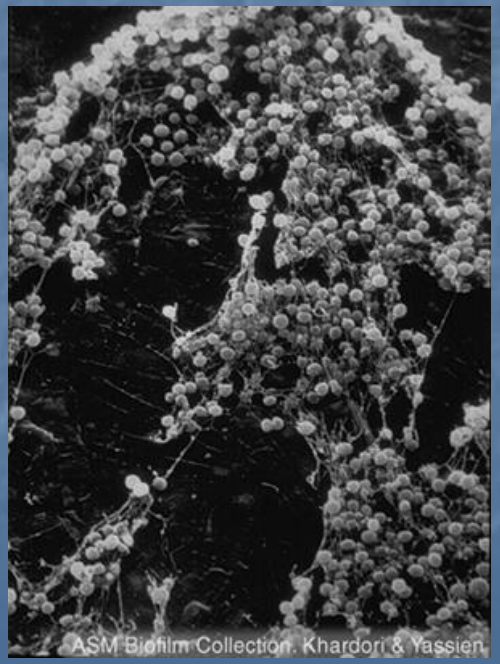
- Heridas

- ITU

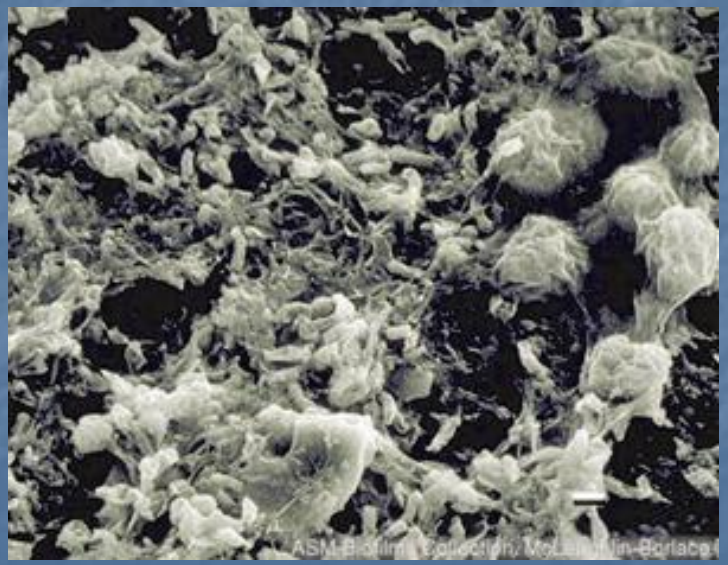
Biofilms en implantes

- Representan 60 % 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)

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

Darouiche, 2001

Durante la pandemia de COVID-19 se observó un aumento dramático de infecciones relacionadas a catéteres intravenosos.

Es necesario desarrollar estrategias preventivas en base a aproximaciones clásicas y modernas

El aumento en ITU asociadas a cateterizacón no fue significativo

Pérez-Granda et al. 2021; Fakih et al. 2021; Ellison et al. 2021

Infecciones urinarias asociadas a catéteres (ITU-C)

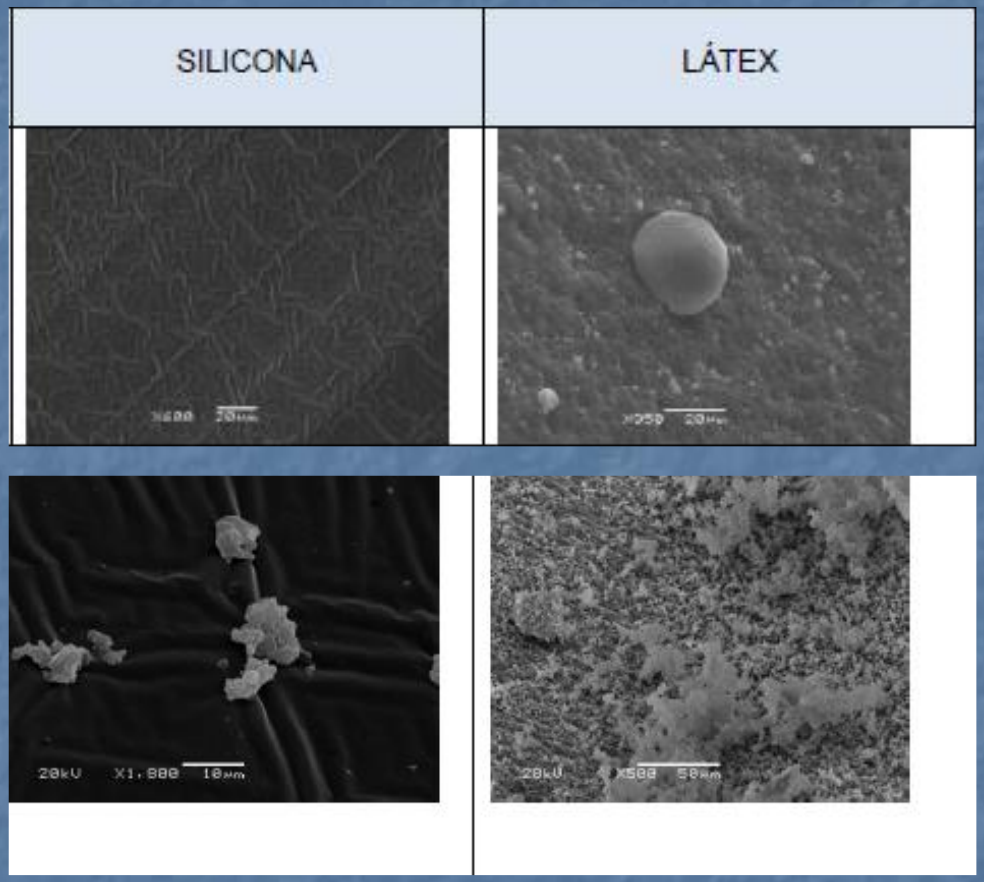
- Vinculadas a la formación de biofilms en la superficie de los catéteres
- Infecciones nosocomiales más comunes
- Costos (USA): U$S 450 millones/año

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)

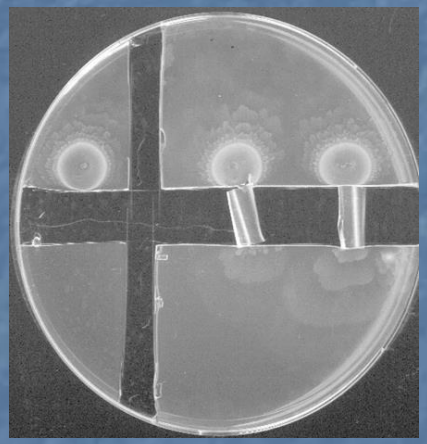


Secciones de catéteres

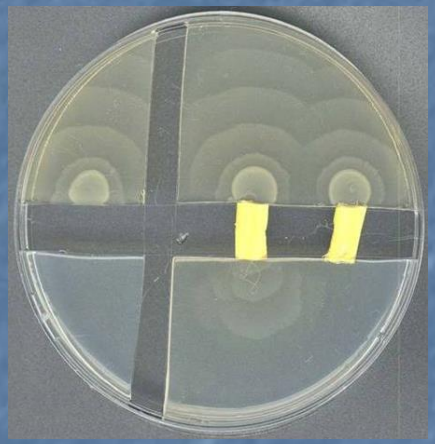
Biofilms de *P. mirabilis*

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

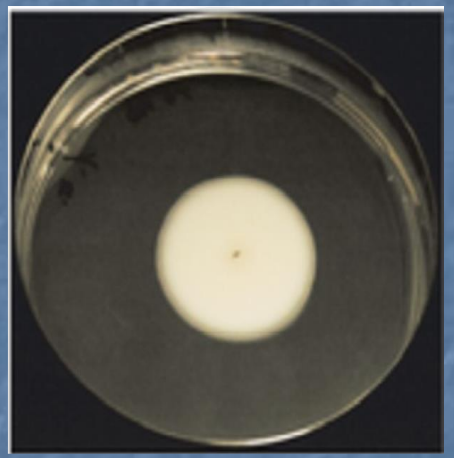
Migración de *P. mirabilis* sobre secciones ("puentes") de catéteres urinarios



silicona



látex



swimming

swarming

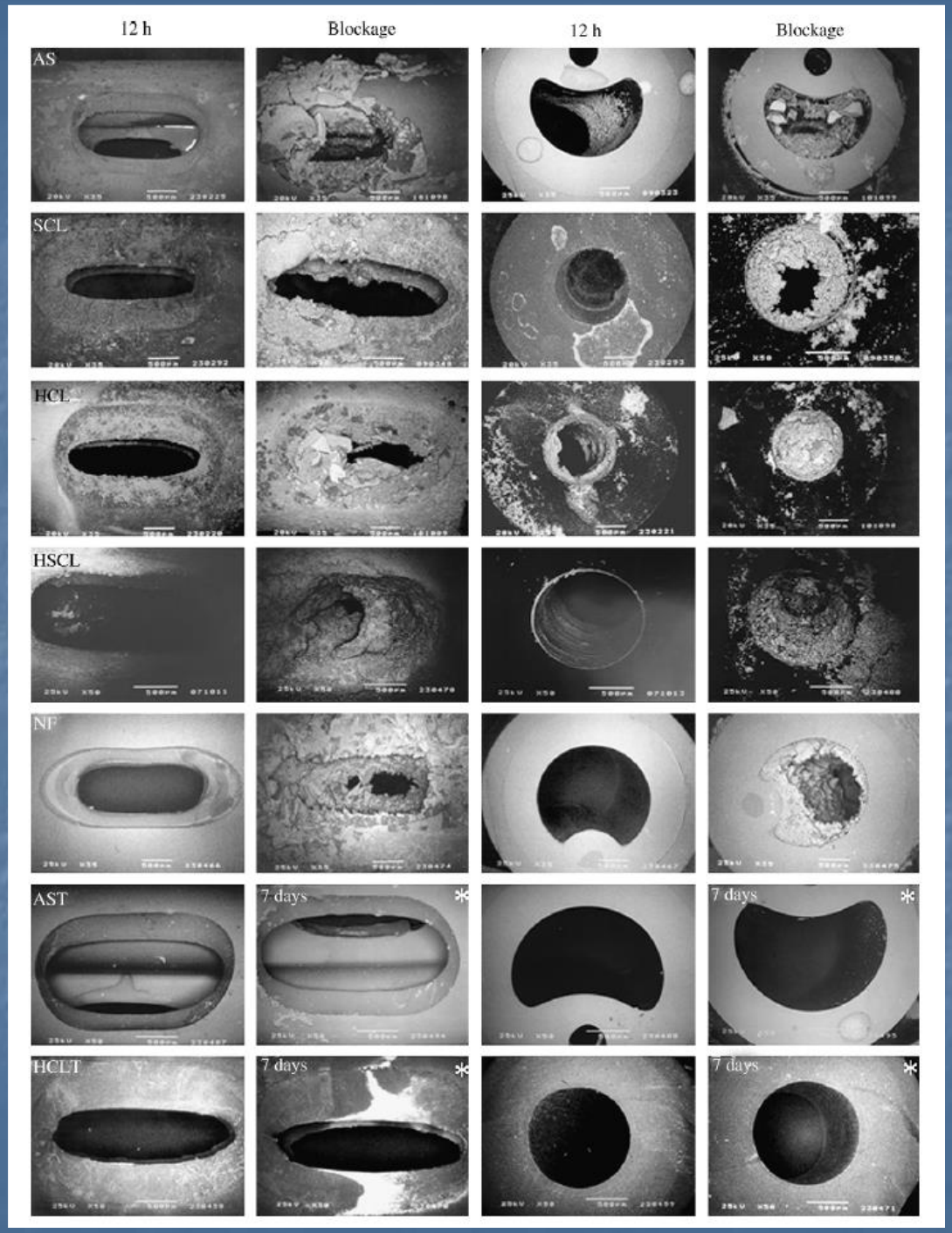
Strain	Latex (%, P)	Silicone (%, P)	Swimming (cm^2) (P)*	Swarming (cm^2) (P)*
Pr2921	15/15 (100%)	15/15 (100%)	49.23±7.79	50.27±0.10
MSD2	11/15 (73%, 0.03)	8/15 (53%, 0.0025)	30.27±16.09 (0.28)	31.37±19.29 (0.14)
P2	5/15 (33%, 0.0001)	9/15 (60%, 0.0062)	42.73±12.83 (0.34)	43.01±12.70 (0.46)
UM1	11/15 (73%, 0.03)	9/15 (60%, 0.0062)	37.99±18.42 (0.18)	28.27±0.10 (0.06)
A4	2/15 (13%, <0.0001)	10/15 (66%, 0.014)	39.81±14.55 (0.31)	34.21±12.70 (0.17)

Mutante aflagelada: 0/15

Scavone et al., 2016

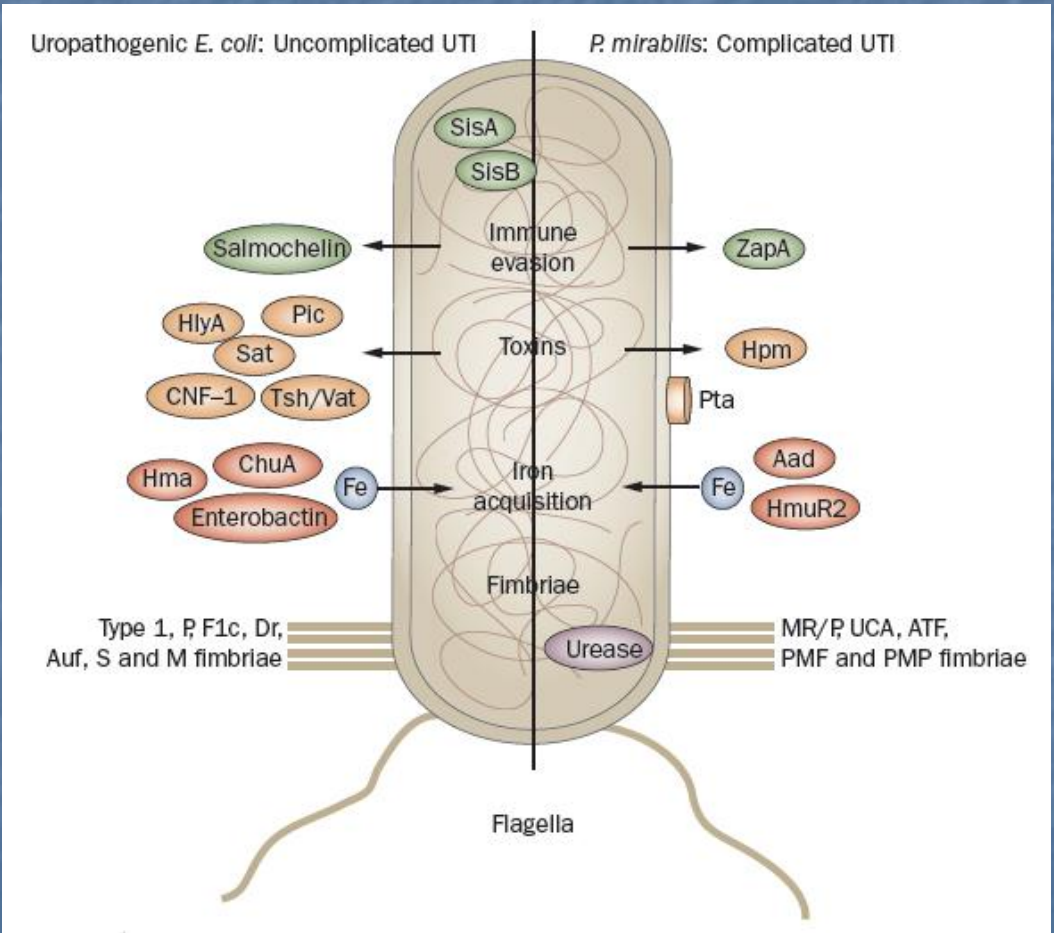
Biofilms cristalinos de *P. mirabilis* en la luz de catéteres urinarios

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

Potenciales factores de virulencia de *E. coli* y *P. mirabilis*



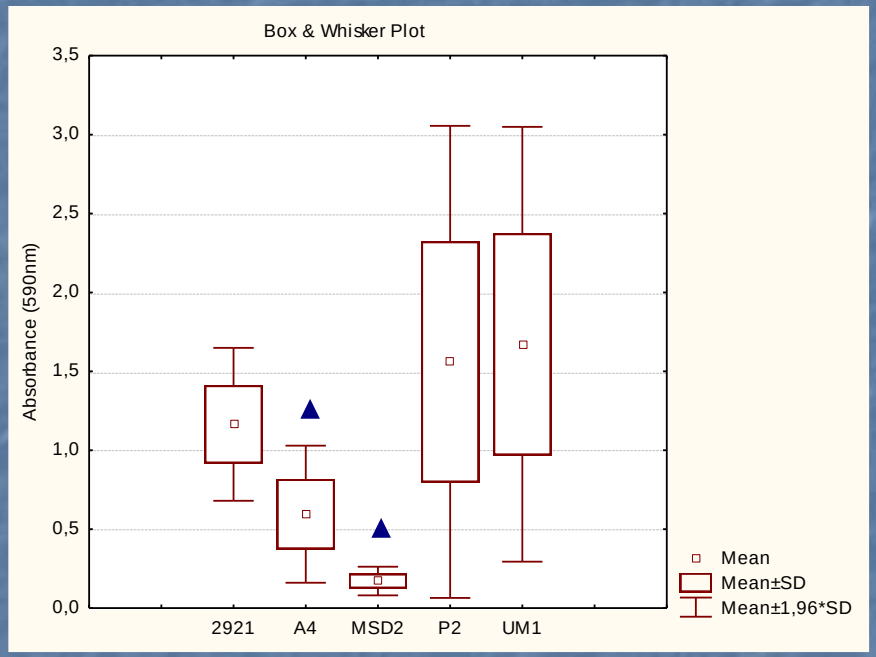
Nielubowicz y Mobley, 2010

Mutantes de *P. mirabilis* (transposición) con capacidad alterada para formar biofilms

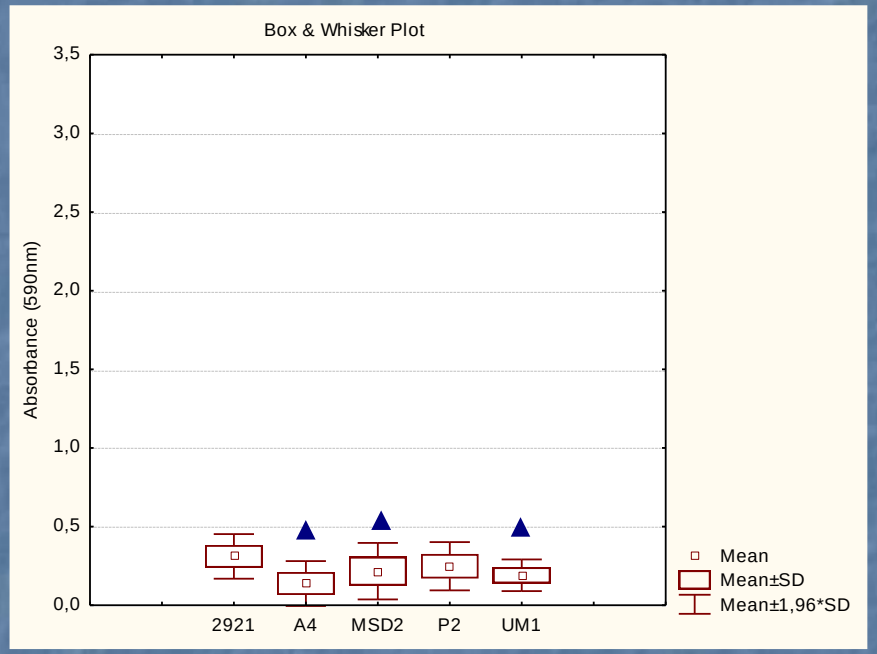
Función	Cepa mutante	Gen mutado
Adhesión (proteínas fimbriales)	41 94 106	Fimbrial protein SteB Fimbrial protein Major fimbrial subunit
Sistemas de hierro	50 72 110	Ferritin Peptide synthetase Ton-B-dependent receptor
Transportadores	40 74 104	MFS family transporter Two partner secretion system (SSTV) PitA
Sistemas de reparación	52 91	Chemical-damaging agent resistance UvrABC system protein B
Metabolismo	45 55 113	Sensor protein KdpD GntR Nitrite reductase nirD
Otros	60	outer membrane porin protein C

Genes de distintas familias y funciones afectan la formación de biofilms

Capacidad de formación de biofilms en superficies abióticas: influencia de fimbrias y flagelos de *P. mirabilis:* "ensayo del cristal violeta"

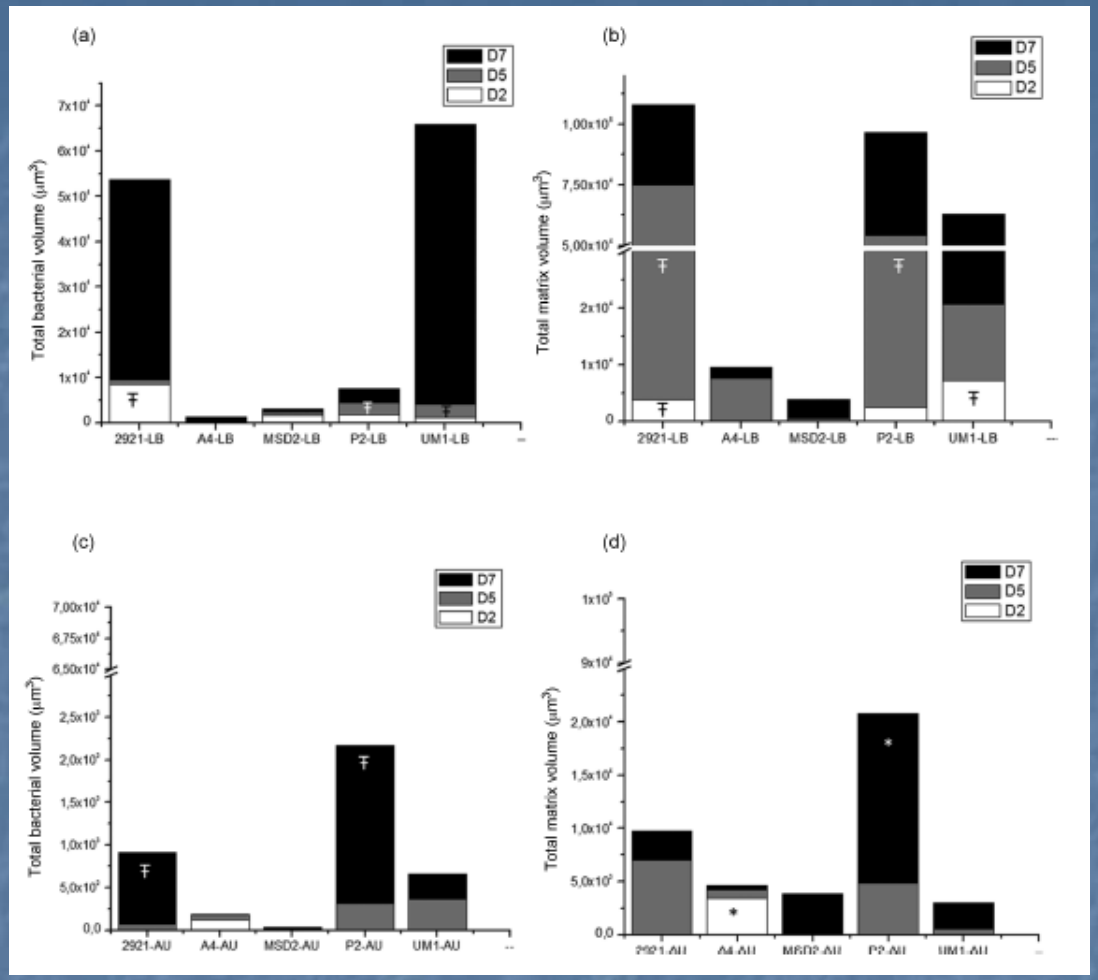


Caldo LB



Orina artificial

▲ Valores significativamente diferentes con respecto a la cepa salvaje(Mann-Whitney, P<0.05)

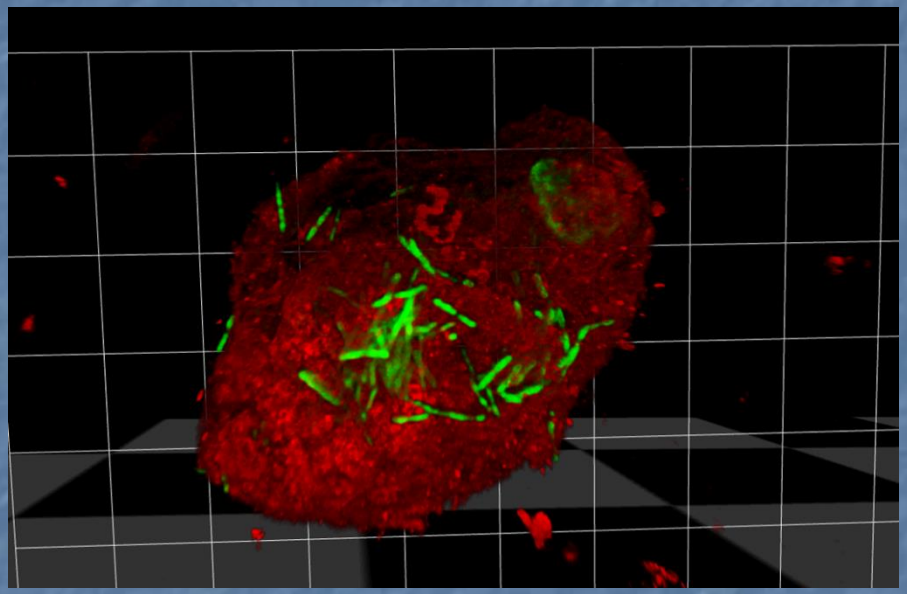


Total de bacterias y volumen de matriz en biofilms de *P. mirabilis* en caldo LB y en orina artificial (AU).

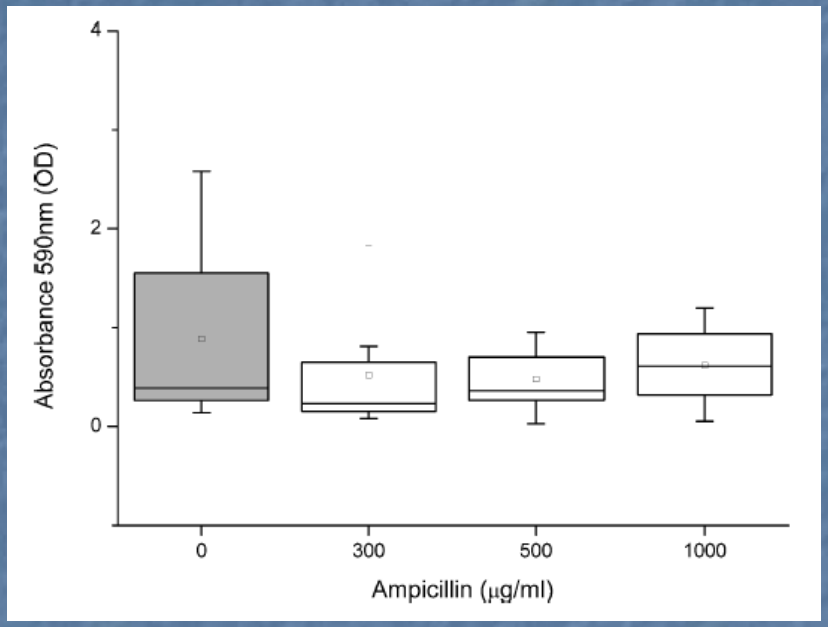
Pr2921 (WT) y mutantes fimbriales UCA, PMF, ATF y MR/P (UM1, P2, A4 y MSD2, respectivamente) en caldo LB (a y b) y en AU (c y d).

* Indica diferencias significativas en comparación con todas las otras cepas. ¯T indica diferencias significativas en comparación con las otras cepas pero no entre ellas

UPEC, biofilms y comunidades intracelulares



Anderson et al., 2003, Robino et al, 2013; Robino et al, 2014



No hay relación entre cepas formadoras de biofilms y CBI

Se relacionaron genes de las fimbrias P y la formación de biofilms

Cepas susceptibles a ampicilina no disminuyen la biomasa del biofilm ante el tratamiento con el antibiótico

González et al., 2017

Métodos básicos de estudio de los biofilms

Biofilms estáticos

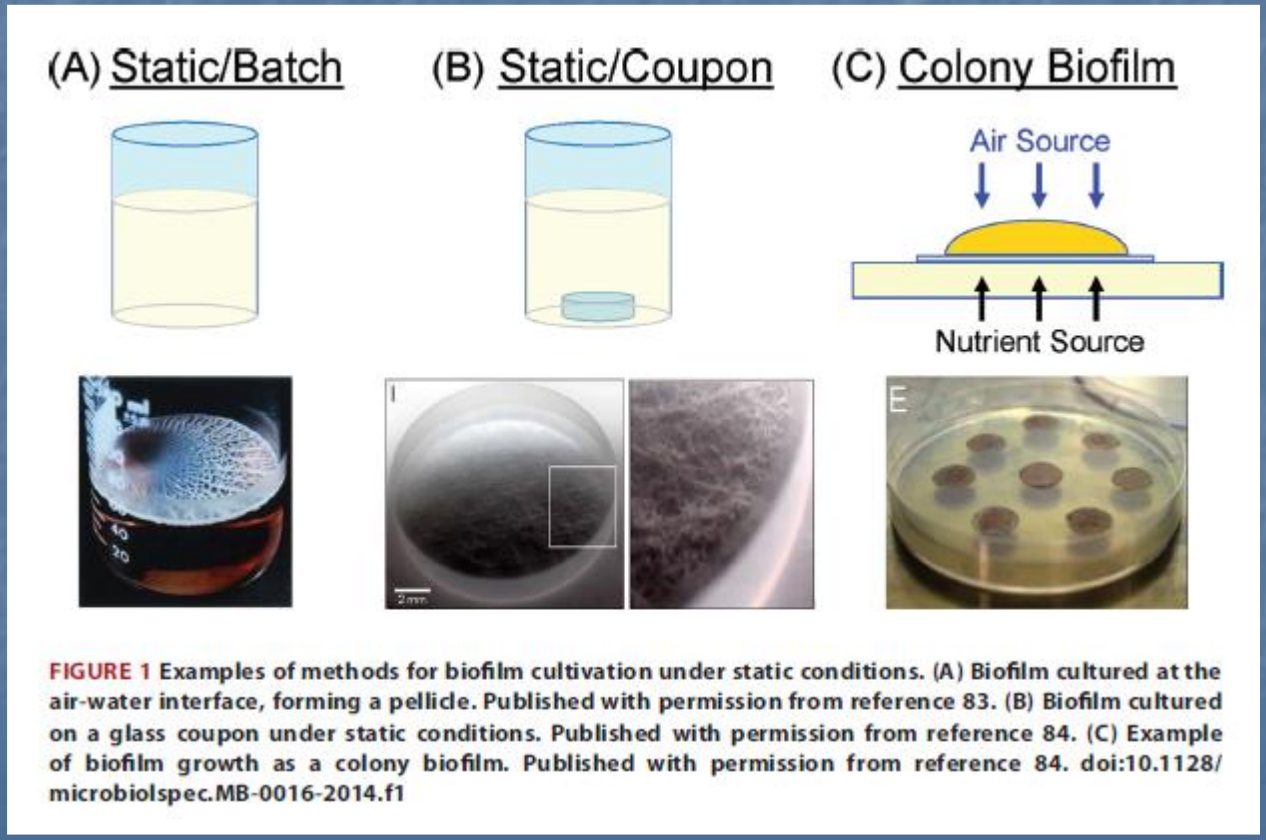


FIGURE 1 Examples of methods for biofilm cultivation under static conditions. (A) Biofilm cultured at the air-water interface, forming a pellicle. Published with permission from reference 83. (B) Biofilm cultured on a glass coupon under static conditions. Published with permission from reference 84. (C) Example of biofilm growth as a colony biofilm. Published with permission from reference 84. doi:10.1128/microbiolspec.MB-0016-2014.f1

Biofilms en sistemas de flujo