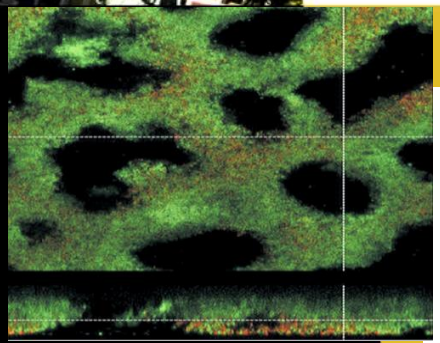
Formación de biofilms:

Let´s stick together

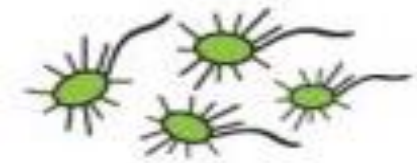
Zaragoza,
27- Abril- 2022

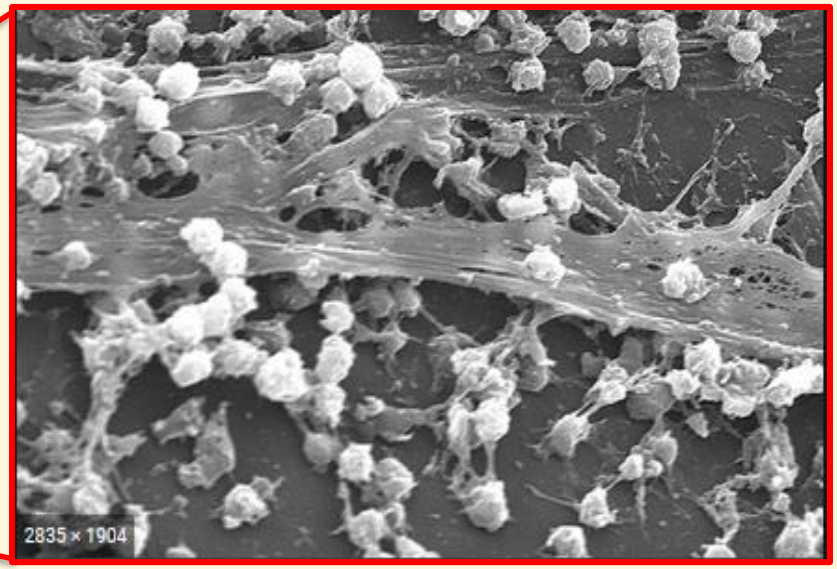
Jesús Arenas
jaarenas@unizar.es



1542
Facultad de Veterinaria
Universidad Zaragoza

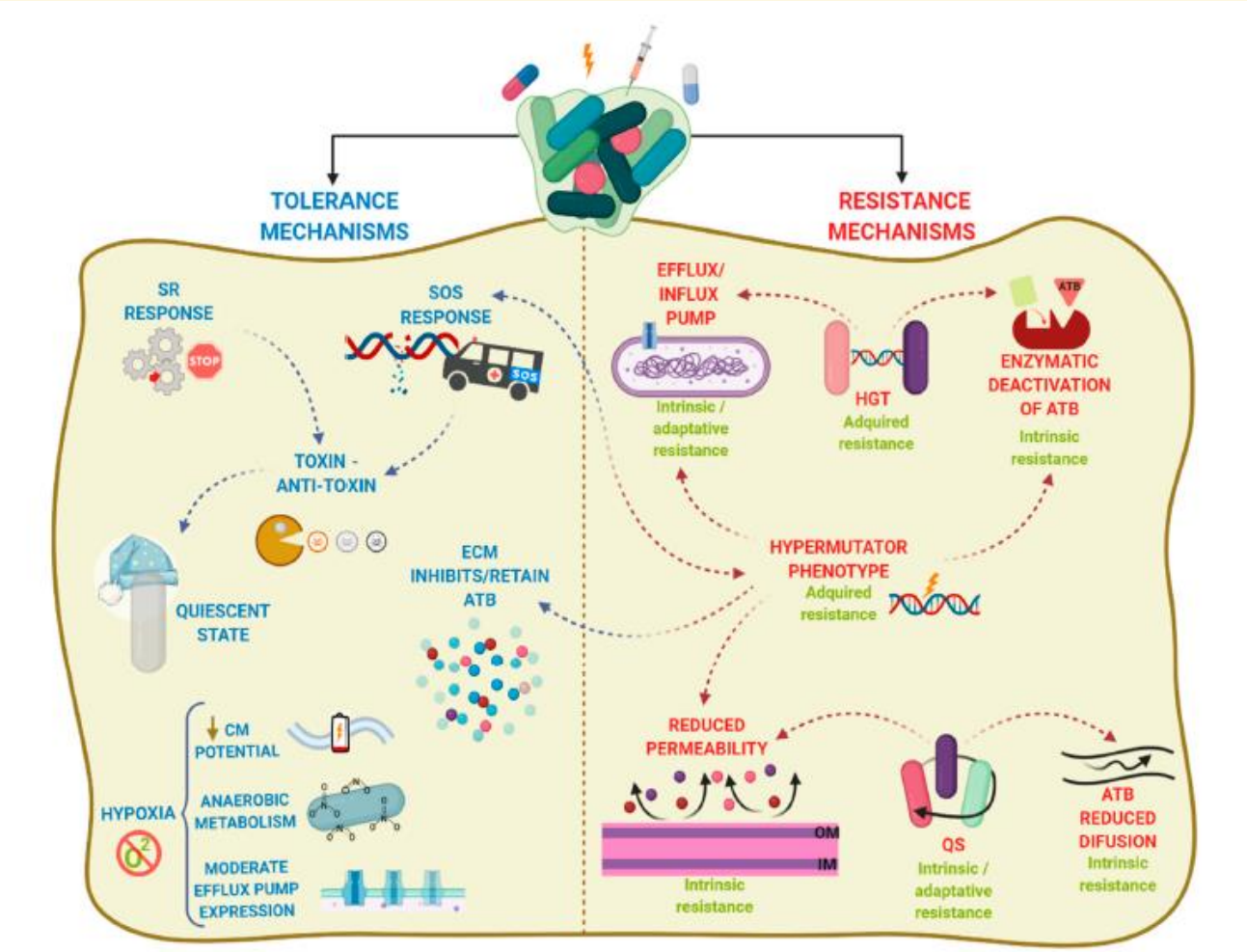
Biofilms





Comunidad microbiana embebida en una **matriz extracellular** (ECM) producida por sí misma.

Biofilms



Urién C,…, **Arenas**. 2021. *Antibiotics,* IF 4.2

Biogenesis de Biofilms

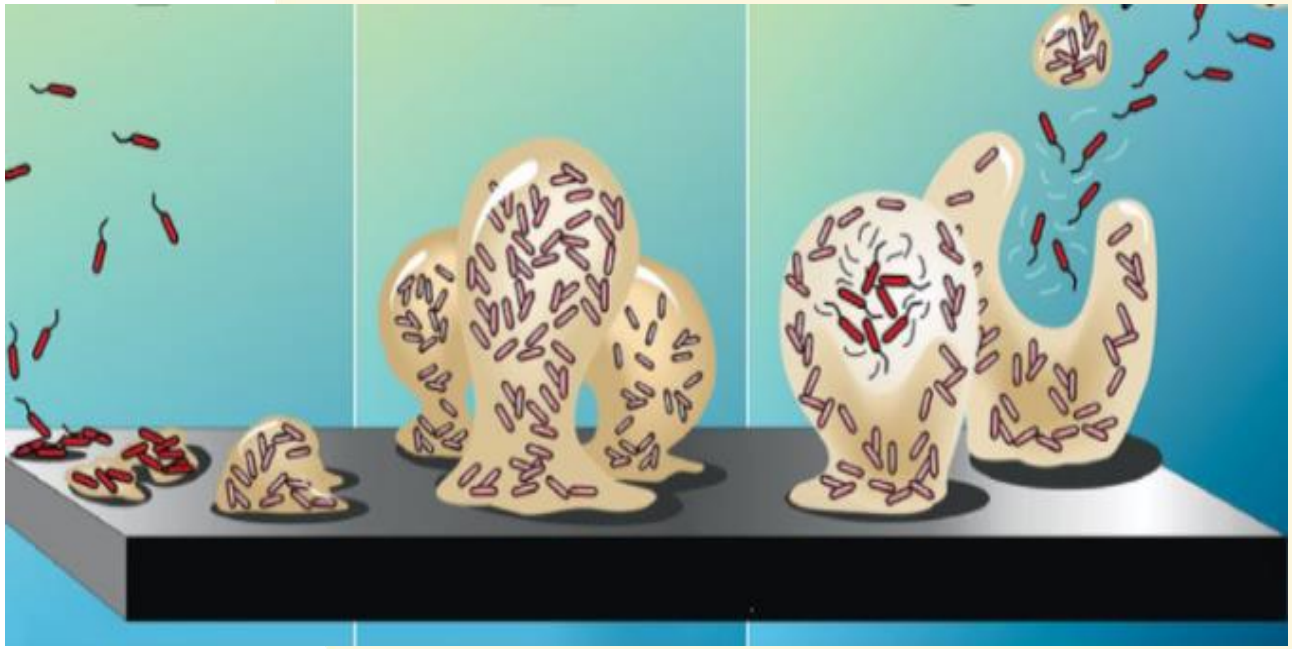
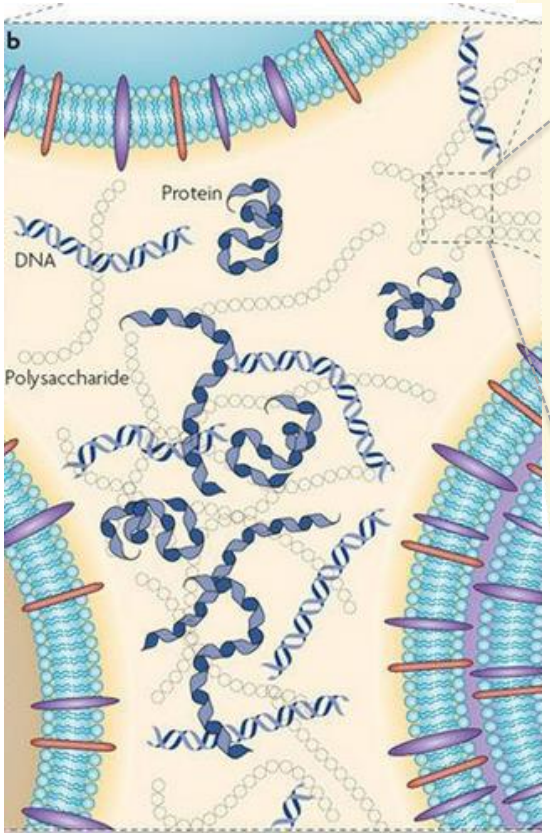
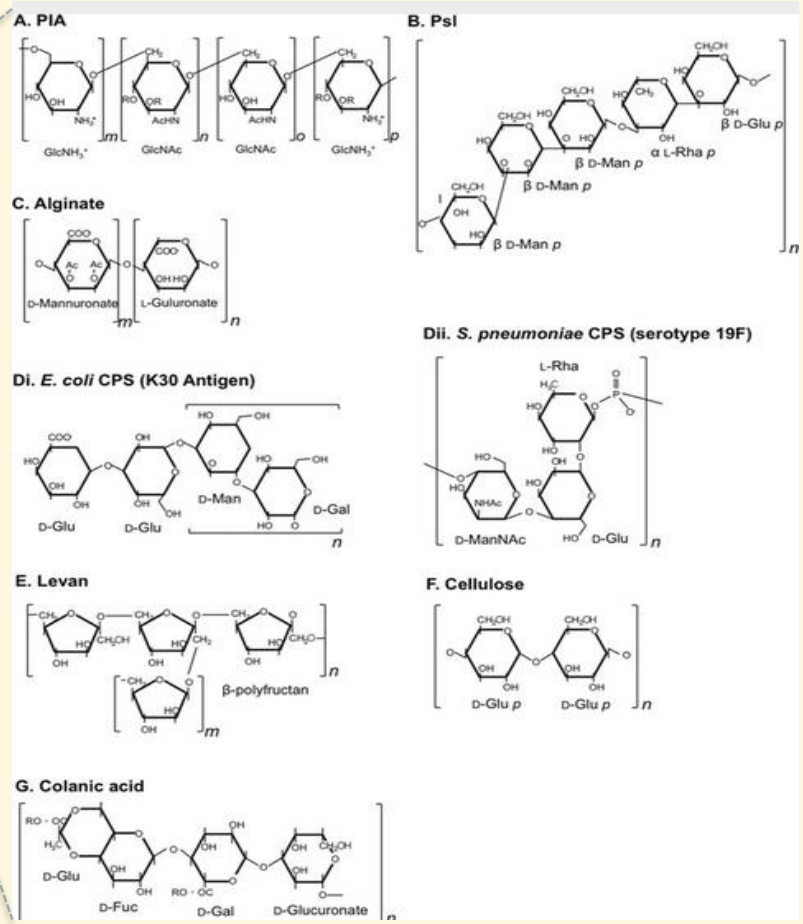


Figura. Proceso de formación de biofilms (Reichling, 2020)

Biogenesis de Biofilms

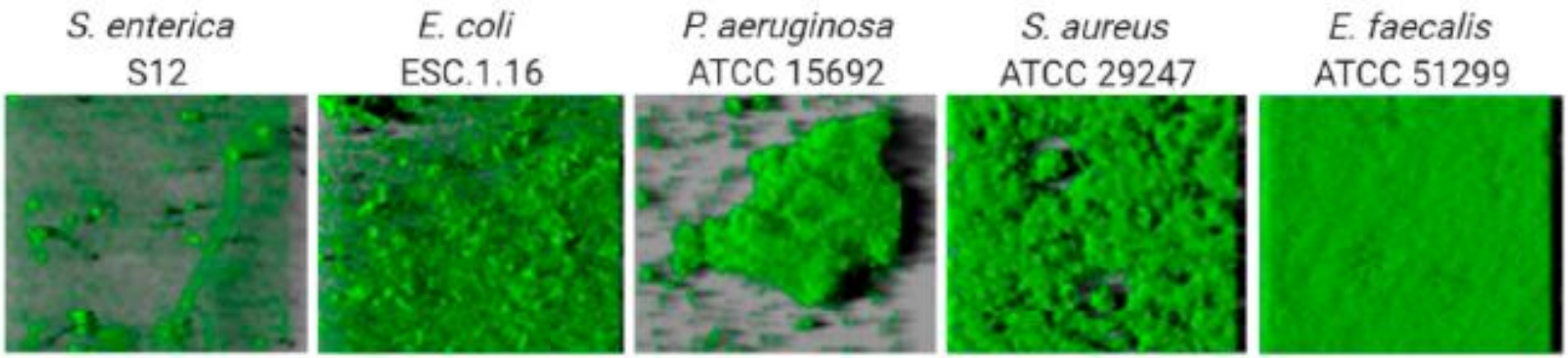
La matriz extracelular





Biogenesis de Biofilms

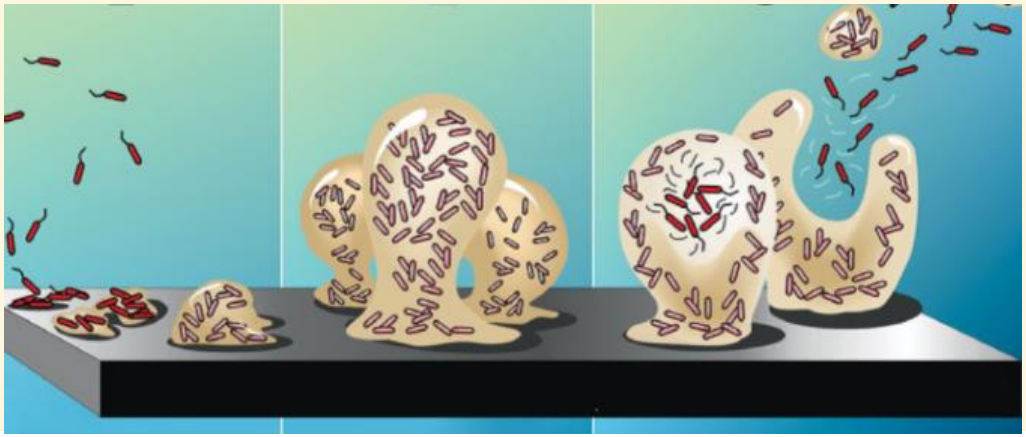
Arquitectura del biofilm

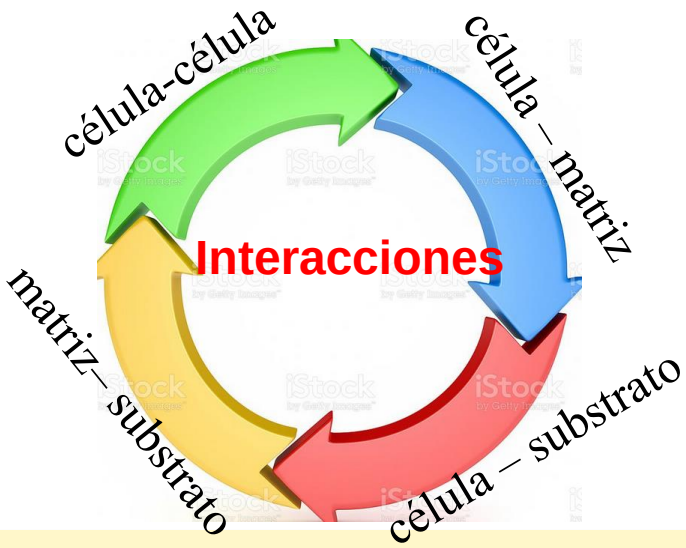


Urién C,…, **Arenas**. 2021.
Antibiotics, IF 4.2

Biogenesis de Biofilms

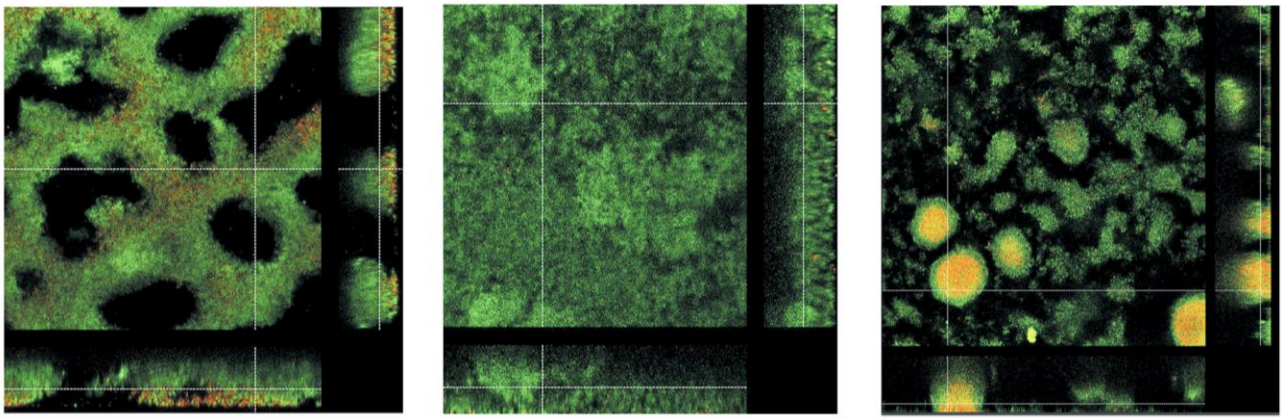
Arquitectura del biofilm:





Biogenesis de Biofilms

Arquitectura del biofilm: *Neisseria meningitidis* como microorganismo modelo



Variable Architecture of Meningococcal Biofilms

Arenas and Tommassen, 2017.
Trends in Microbiology. IF 11,7

Biogenesis de Biofilms

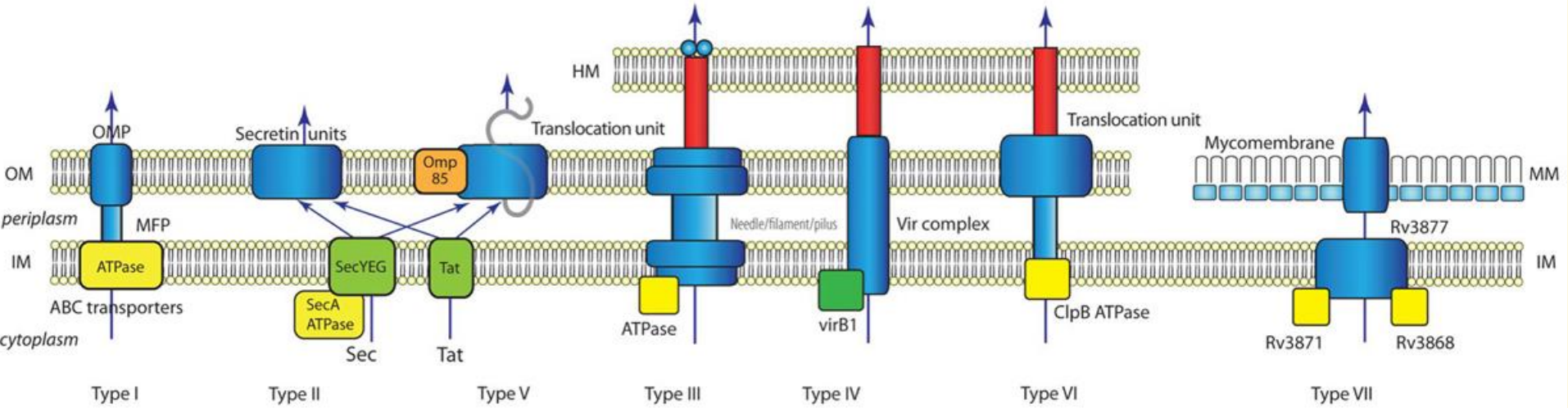
Neisseria meningitidis

Sim et al. 2000.
Lancet ***IF 18,2***

- Gram-negative diplococcus.
- Colonizes the human nasopharynx asymptomatically.
- Occasionally, it causes meningitis and/or sepsis.
- Disease is known for its rapid onset.

Biogenesis de Biofilms

Sistemas de Secreción en Bacterias Gram negativas

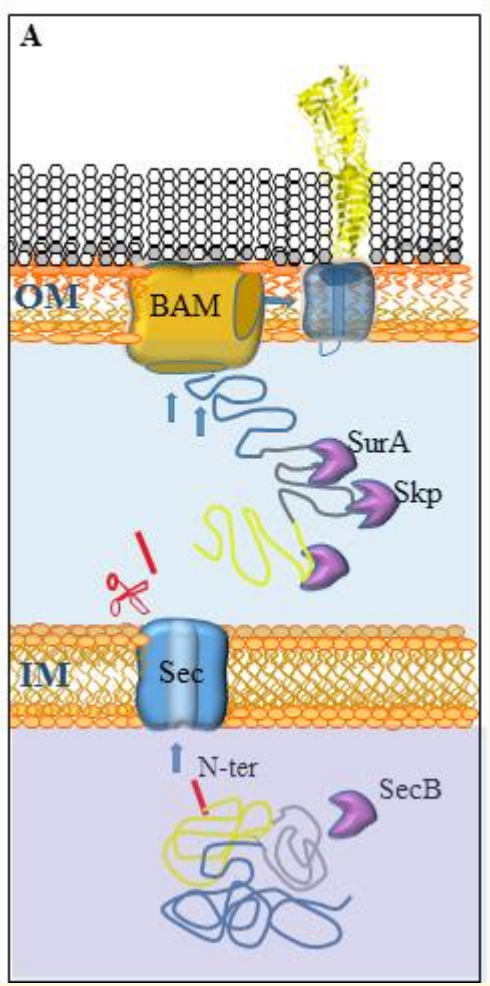


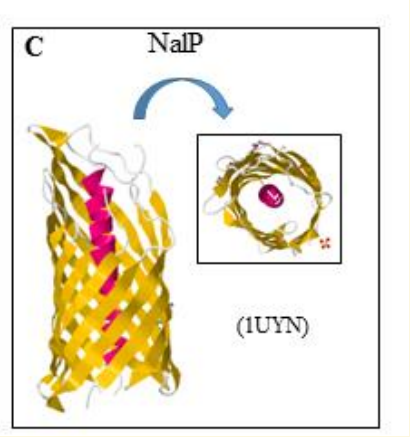
***Neisseria Secretion Systems*:** Type I and Type V

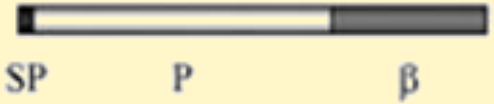
Biogenesis de Biofilms:
Sistema de secreción tipo V

Type V Secretion Systems (a-e)

a) **Autotransportadores Clásicos**
b) **Two partner secretion system**
c) **Autotransportadores trimericos**







Tommassen and **Arenas**, 2017
Frontiers Cell Infection in Microbiology IF4,2

Biogenesis de Biofilms

Sistema de Secrecion tipo V en *N. meningitidis*

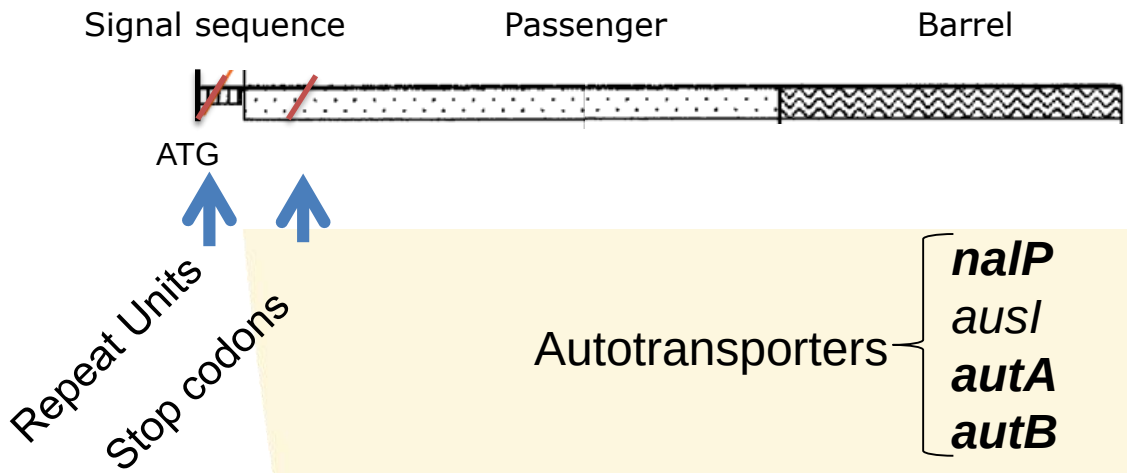
Protein	Function	Secretion System
IgAp	Proteasa (Autocleavage Inmunomodulation)	Classical ATs
AusI	Proteasa y Adhesina (Epithelial and endothelial cells)	Classical ATs
App	Proteasa and Adhesina (Epithelial cells)	Classical ATs
NalP	Proteasa	Classical ATs
AutA	pseudogene?	Classical ATs
AutB	pseudogene?	Classical ATs

Grijpstra, **Arenas**, *et al*. 2013.
Res Microbiol, IF 3,5

Biogenesis de Biofilms

Sistema de Secrecion tipo V en *N. meningitidis*

Regulation de ATs



Autotransporters
- ***nalP***
- *ausI*
- ***autA***
- ***autB***

```
                   aagc
Alpha153   1  atgaaactcg aagcaagcaa gcagaagttt aaaaaatcat ttattataag tctatttttt tctattcttt atacctctcc gcttttggct gttg
               M  K  L   E  A  S   K  Q  K  F   K  K  S   F  I  I   S  L  F  F   S  I  L   Y  T  S   P  L  L  A   V

9757       1  atgaaactcg aagcaagcaa gcaagcaagc aagcaagcag aagtttaaaa aatcatttat tgtaagtcta tttttttcta ttctttatac ctct
               M  K  L   E  A  S   K  Q  A  S   K  Q  A   E  V  -   K  I  I  Y   C  K  S   I  F  F   Y  S  L  Y   L
```

Tommassen and **Arenas**, 2017
Frontiers Cell Infection in Microbiology, IF 3,5

Objectivos

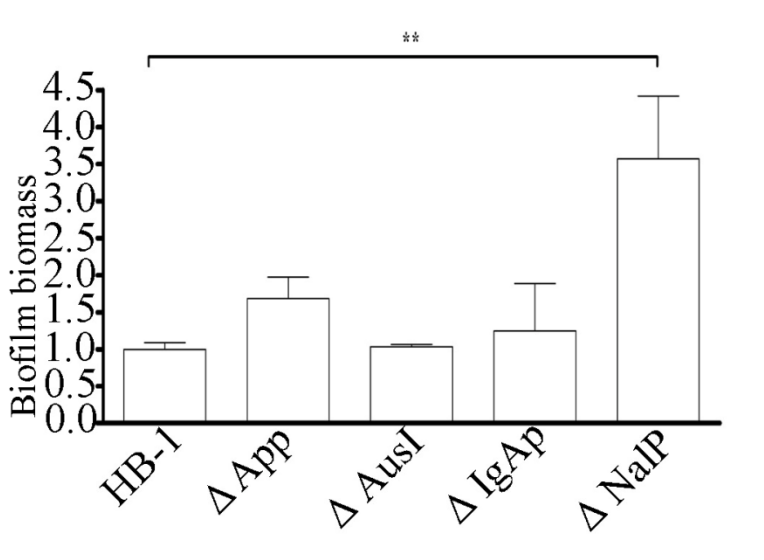
Determinar el papel de autotransportadores en la formación del biofilm

Fase I. Role of proteases (AusI, App, IgA protease, y NalP)

Fase II. Role of novel ATs (AutA, AutB)

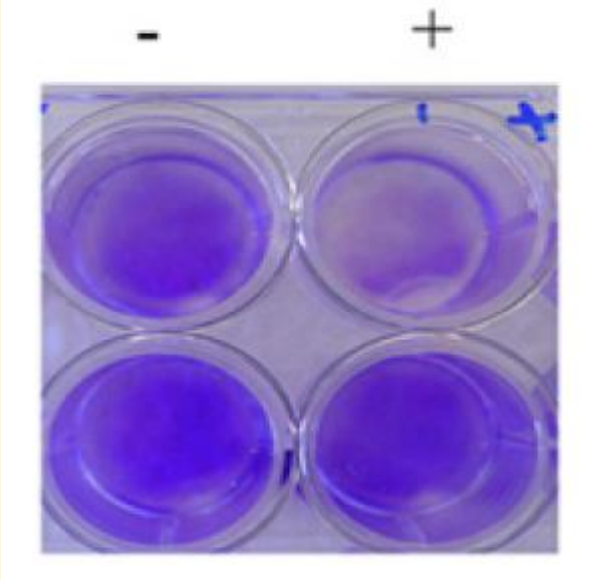
Biogenesis de Biofilms: *N. meningitidis*

HB-1



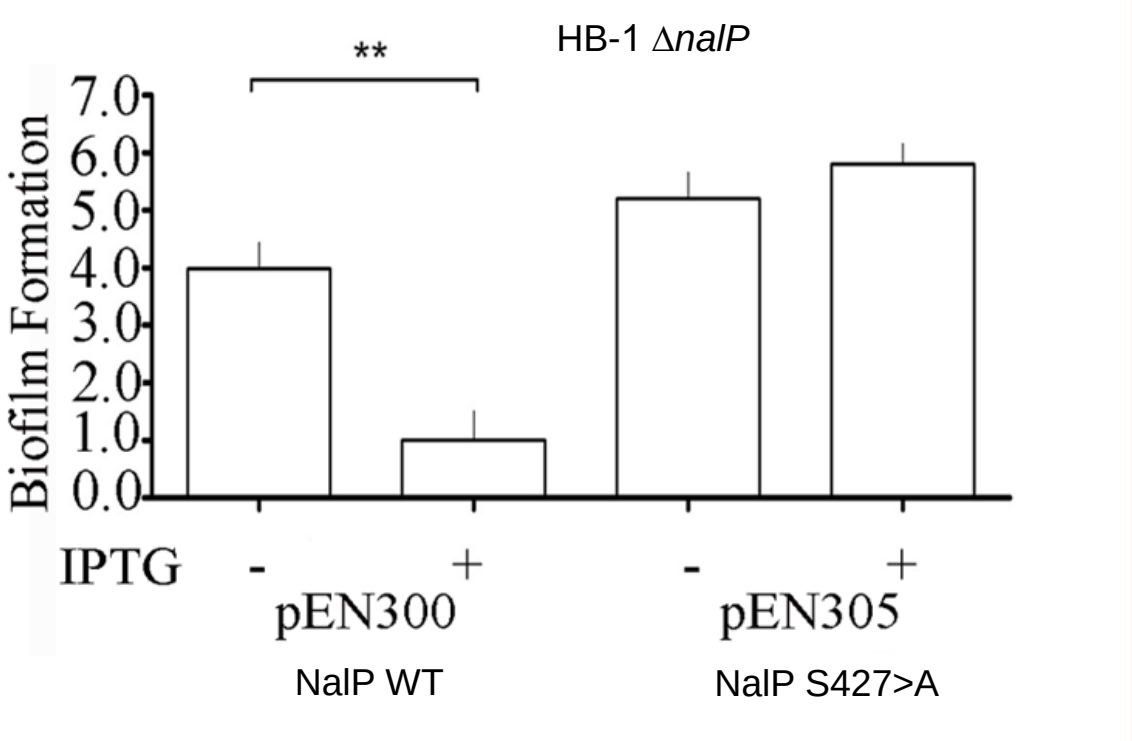
Biofilms de 1 h

Biofilms en condiciones estáticas



Arenas *et al*., 2013.
Mol Microbiol, IF 5,2

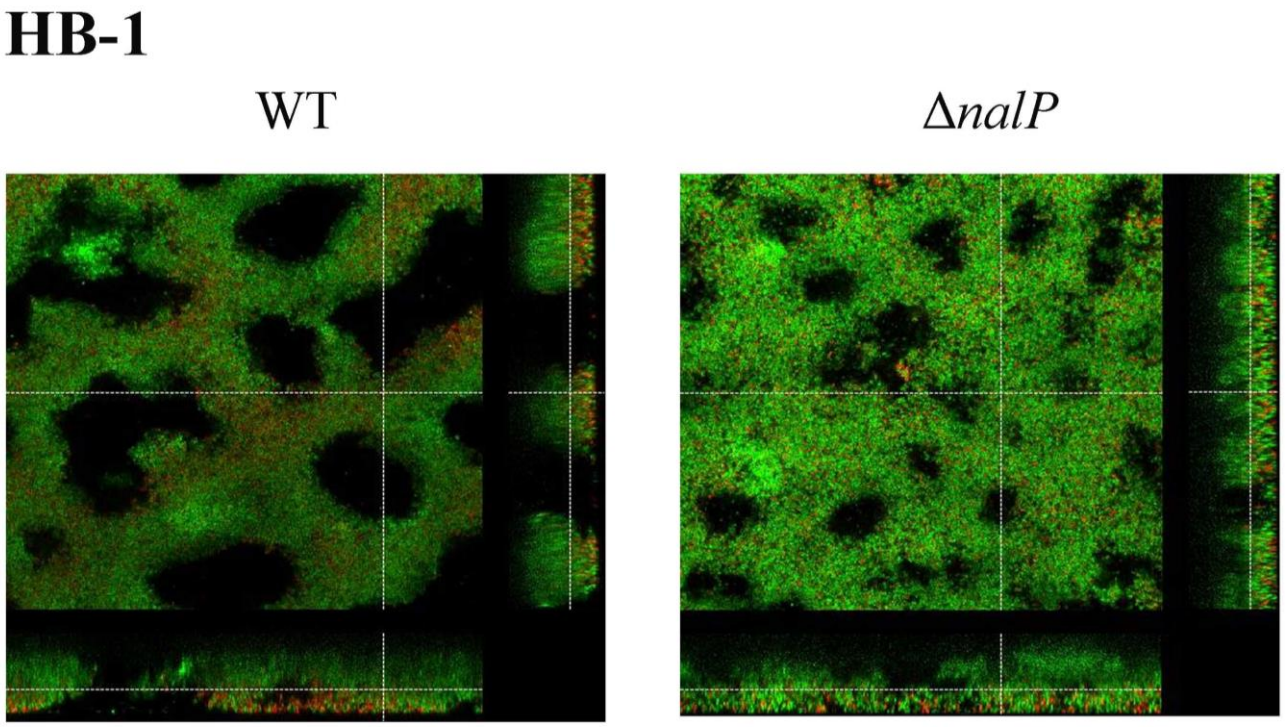
Biogenesis de Biofilms: *N. meningitidis*



El efecto de NalP depende de su actividad proteasa

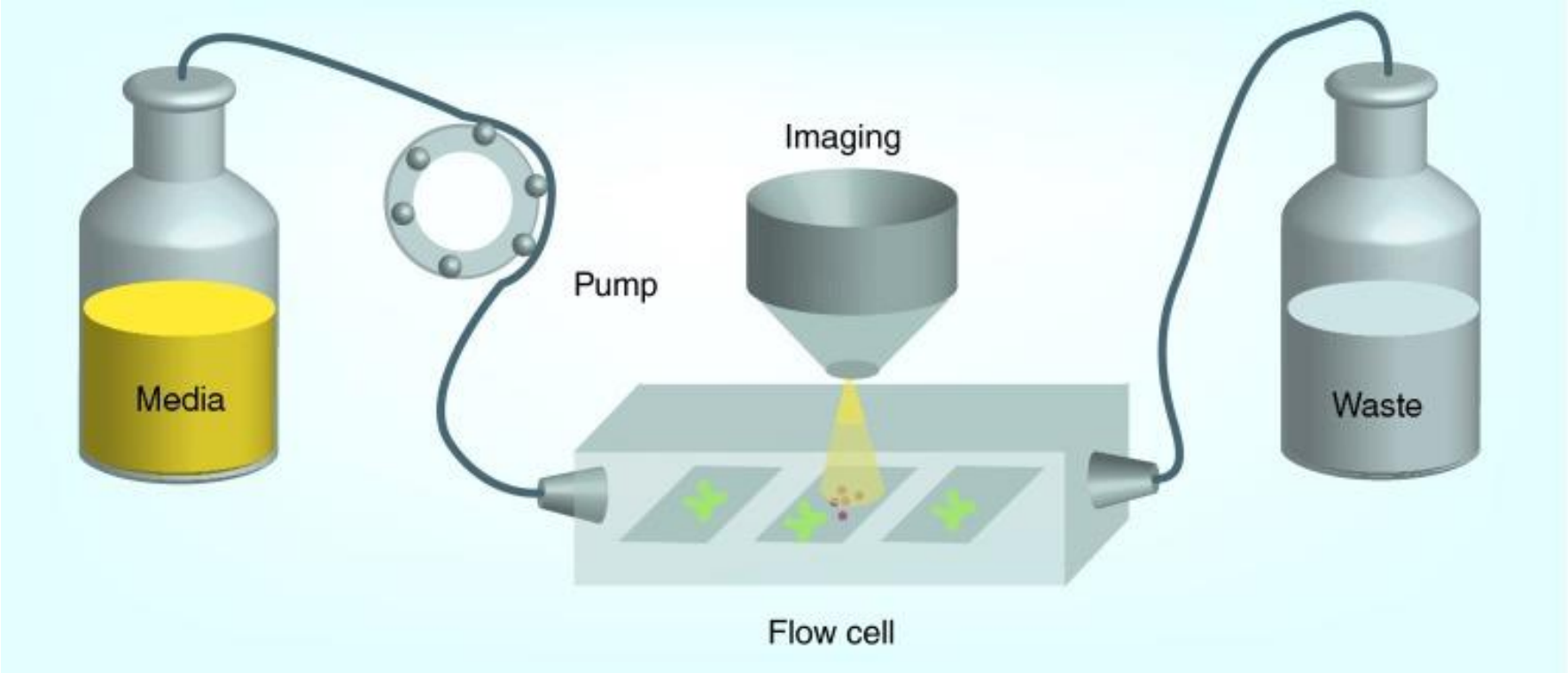
Arenas *et al*., 2013.
Mol Microbiol. IF 5,2

Biogenesis de Biofilms: *N. meningitidis*



Biogenesis de Biofilms: *N. meningitidis*

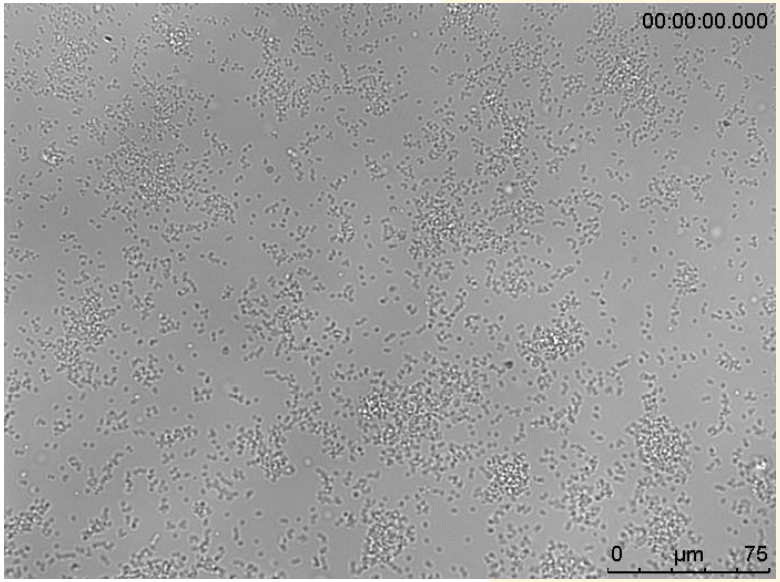
Formación de biofilms en Cámaras de flujo



Biogenesis de Biofilms: *N. meningitidis*

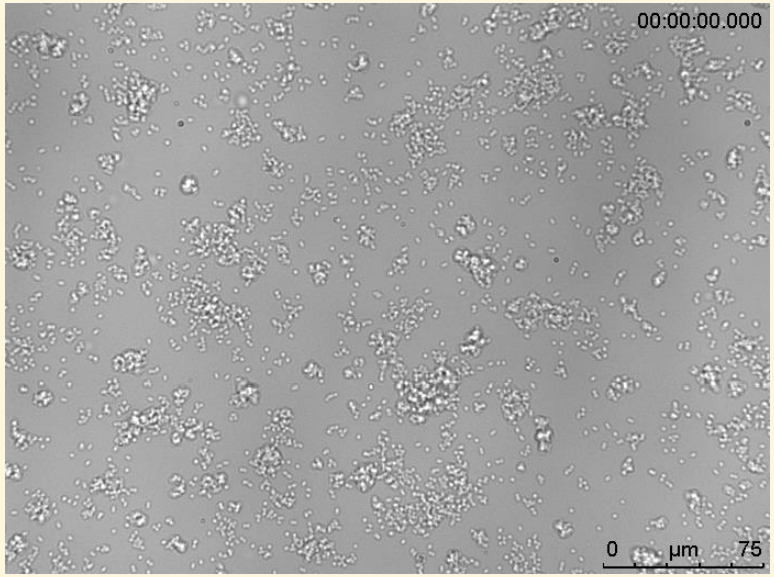
Biofilm development

HB-1 wildtype



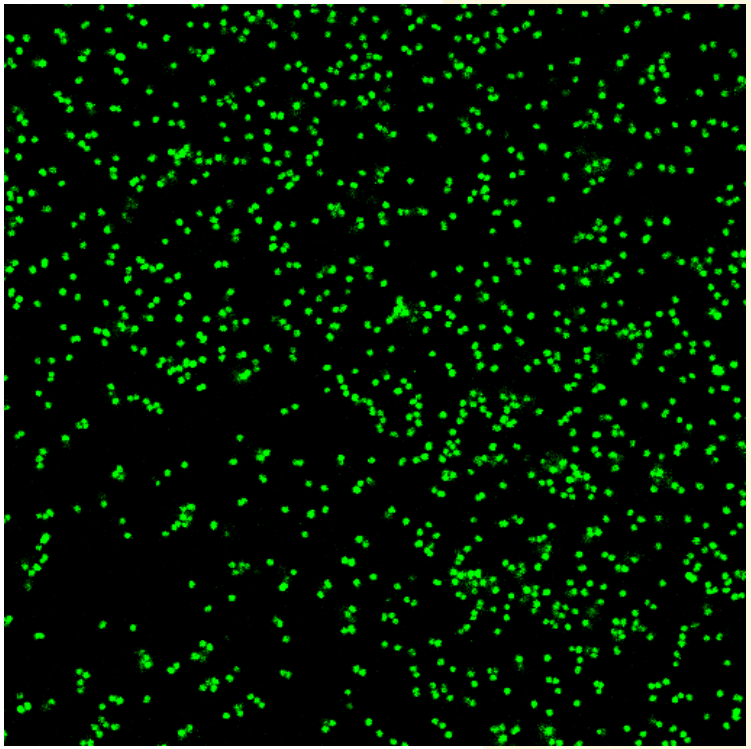
HB-1_WT-Pos003(1).mov

HB-1 *nalP*::*kan*

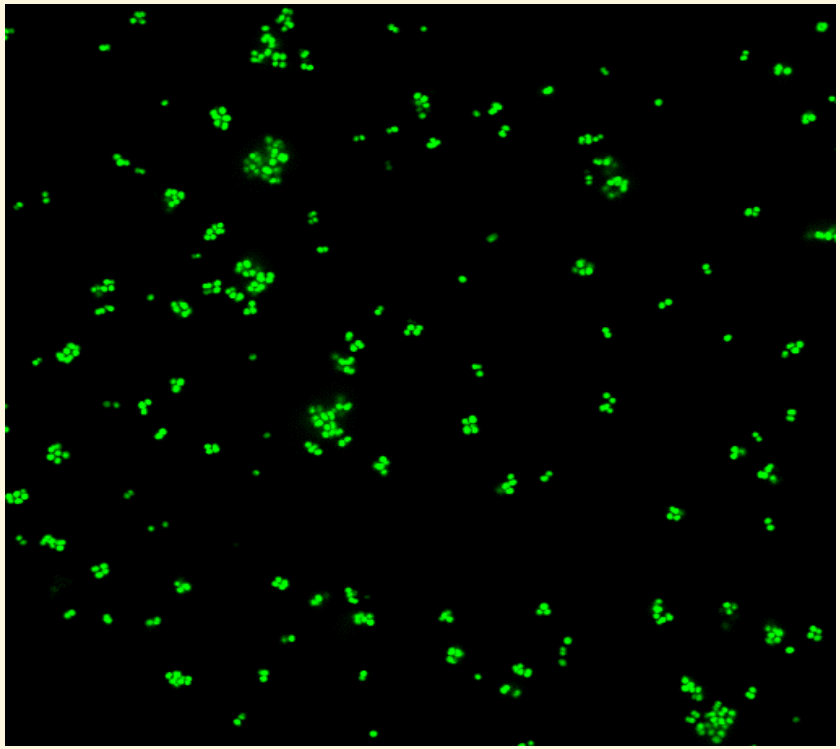


HB-1_nalP-Pos007.mov

Biogenesis de Biofilms: *N. meningitidis*



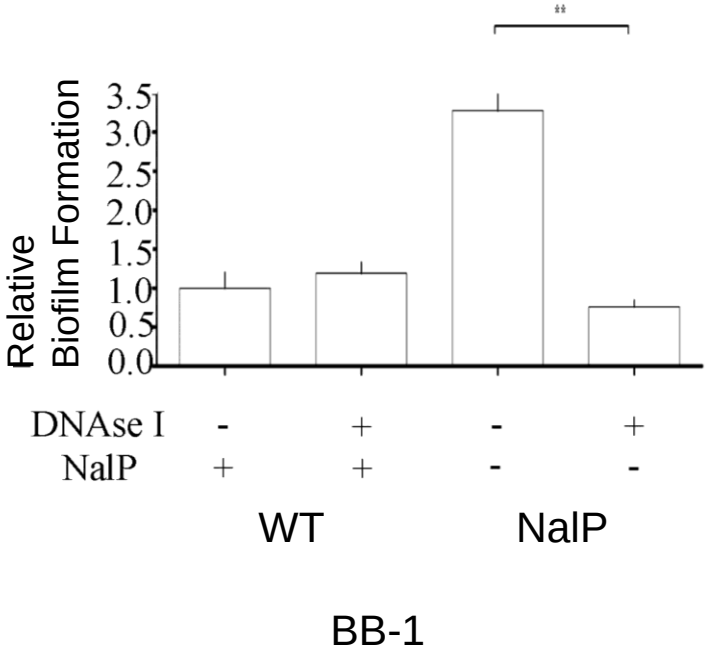
HB-1

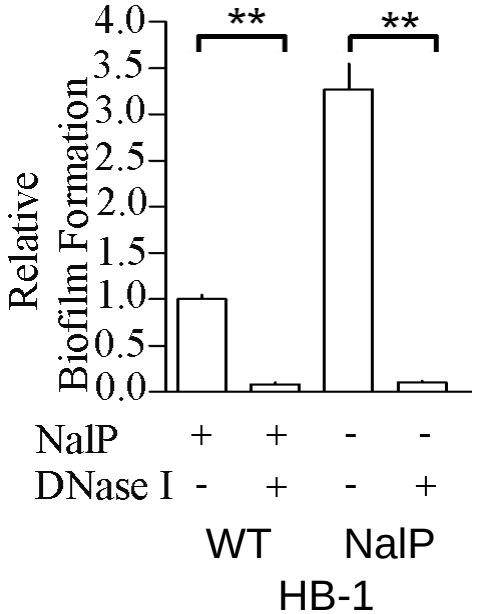


HB-1 Δ*pilE*

Sixty images were taken at 0.1-ms.

Biogenesis de Biofilms: *N. meningitidis*





El efecto de NalP en la formación de biofilm está relacionado con ADN extracellular

Function of NalP

Expression is prone to phase-variation by slipped-strand mispairing

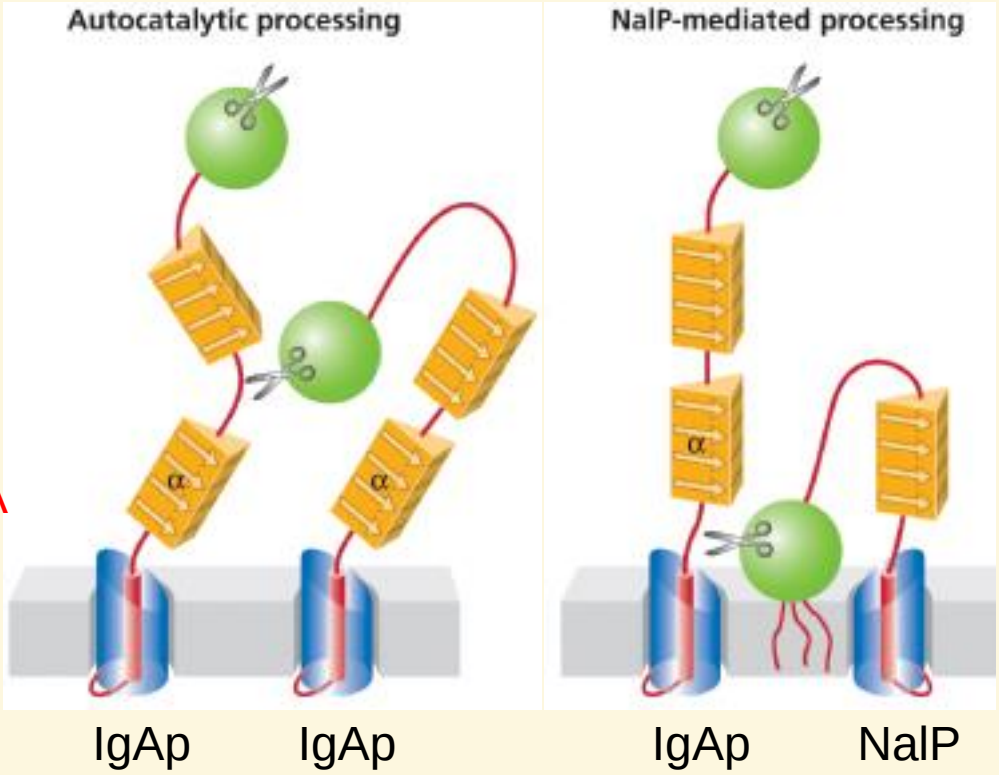
Protease that cleaves:

Substrates

- Autotransporters
  - App
  - IgAp
  - AusI

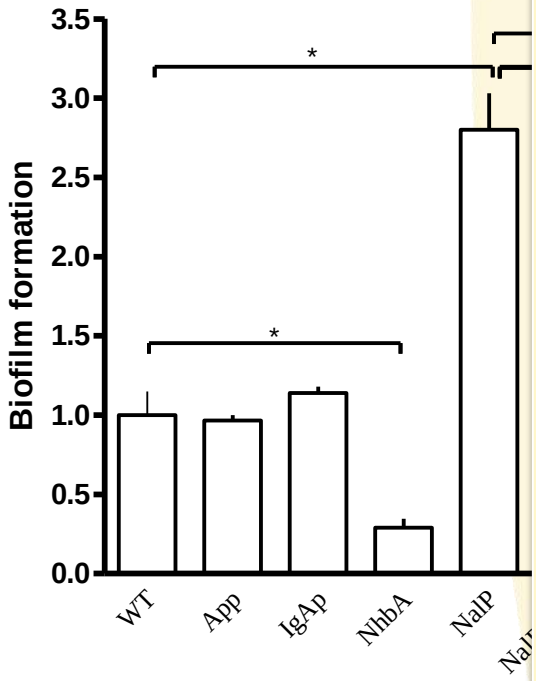
  van Ulsen et al, 2003 and 2006
- Lipoproteins
  - NhbA
  - LbpB

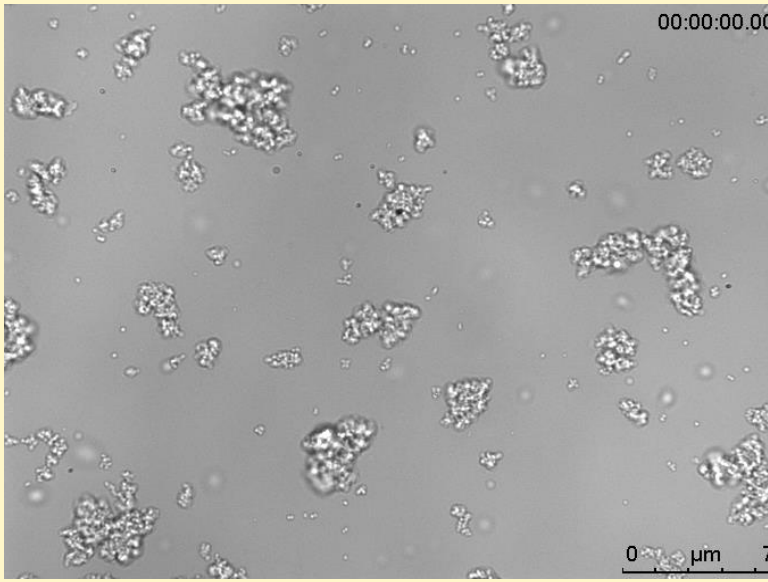
  Serruto et al, 2010
  Roussel-Jazédé V et al, 2010



van Ulsen and Tommassen, 2006. FEMS

Biofilm formation in strain HB-2 (markerless capsule knockout)

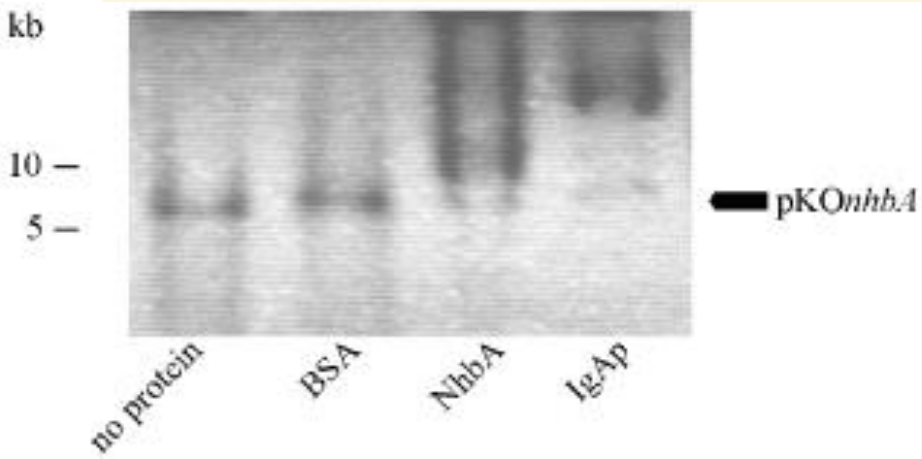




HB-1_nhbA-Pos012(1).mov

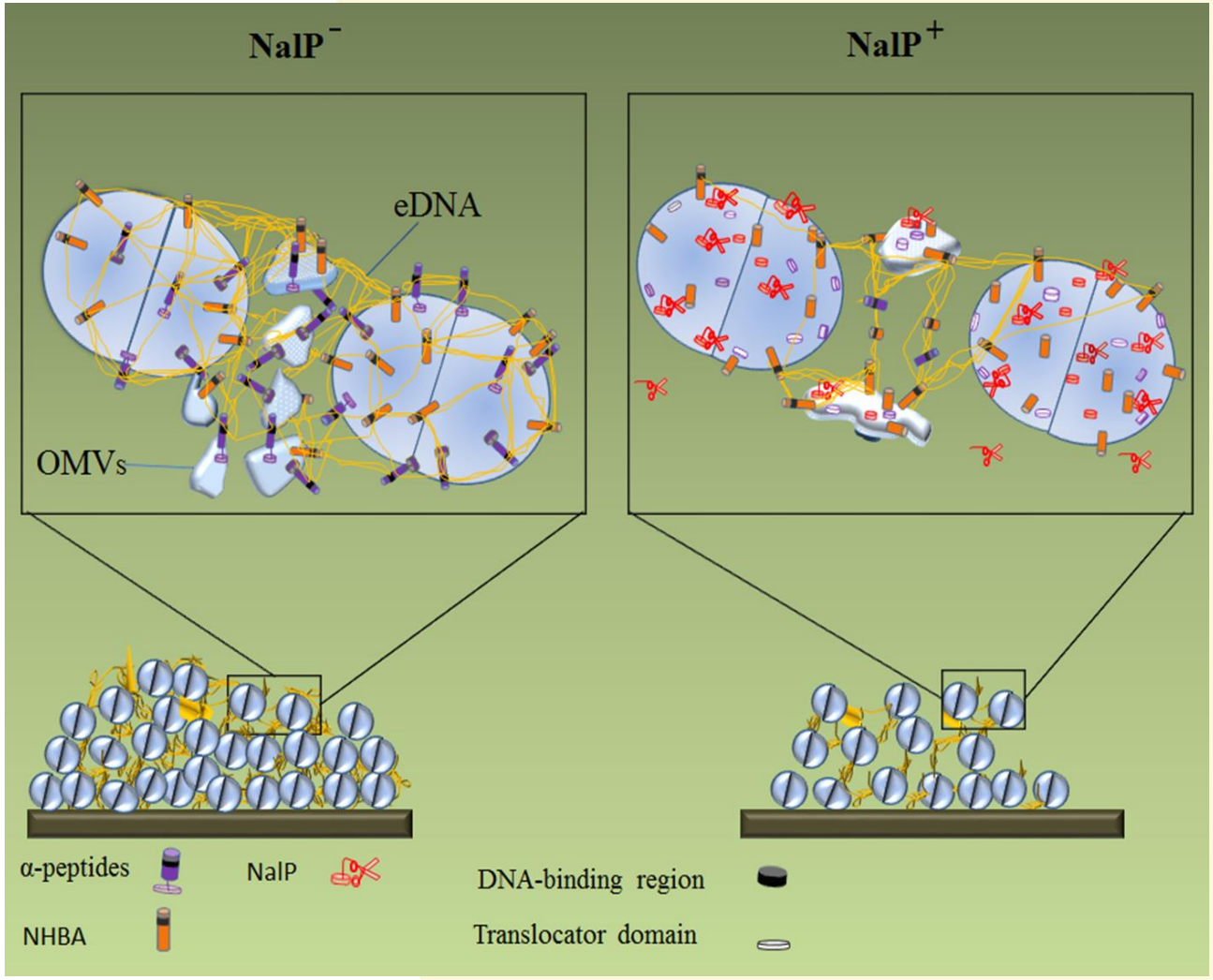
NhbA y IgaP unen DNA y heparina

EMSA- DNA binding assays



Arenas *et al*., 2013.
Mol Microbiol. IF 5,2

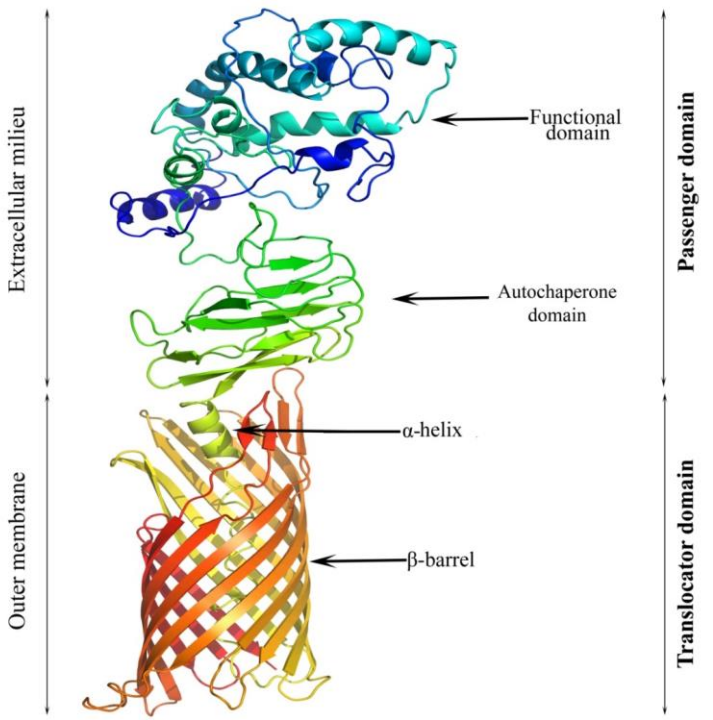
Biofilm Formation Model

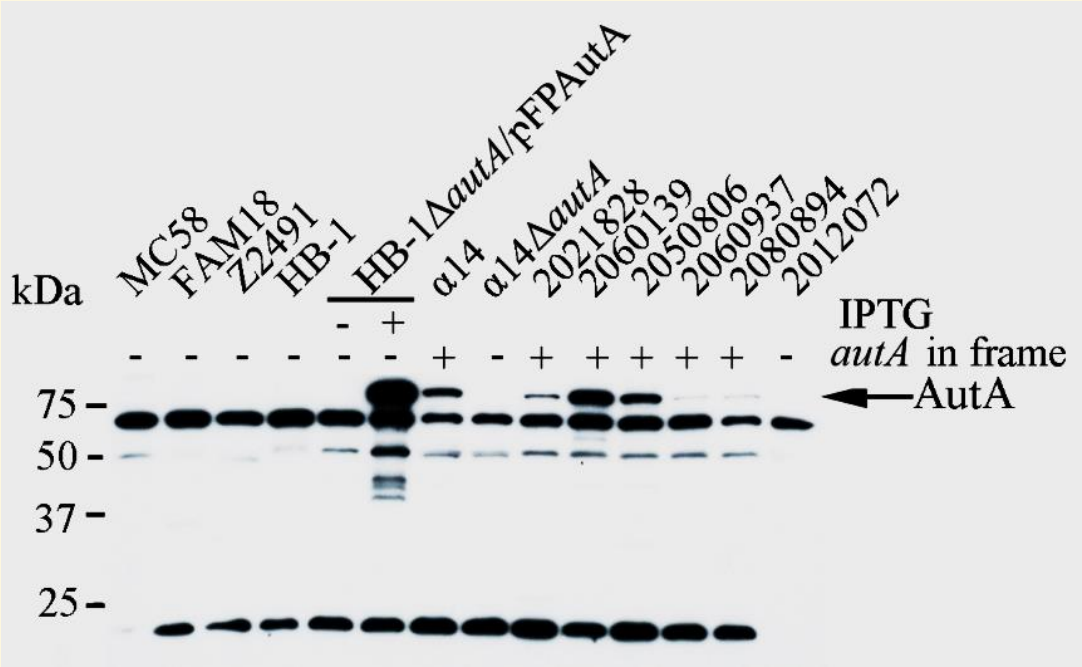


Arenas and Tommassen, 2017. *Trends Microbiol.*

Biogenesis de Biofilms: *N. meningitidis*

Estructura de AutA



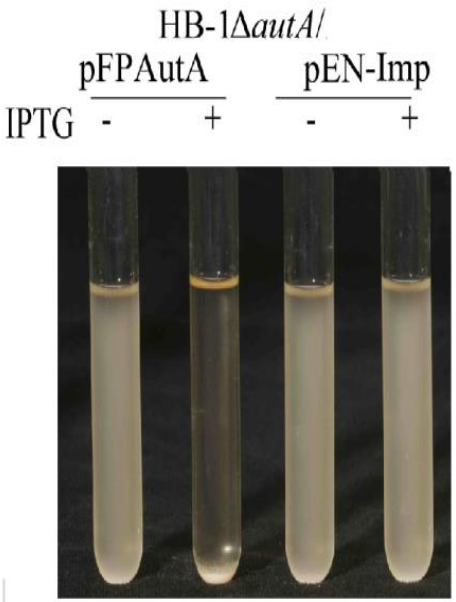


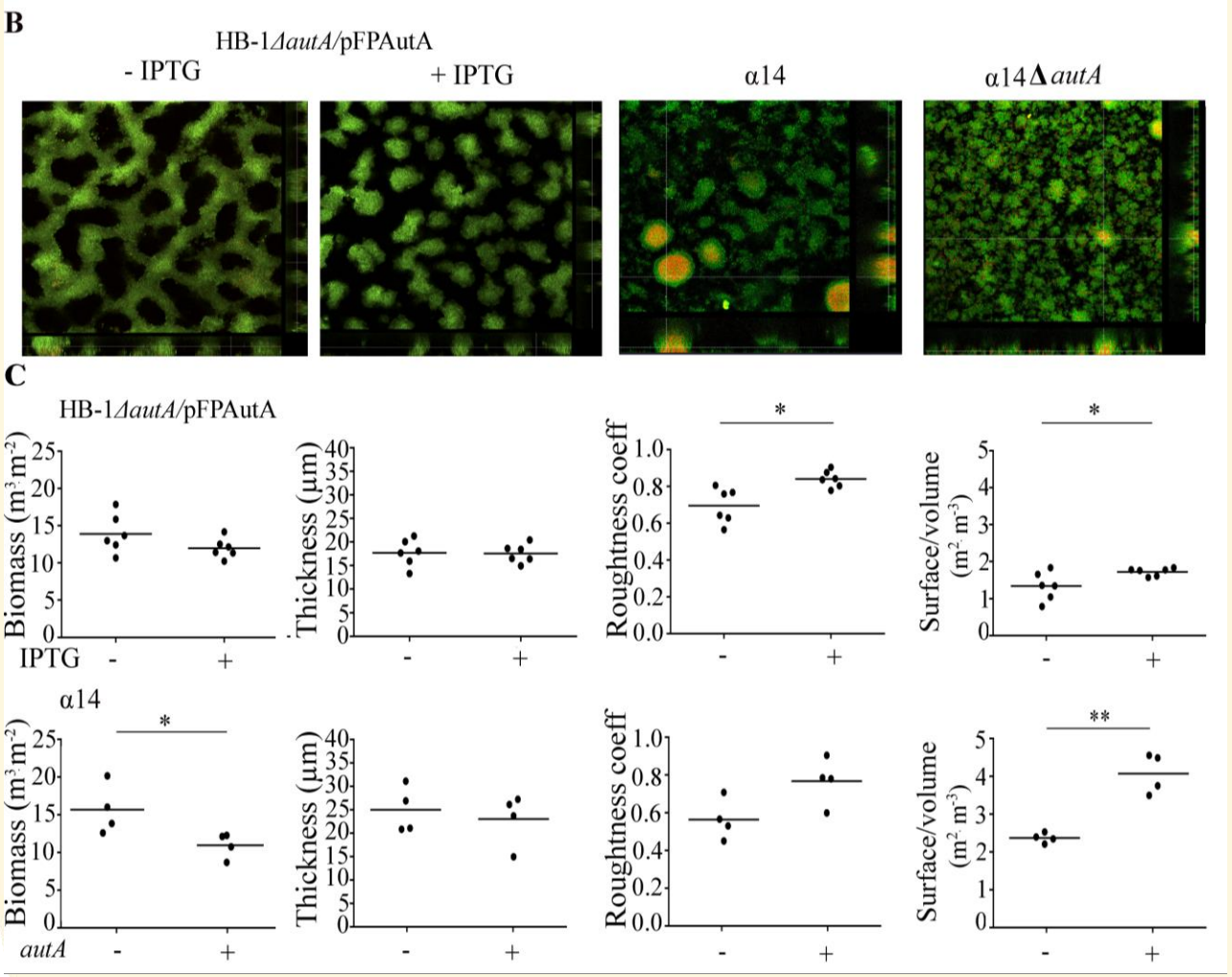
Western blot Anti-AutA

AutA es expresado pero su expresión es limitada a ciertas cepas

Arenas *et al.*, 2014,
Environ. Microbiol. IF 6,4

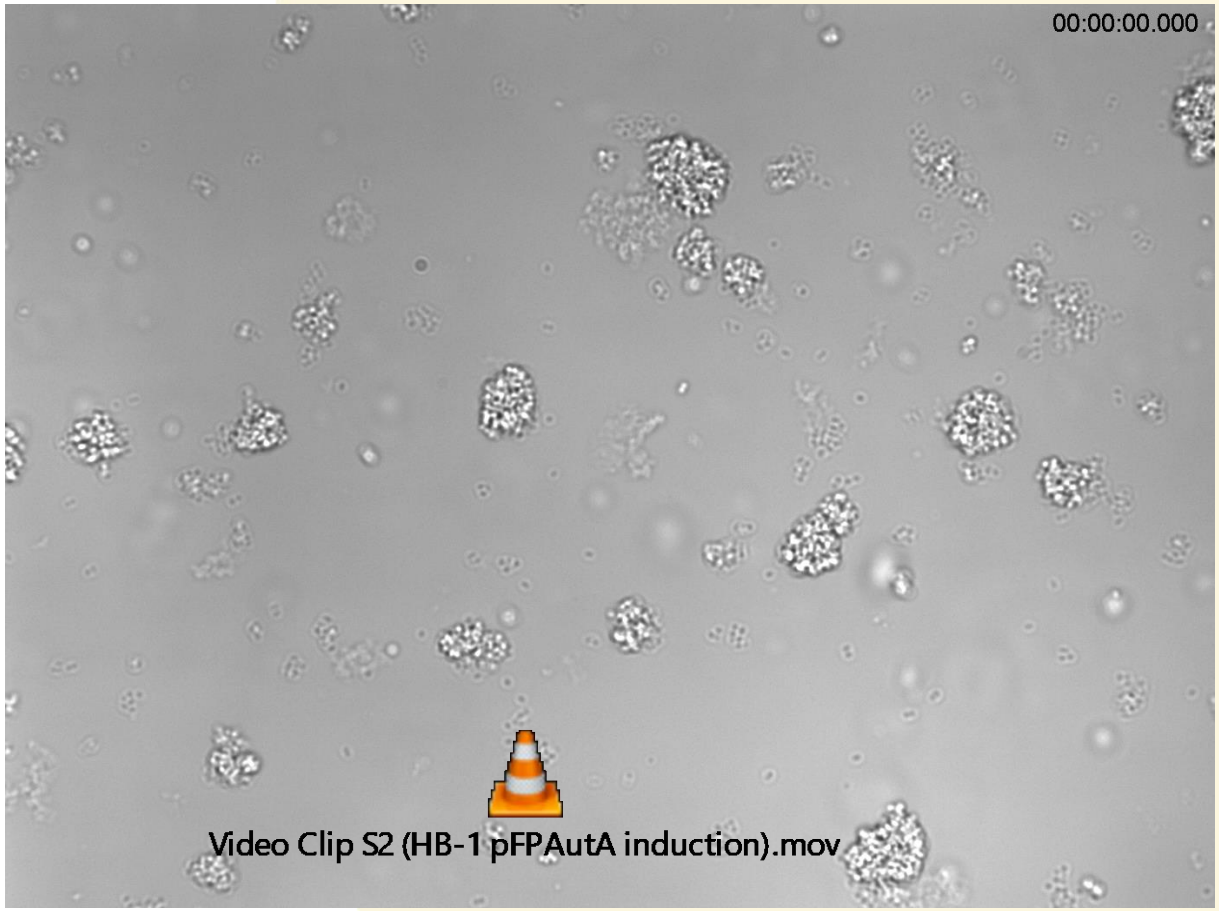
Biogenesis de Biofilms: *N. meningitidis*



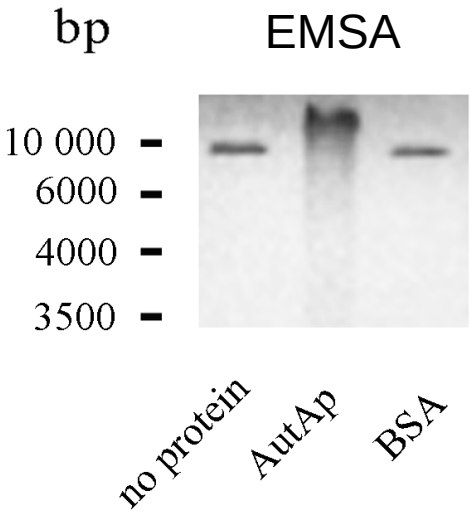


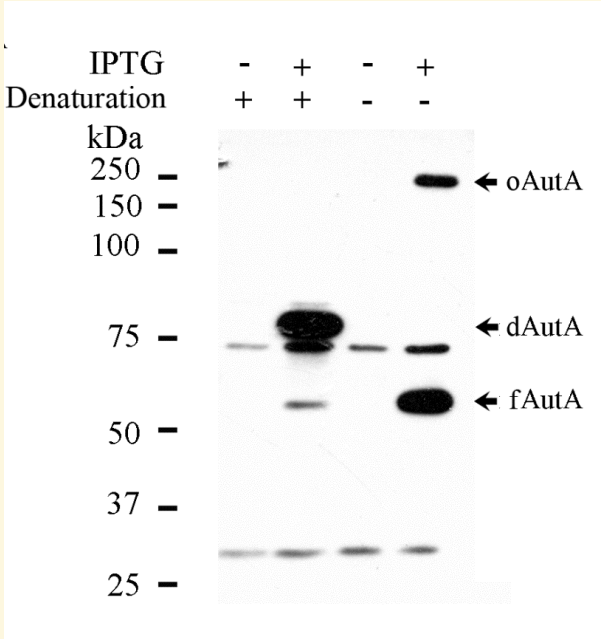
AutA induce autoagregacion y varia la estructura del biofilm

AutA alters meningococcal biofilm development

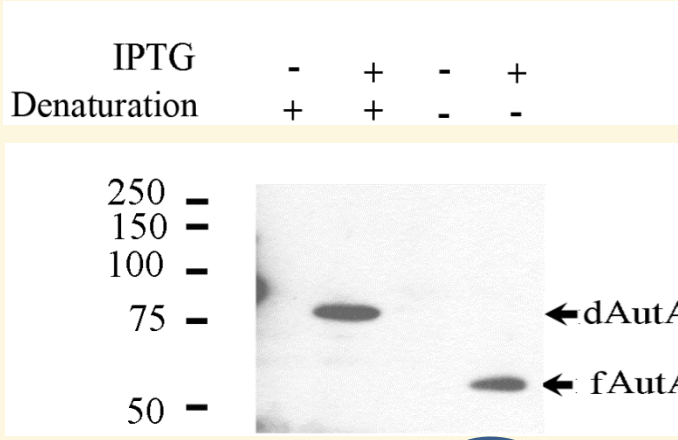


Molecular interactions of AutA





Seminative
Western blotting
Anti-AutA

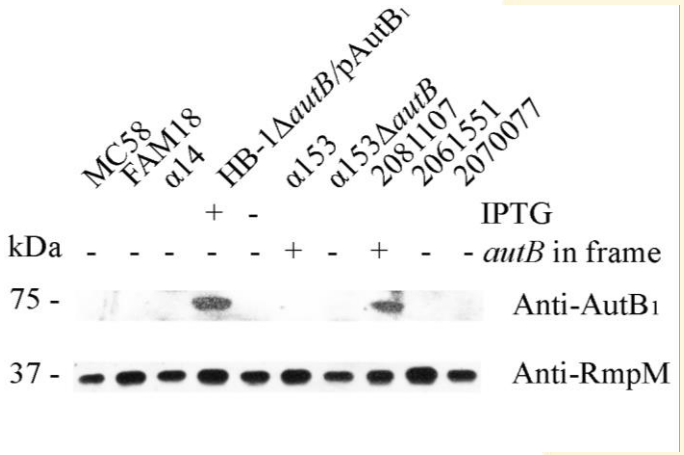


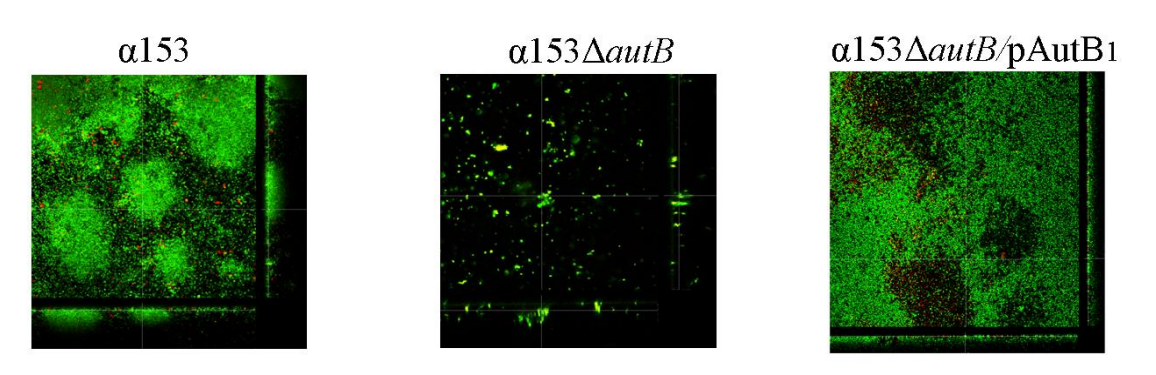
rAutAp

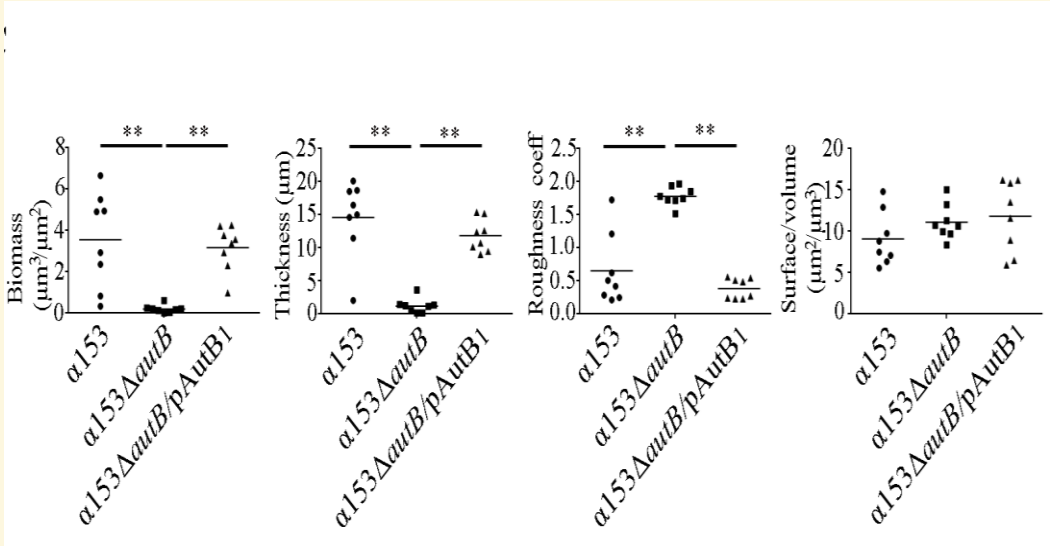
Far-Western blotting
Anti-His

AutA binds DNA and is a self-associating autotransporter

Expression of AutB



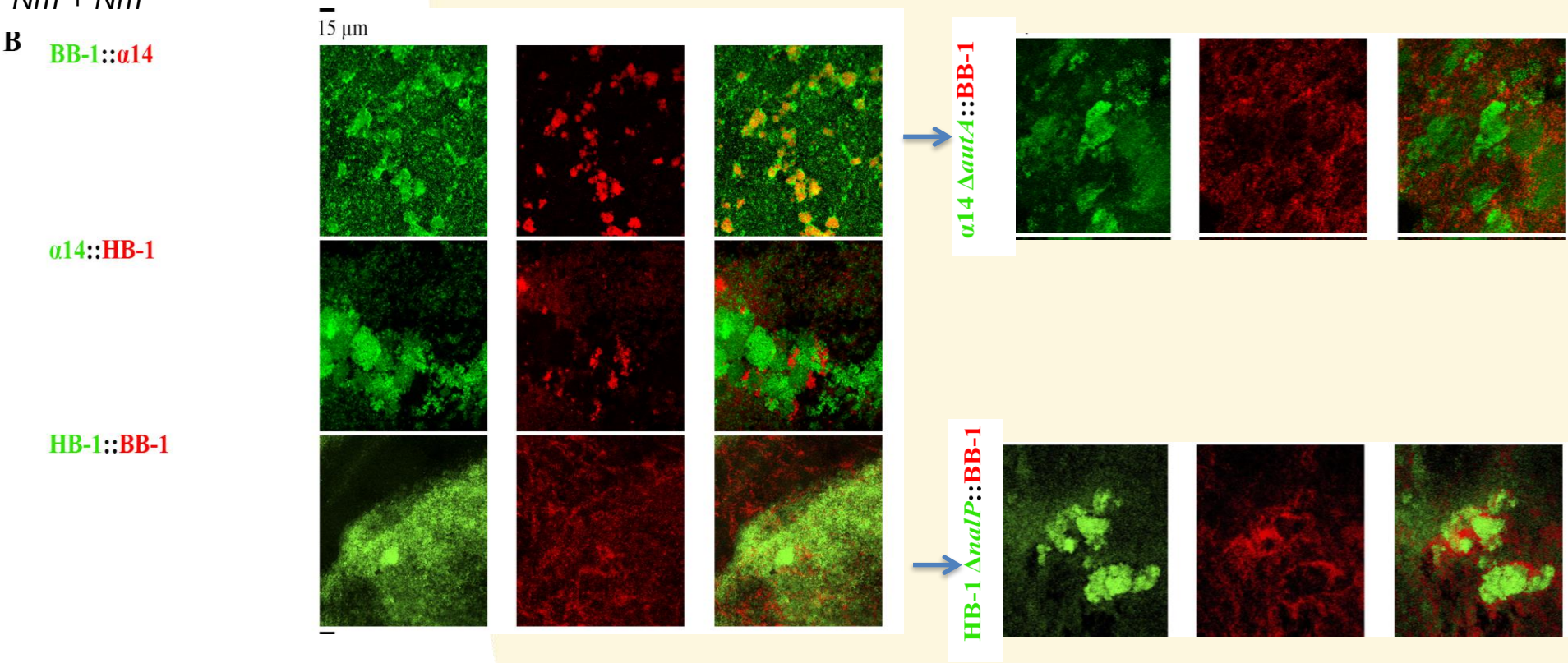




Arenas *et al*. 2016.
Front Cell Infect Microbiol. IF 4,3

Inter-strain interactions in biofilms: Role of NalP and AutA

Nm + Nm



> AutA and NalP are involved in inter-strain interaction during biofilm formation

Rodriguez, …, **Arenas**. 2017, *Front Microbiol IF 4,2*

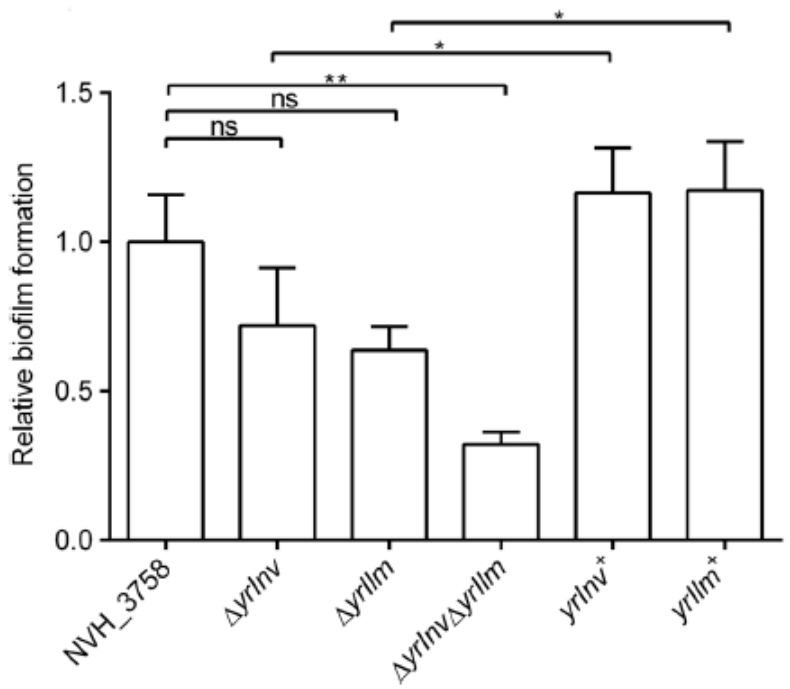
Lecciones de *Neisseria meningitidis*: ¿son válidas para otros microorganismos?

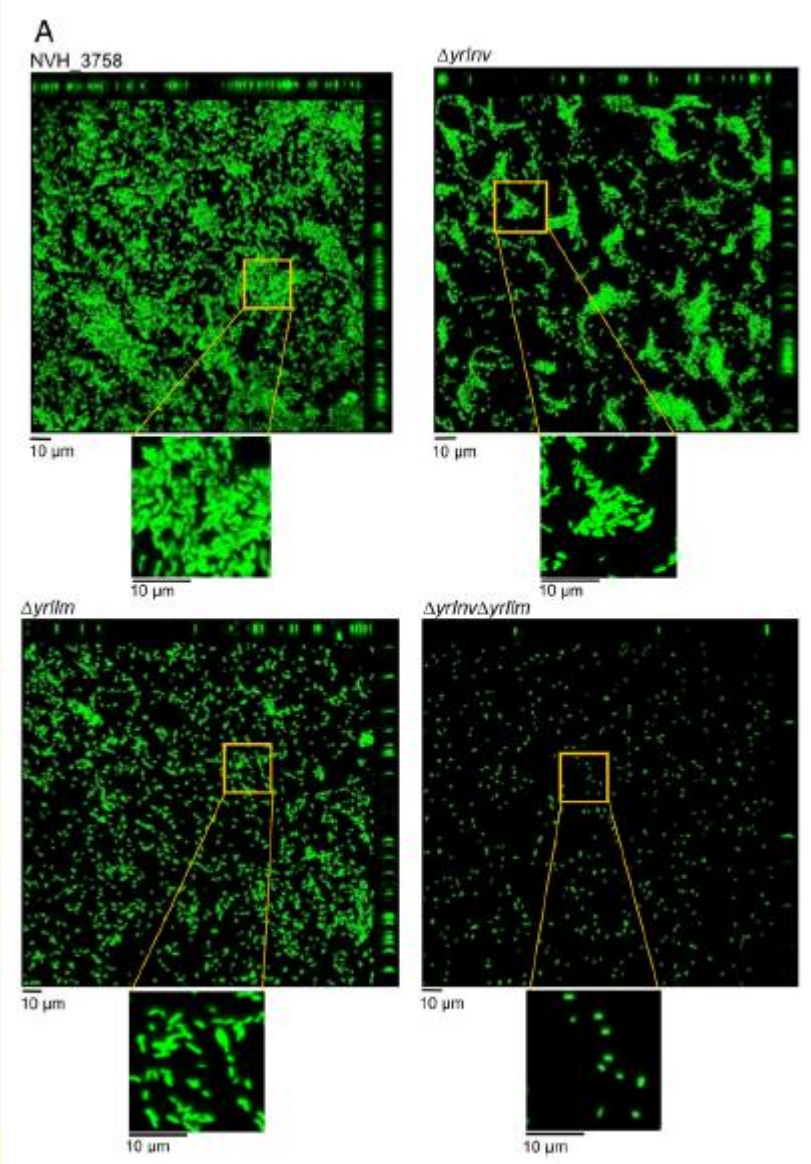
Specie	Biofilm	Protein(s)	Characteristics
B. pertussis	eDNA dependent	FHA surface	binding heparin (arginine-rich)
L. monocytogenes	eDNA dependent	ActA	binding heparin
S. pneumoniae	eDNA dependent	PsrP	DNA binding
M. tuberculosis	eDNA dependent	MDP1, HBHA	heparin binding
C. albicans	eDNA dependent	Int1	heparin binding

Anclar el DNA extracellular a la superficie de la célula a través de
Regiones de union al ADN es un mecanismo universal extendido para formar biofilms

Arenas and Tommassen, 2017.
Trends in Microbiol. IF 11,7

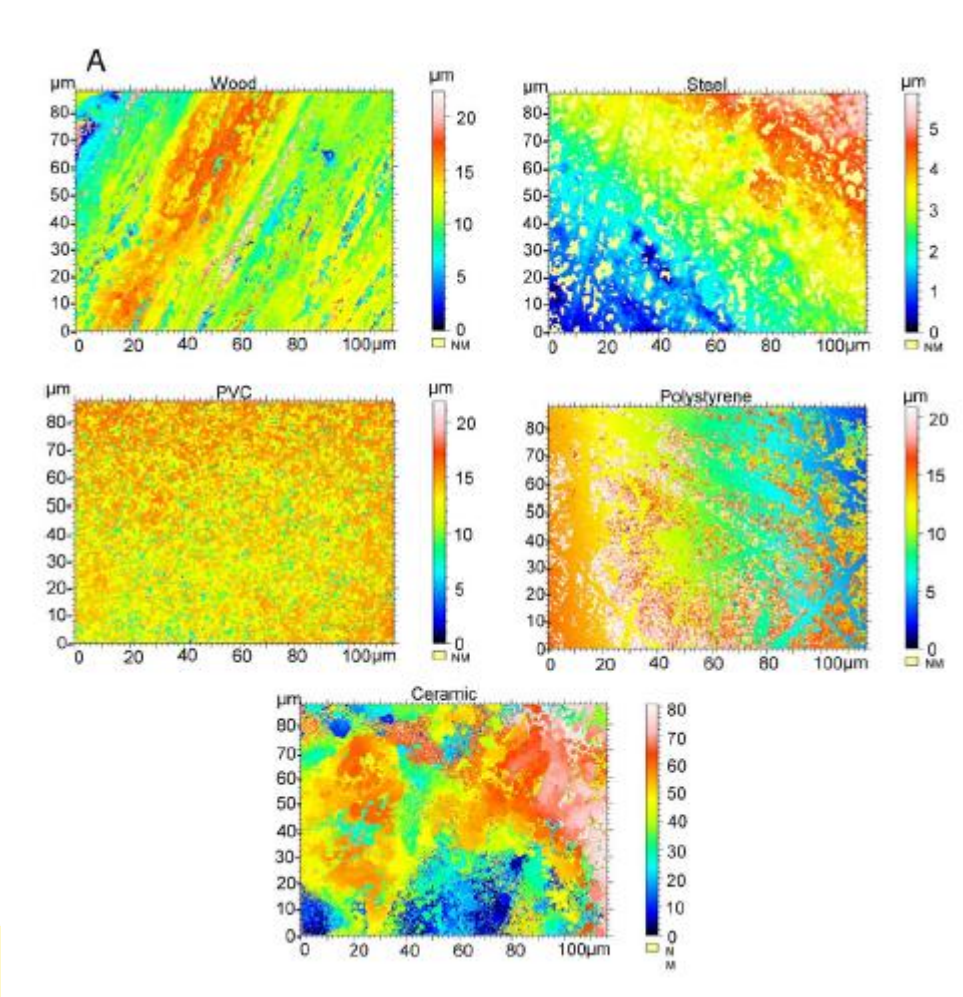
Biogenesis de Biofilms: *Yersini ruckeri*

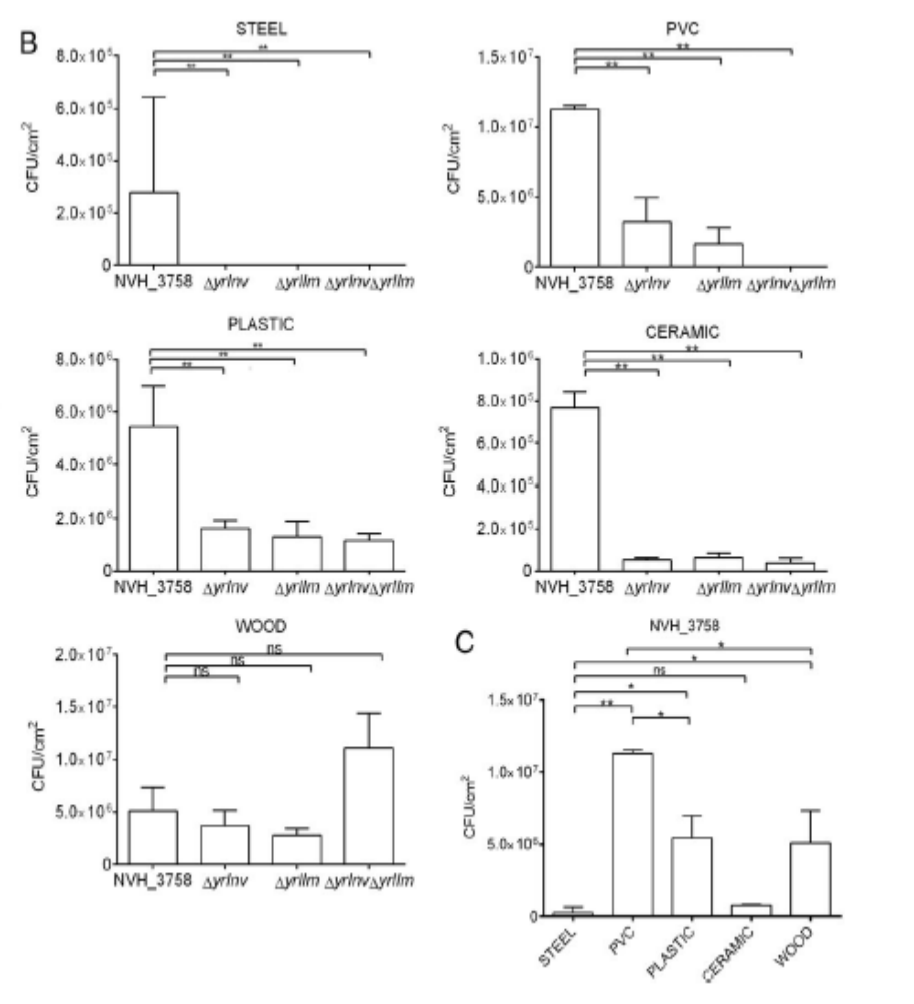




Wrobel A, …, **Arenas**. 2020,
Envirom Microbiol. IF: 5,6

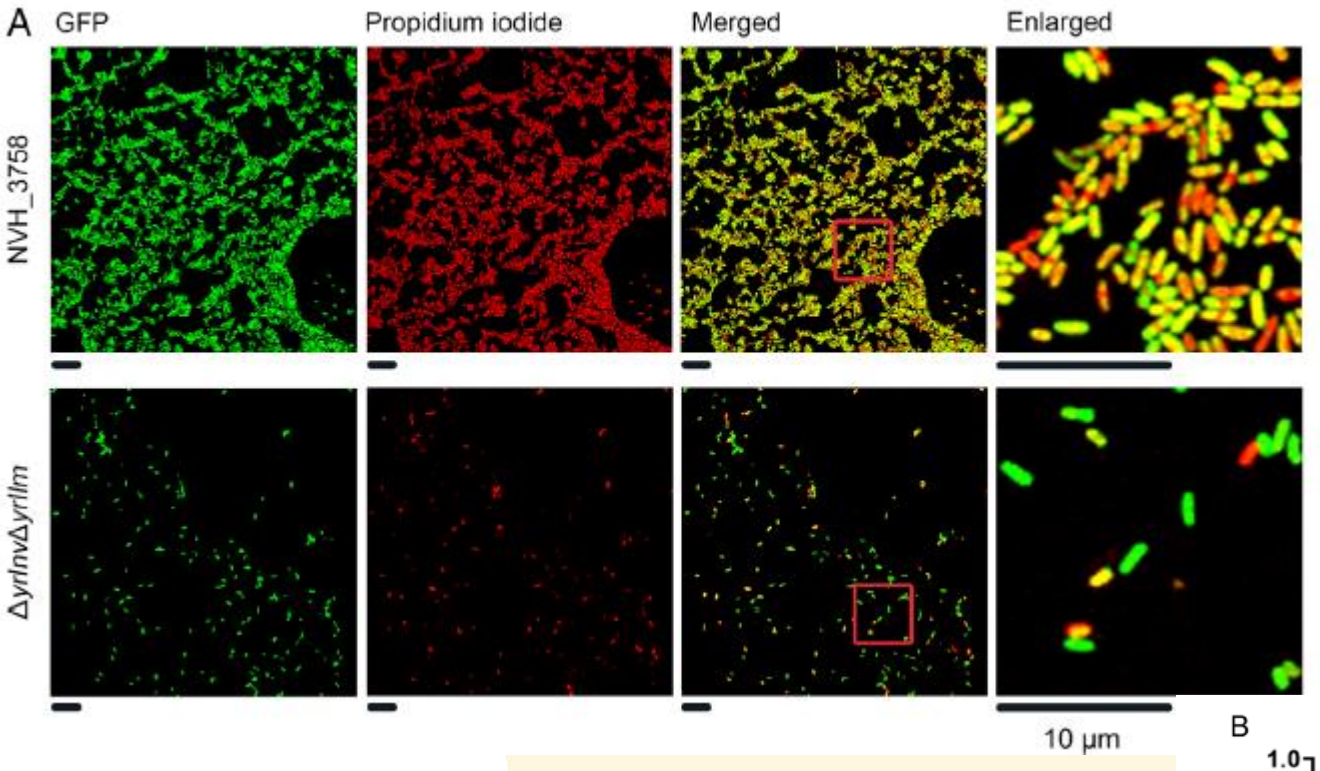
Biogenesis de Biofilms: *Yersinia ruckeri*

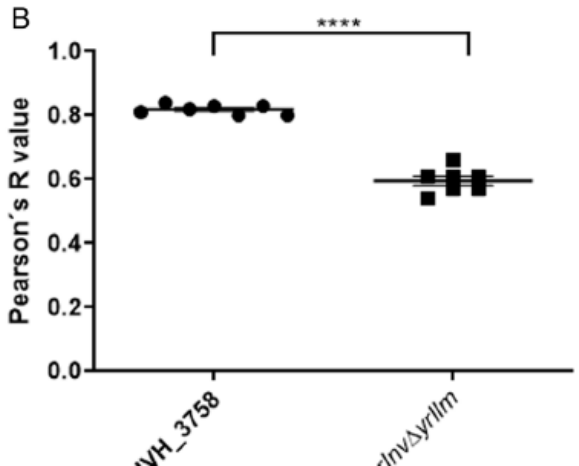




Wrobel A, …, **Arenas**. 2020, *Envirom Microbiol.* IF: 5,6

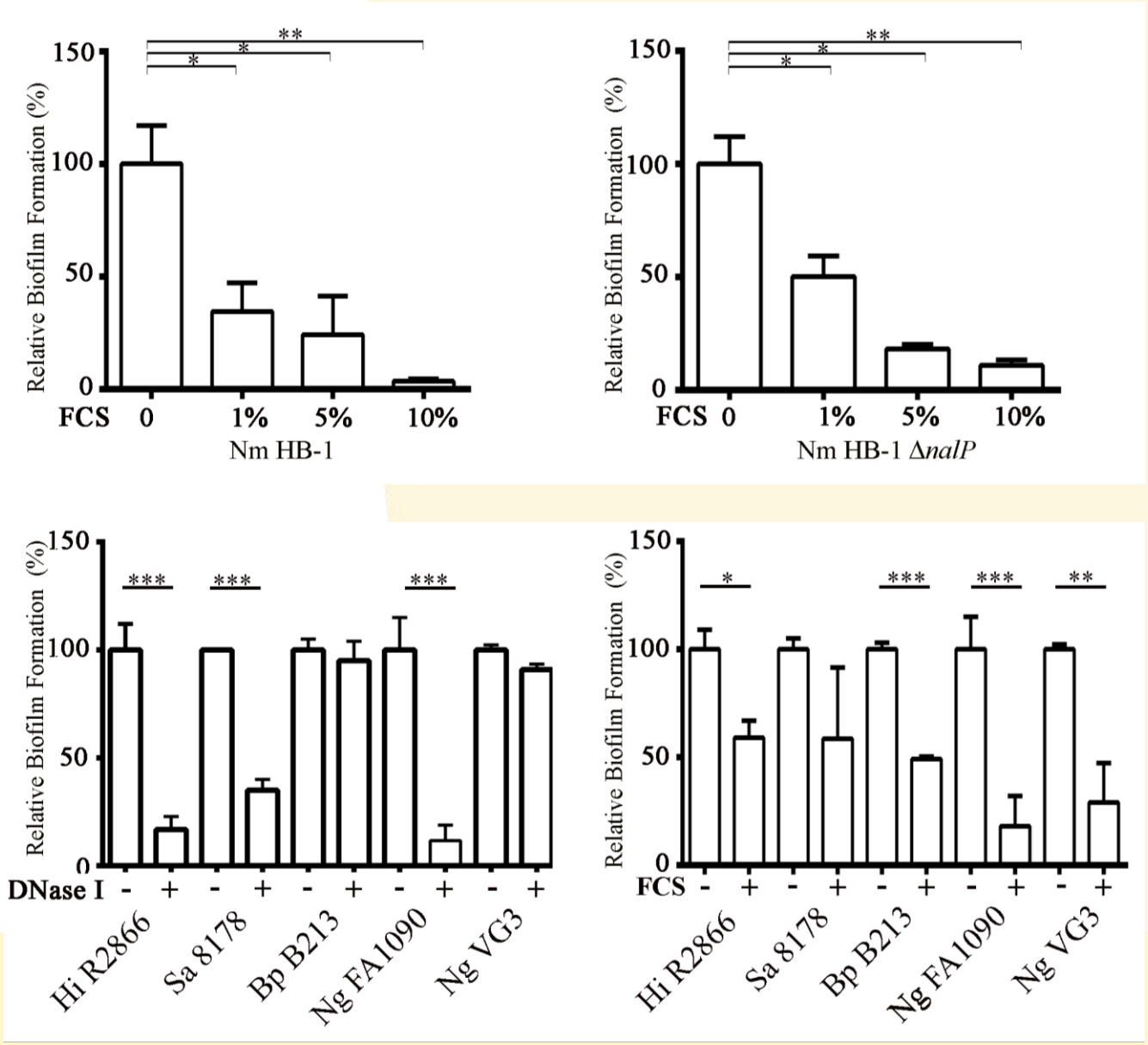
Biogenesis de Biofilms: *Yersinia ruckeri*





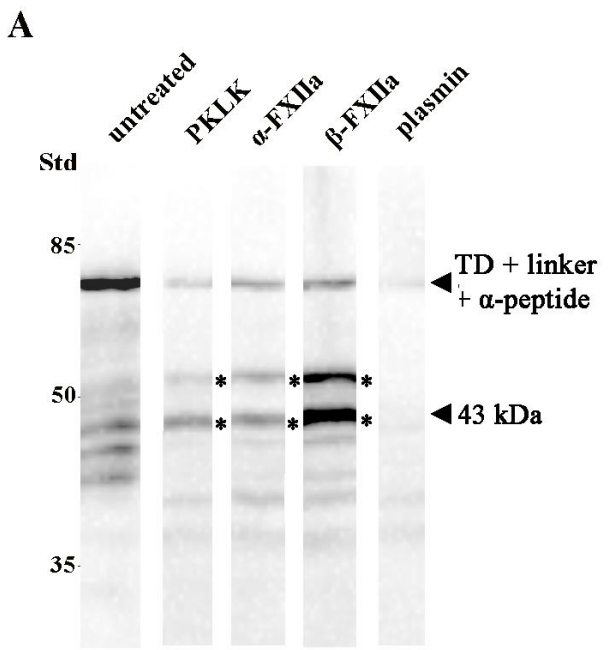
Wrobel A, …, **Arenas**. 2020, *Envirom Microbiol.* IF: 5,6

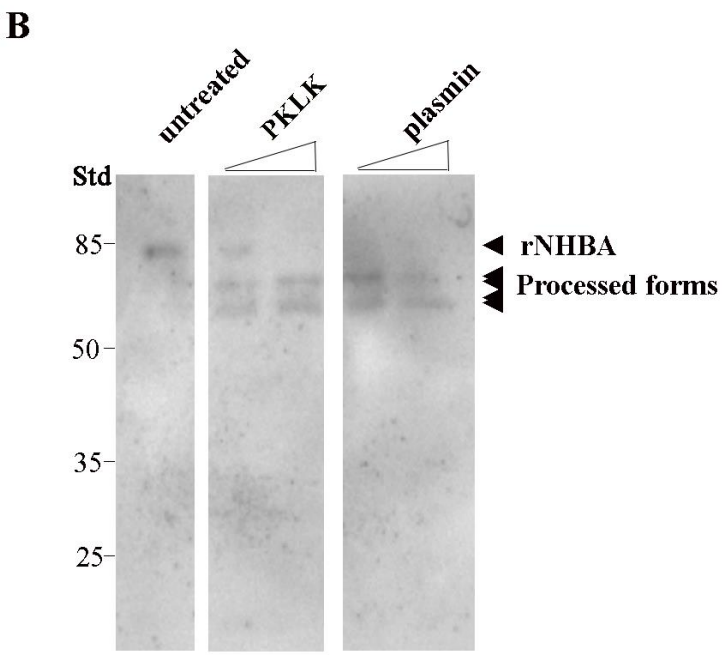
Inhibición del biofilm por Suero

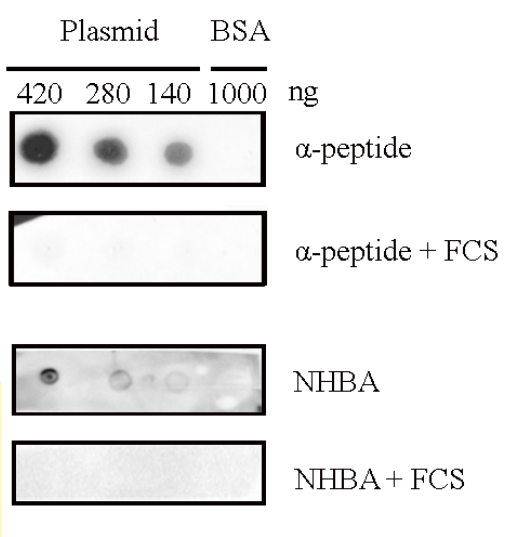


Arenas et al, 2021.
Virulence IF5,8

Inhibición del biofilm por suero







Arenas et al, 2021.
Virulence IF5,8

Conclusiones

- *Neisseria meningitidis* expresa diferentes proteinas expuestas en superficie cuya expression es variable y afecta drásticamente a la arquitectura del biofilm

- Anclar el DNA extracellular a la superficie de la célula a través de Regiones de union al ADN es un mecanismo universal extendido para formar biofilms

- Proteínas presentes en el suero de mamiferos constituyen un mecanismo de defensa contra biofilms

Gracias!

jaarenas@unizar.es