



UNIVERSITAT DE
BARCELONA

Facultat de Farmàcia i Ciències de l'Alimentació

GLICOSILACIÓ DEL FLAGELO EN *AEROMONAS SPP.*

Seminaris de Recerca i Seminaris Tecnològics

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Departament de Biologia, Sanitat i Medi Ambient
Secció de Microbiologia

21 MARÇ 2024

Me presento

2004 2005 2007 2008 2009 2012 2014 2020 2023

Formación académica

 **Licenciada en Biología**
 Programa de Premedicina
 

 **DEA**
 Bioquímica, Biología Molecular y Biomedicina
 Universidad Autónoma de Madrid

 **Doctorado Europeo** 
 Bioquímica, Biología Molecular y Biomedicina
 Universidad Autónoma de Madrid
 "Activation mechanisms of the innate immune system: Structure-function studies of interactions between death domains of MyD88 and IRAK proteins"



Experiencia profesional

Bióloga
 Hospital Universitario La Paz
 Hospital Carlos III Hospital Cantoblanco

Investigadora predoctoral
  

(Voluntaria)
 IdiPAZ
 Instituto de Investigación Hospital Universitario La Paz

Investigadora postdoctoral
 Genética, Microbiología y Estadística
 UNIVERSITAT DE BARCELONA  Bosch i Gimpera

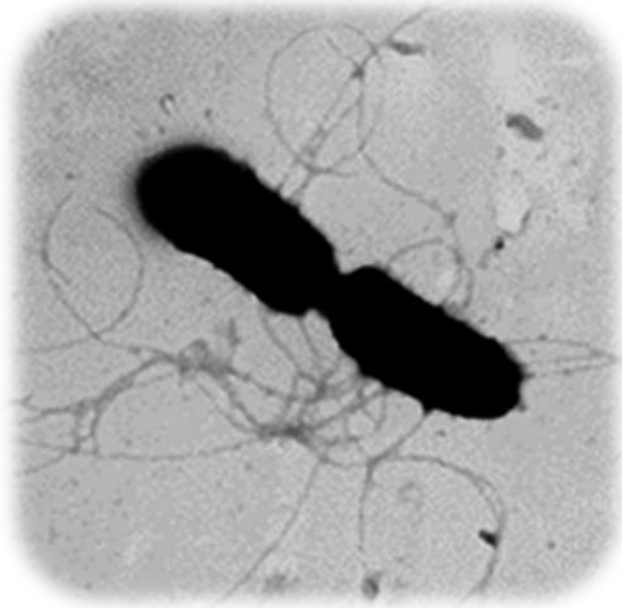
 UNIVERSITAT DE BARCELONA
Profesora asociada
 Genética, Microbiología y Estadística

 UNIVERSITAT DE BARCELONA
Profesora lectora
 Biología, Sanidad y Medio Ambiente



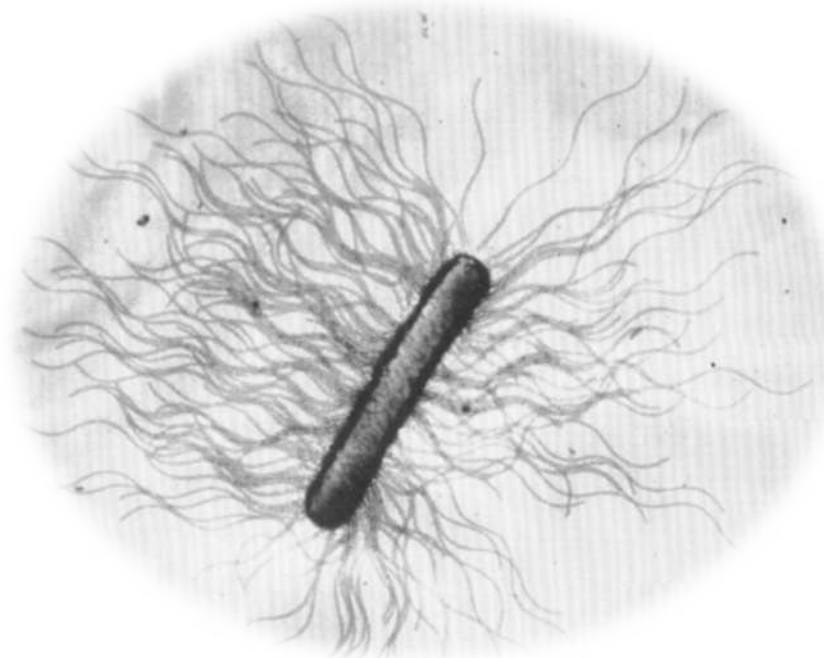
Patogenicidad de gramnegativas

Edwardsiella tarda



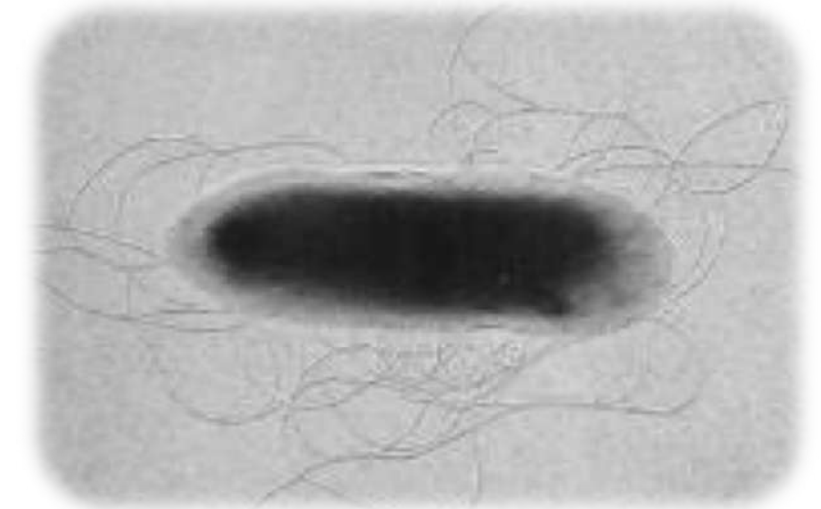
(He et al. 2012)

Proteus mirabilis



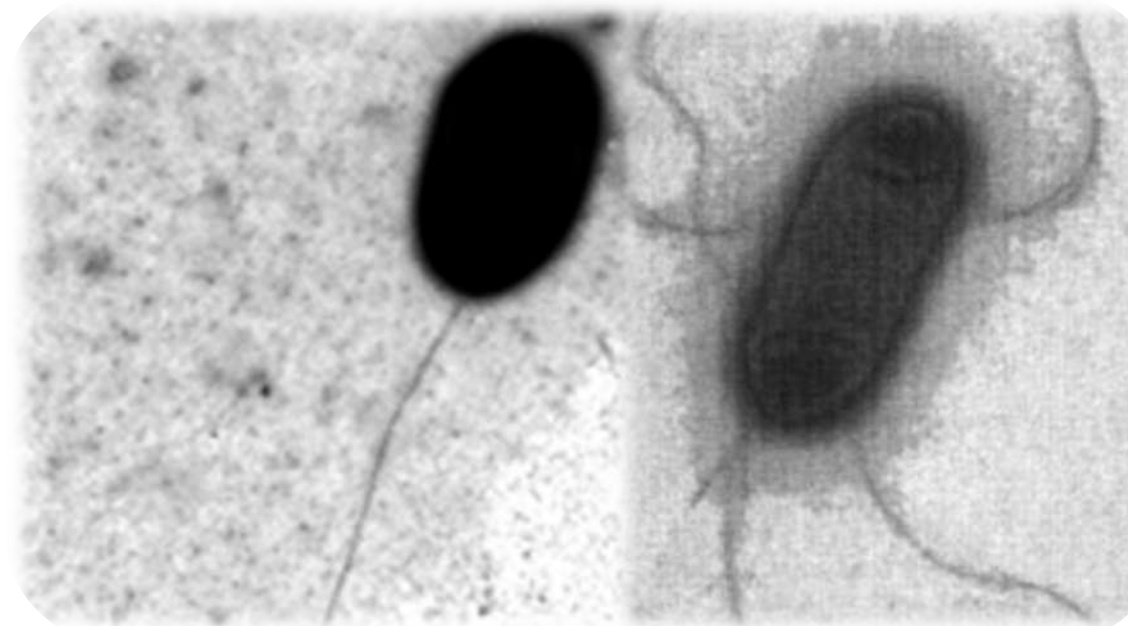
(Hoeniger, 1965)

Erwinia amylovora



(Van der Zwet et al. 2012)

Aeromonas spp.



(Merino et al. 2014)

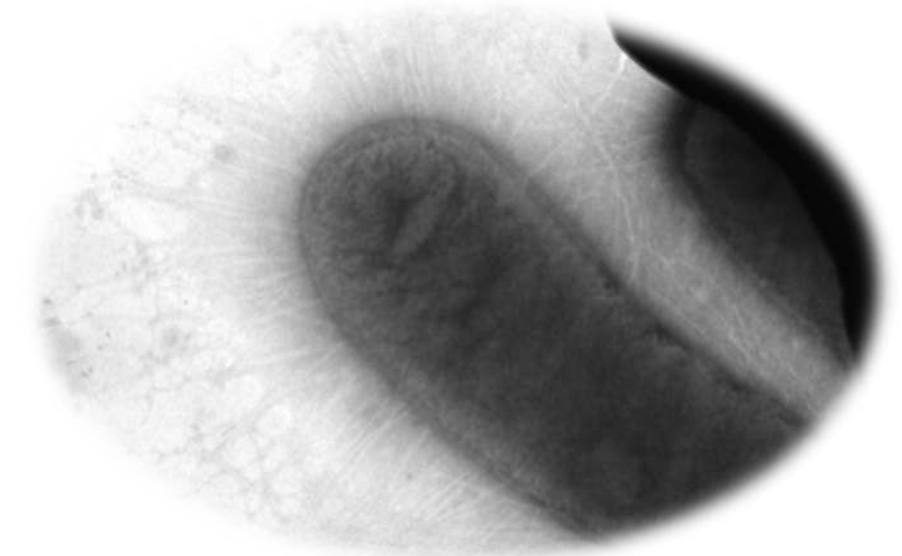
(Canals et al. 2006)

Plesiomonas shigelloides



(Merino et al. 2015)

Klebsiella pneumoniae



(Schembri et al. 2005)

Patogenicidad de gramnegativas

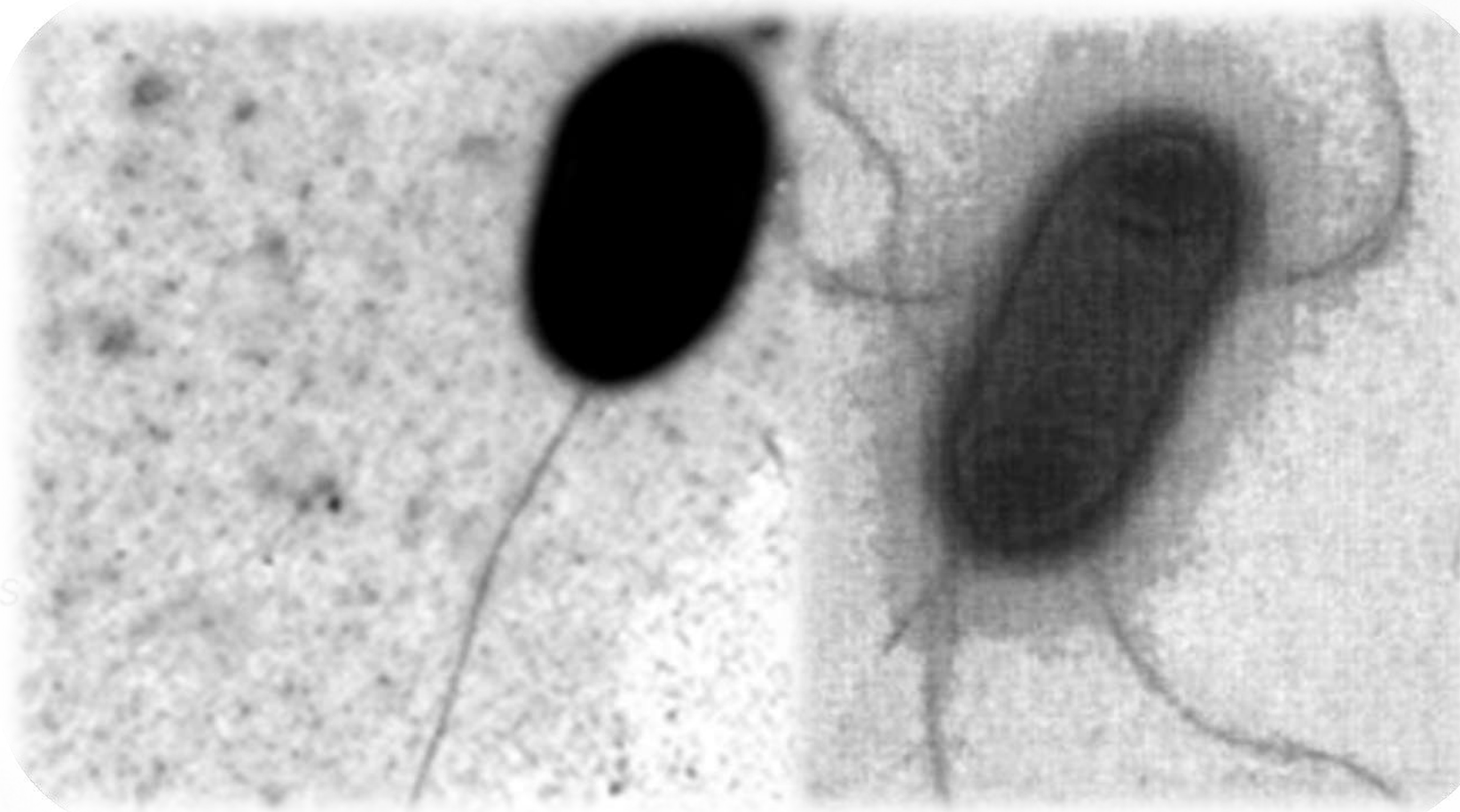
Edwardsiella tarda



(He et al. 2012)

Proteus mirabilis

Aeromonas spp.



(Merino et al. 2014)

(Canals et al. 2006)

Erwinia amylovora



(Van der Zwet et al. 2012)

Plesiomonas shigelloides



(Merino et al. 2015)

Klebsiella pneumoniae



(Schembri et al. 2005)

Aeromonas spp.

Hábitat



agua potable



algunos alimentos

Patogénesis



humanos

Enfermedades
intestinales
gastroenteritis
diarrea



Enfermedades
extraintestinales
meningitis
septicemia



poiquiloterms



Pérdidas en
acuicultura



animales salvajes



animales domésticos

Aeromonas spp.



(shutterstock.com)

Reino: **Bacteria**
Filo: **Pseudomonadota (Proteobacteria)**
Clase: **Gammaproteobacteria**
Orden: **Aeromonadales**
Familia: **Aeromonadaceae**
Género: **Aeromonas**

(Schoch et al. 2020)

Características generales

Bacilos gramnegativos
Anaerobios facultativos
Oxidasa positivos
Quimioheterótrofos
Productores de ácidos y gases
Reductores de nitratos

Aeromonas mesófilas

35 – 37 °C

Móviles (con flagelos)

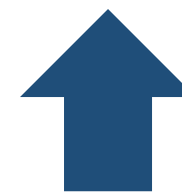
Ej. *A. hydrophila*

Aeromonas psicrófilas

22 – 25 °C

No móviles (sin flagelos)

Ej. *A. salmonicida*



Flagelo procariota

Distribución flagelar

Flagelos polares

(Expresión constitutiva)



Monótricos



Aeromonas spp.



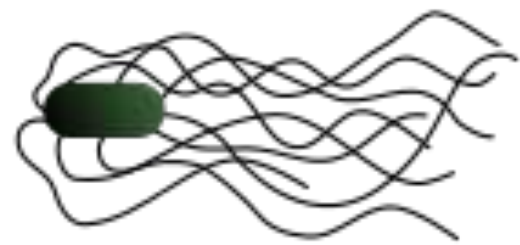
Lofótricos



Anfítricos

Flagelos laterales

(Expresión inducida)

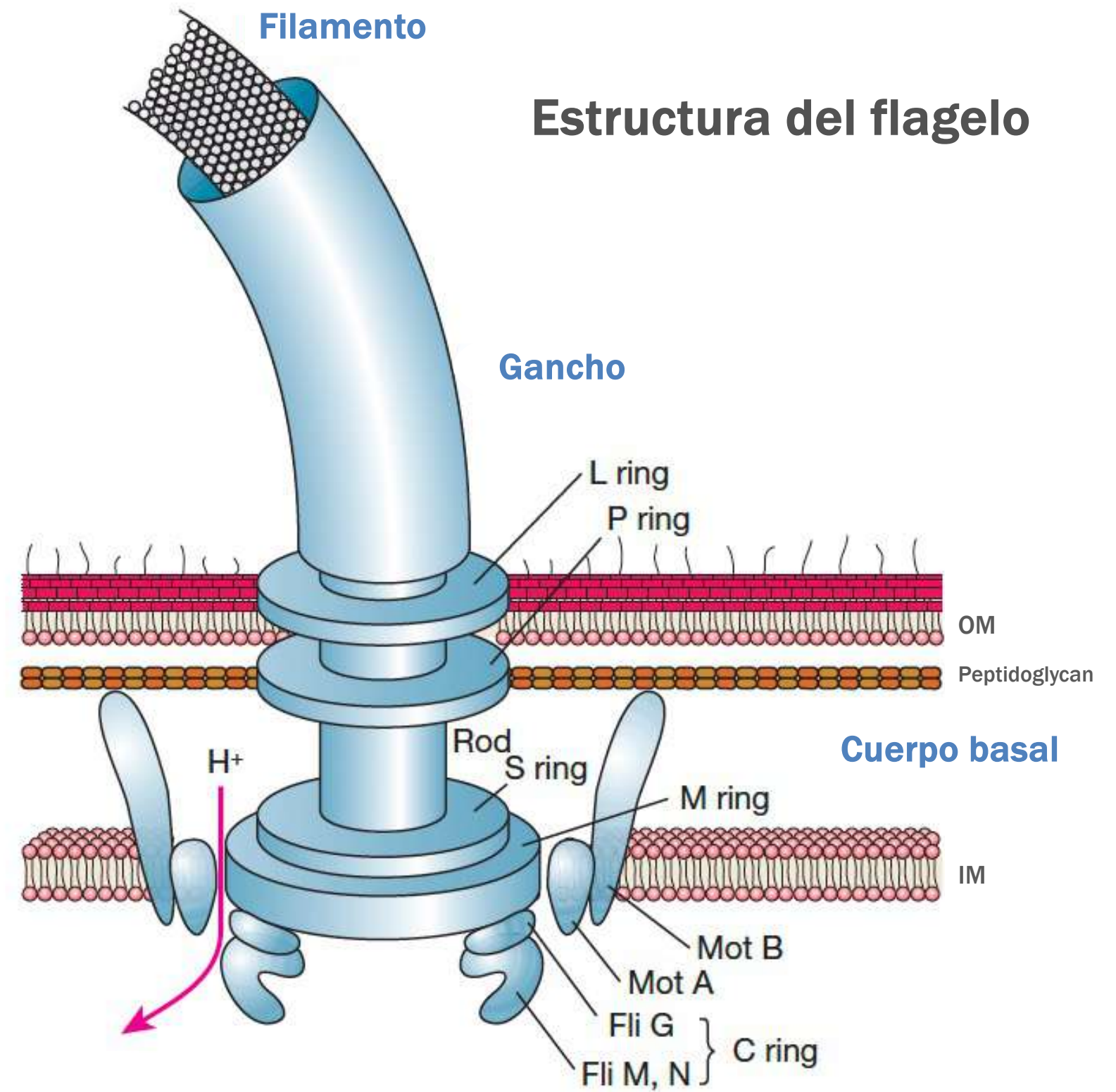


Perítricos



Aeromonas spp.

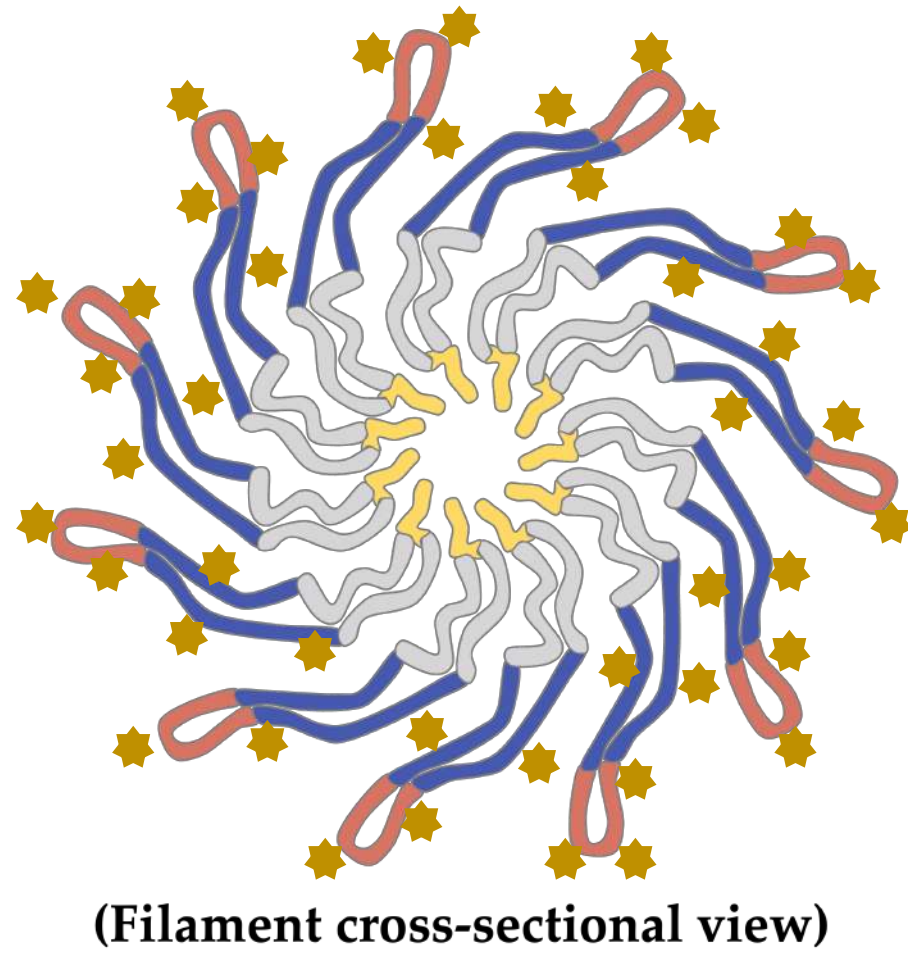
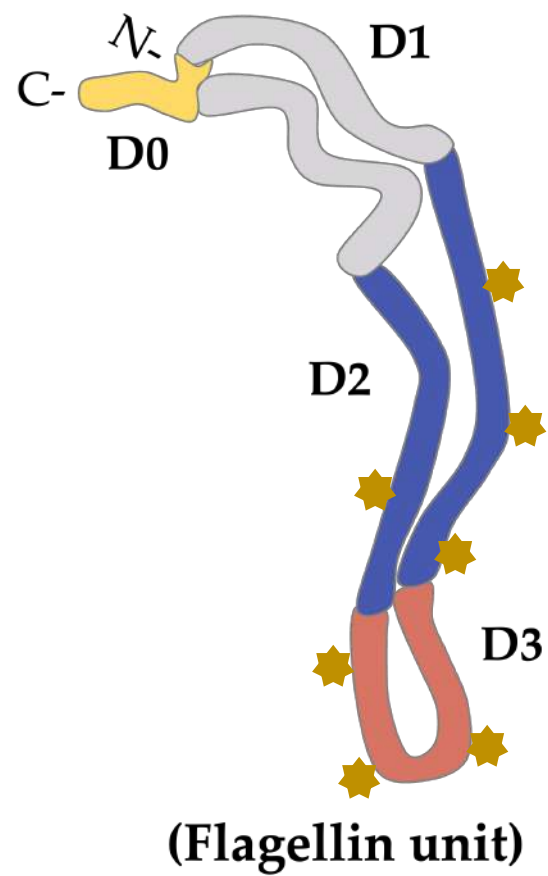
Estructura del flagelo



(Prescott et al. 2020)

Flagelo procariota

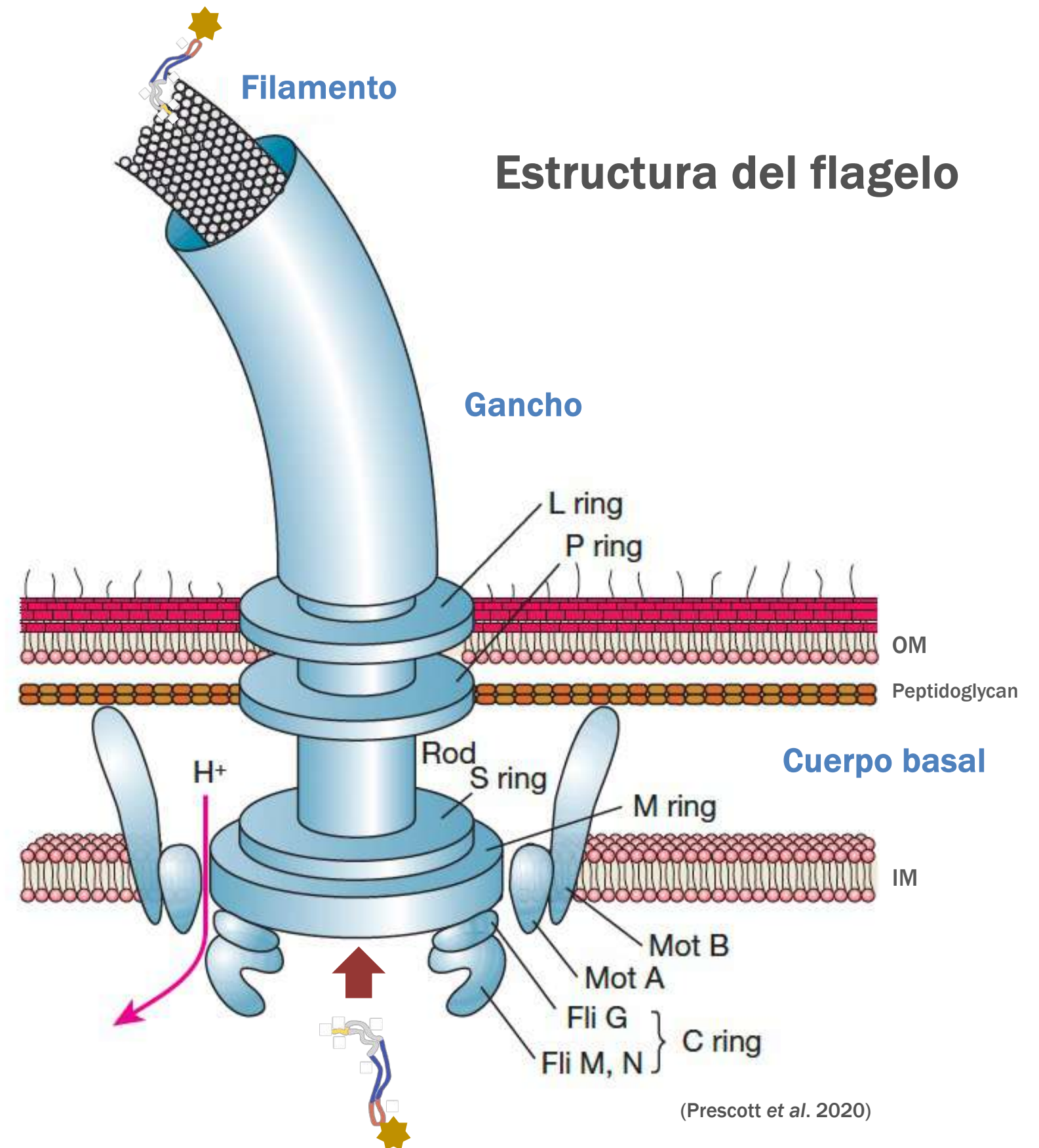
Disposición de las flagelinas



(Mendoza-Barberá et al. 2021)

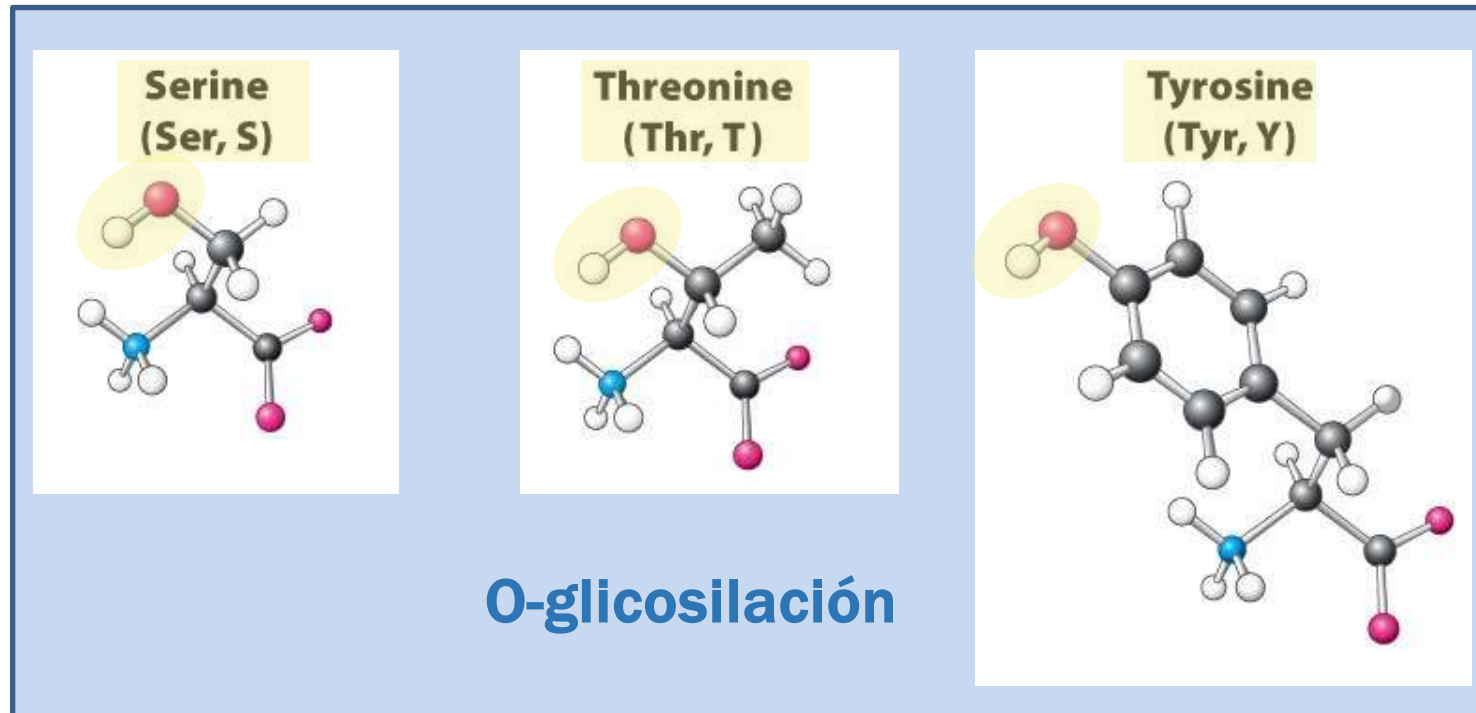
★ Glicosilación de las flagelinas

Estructura del flagelo

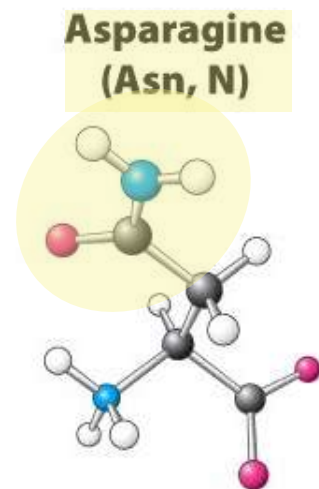


(Prescott et al. 2020)

Glicosilación del flagelo procariota

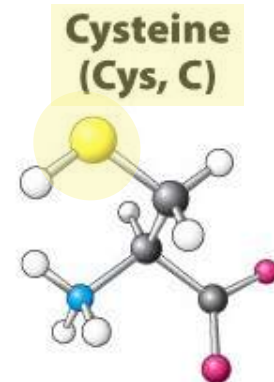


N-glicosilación



Helicobacter pullorum
Campylobacter jejuni
Haemophilus influenzae

S-glicosilación



Lactobacillus plantarum
Bacillus subtilis

Estructura de los O-glicanos en gramnegativas

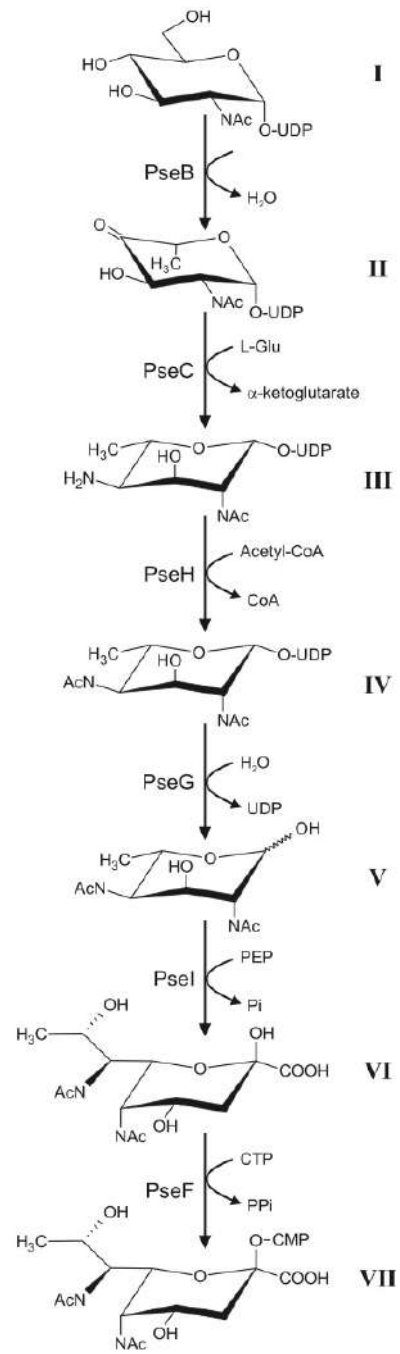
| Organism | Glycoprotein | Number of sites | Glycan structure |
|---|--------------|-----------------|--|
| <i>Aeromonas caviae</i> Sch3N | FlaA/FlaB | 6/7 | Pse5Ac7Ac |
| <i>Aeromonas caviae</i> UU51 | FlaA/FlaB | 6/7 | Pse5Ac7Ac8Ac |
| <i>Aeromonas hydrophila</i> AH3 | FlaA/FlaB | 6FlaB | PseAc derivative-Hex-Hex-HexNAc-HexNAc-HexNAc-102 Da |
| <i>Azospirillum brasilense</i> Sp7 | LafA | 3 | PseAc derivative |
| | Laf1 | ND | (→3)-α-L-Rhap-(1→3)-β-D-Galp-(1→3)-β-D-GlcpNAc-(1→)n = 3-4 |
| <i>Burkholderia pseudomallei</i> K96243 | FliC | 1 | 582.4 Da (2 × 291 Da) |
| <i>Burkholderia thailandensis</i> E264 | FliC | 1 | 342 Da |
| <i>Campylobacter jejuni</i> 81-176 | FlaA | 19 | Pse5Ac7Ac, Pse5Am7Ac, Pse5Ac7Ac8OAc, Pse5Am7Ac8GlnAc, Pse5Pr7Pr |
| <i>Campylobacter jejuni</i> 11168 | FlaA | ND | Pse5Ac7Ac, Leg5Am7Ac, Leg5AmNMe7Ac |
| <i>Campylobacter coli</i> VC167 | FlaA | 16 | Pse5Ac7Ac, PseAm, PseAc/LegAm-deoxypentose, Leg5Ac7Ac, Leg5Am7Ac, Leg5AmNMe7Ac |
| <i>Helicobacter pylori</i> | FlaA/FlaB | 7/10 | Pse5Ac7Ac |
| <i>Pseudomonas aeruginosa</i> PAK | FliC | 2 | 11 residues (pentose, hexose, deoxyhexose, hexuronic) attached via L-rhamnose |
| <i>Pseudomonas aeruginosa</i> JJ692 | FliC | 2 | L-rhamnose |
| <i>Pseudomonas aeruginosa</i> PAO1 | FliC | 2 | L-rhamnose and 209 Da phosphate |
| <i>Pseudomonas syringae</i> pv. <i>tabaci</i> | FliC | 6 | β-D-Quip4N(3-hydroxy-1-oxobutyl)2Me-(133)-α-L-Rhap-(132)-α-L-Rhap |
| <i>Shewanella oneidensis</i> | FlaB | 5 | 274 Da and 274 ± 14 Da |

ND: not determined.

(Merino and Tomás, 2014)

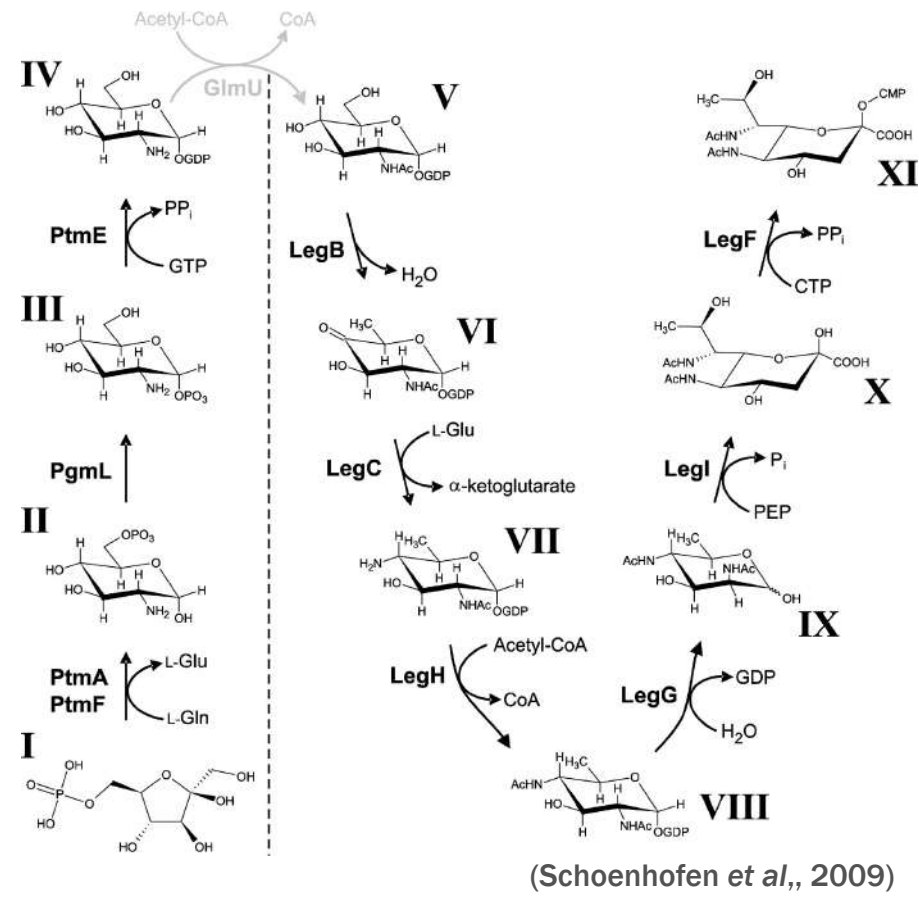
Rutas y mecanismos de glicosilación

H. pylori y *C. jejuni*

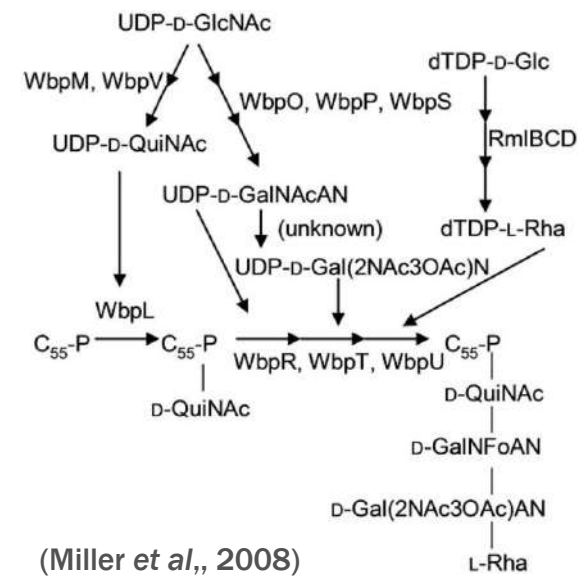


(Schoenhofen et al., 2006)

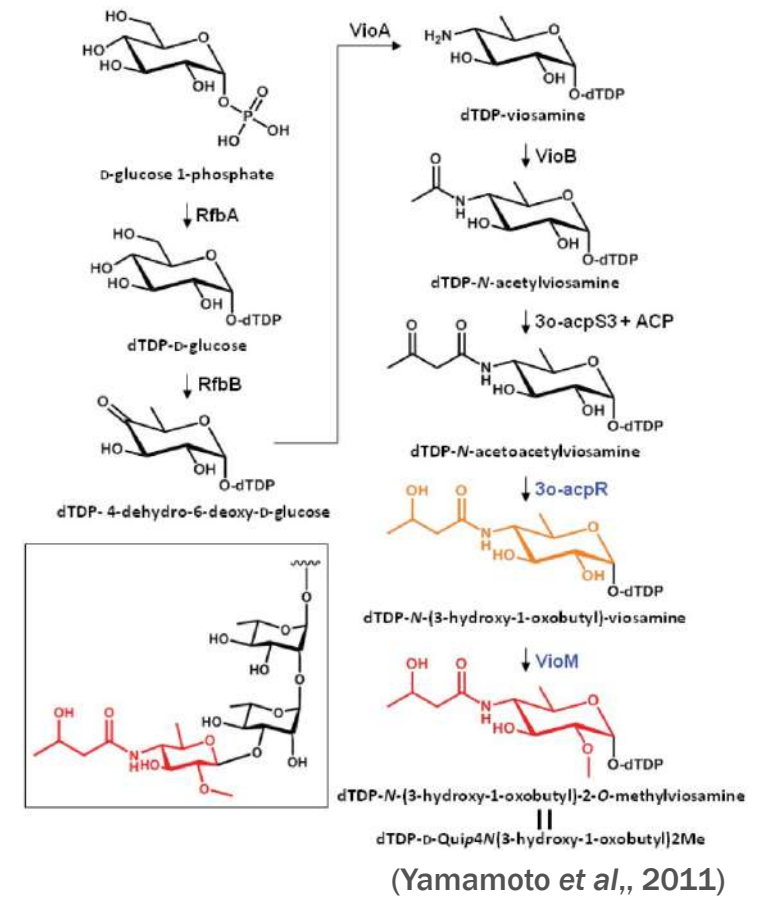
C. jejuni



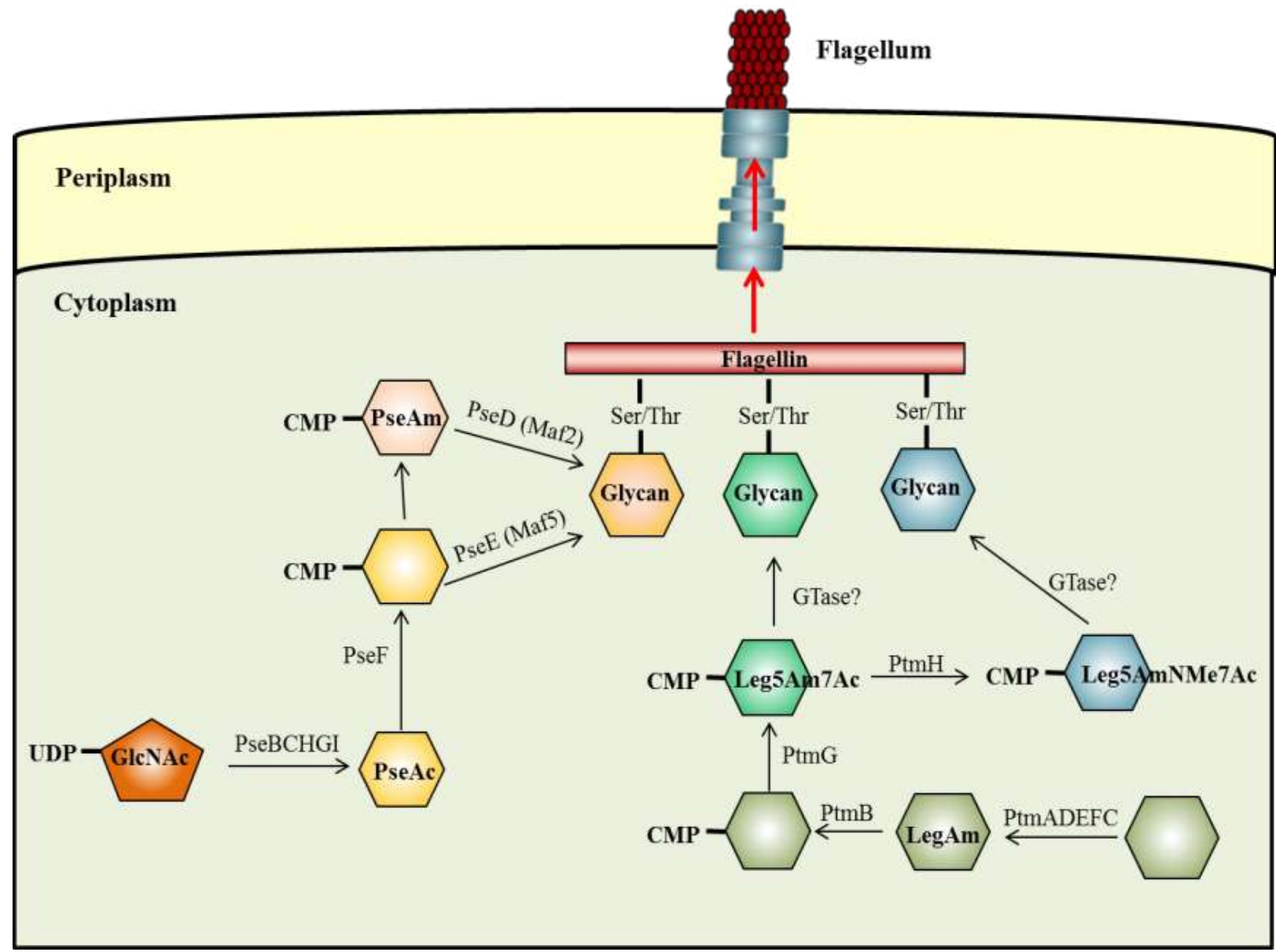
P. aeruginosa



P. syringae

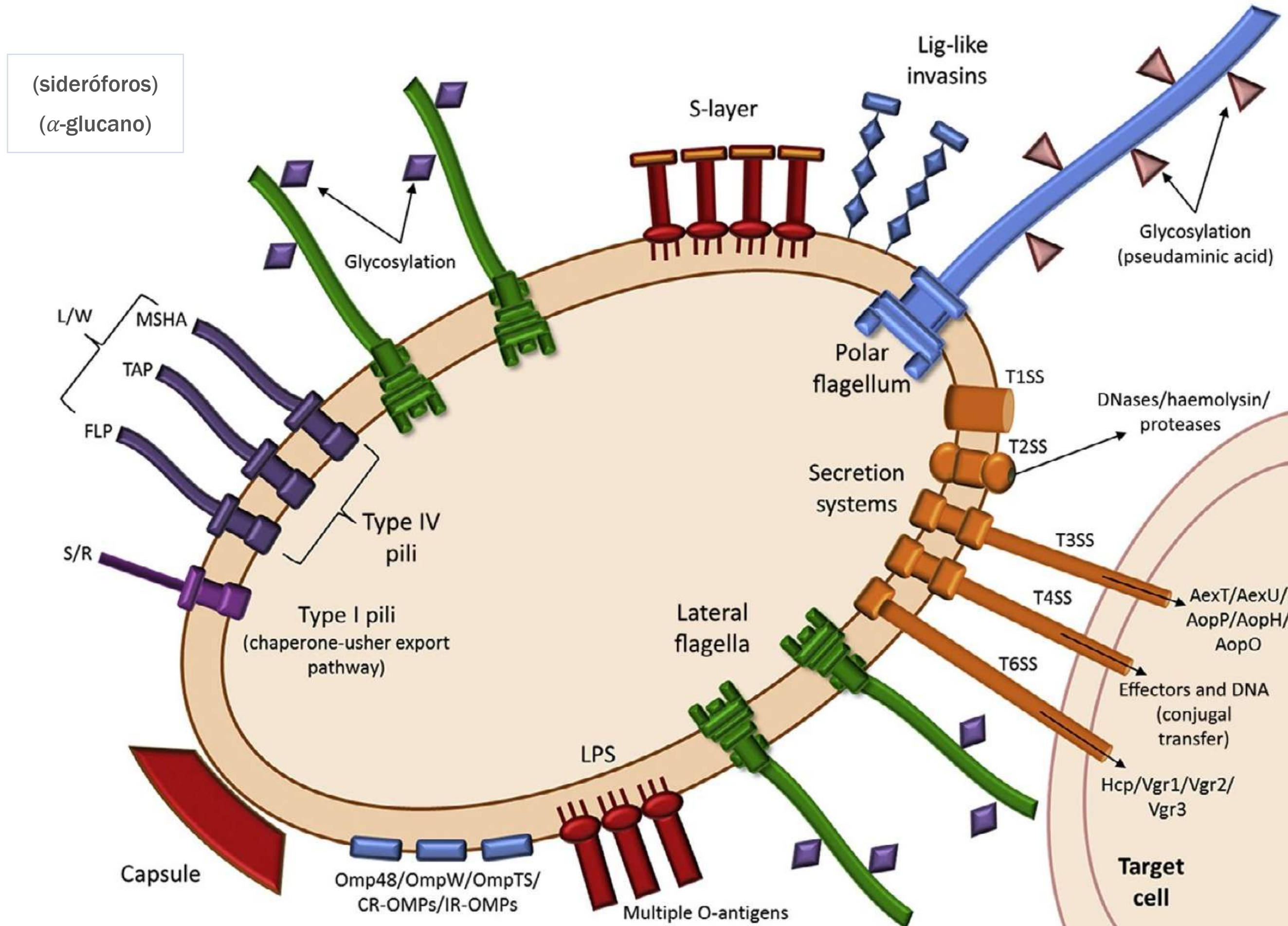


Rutas y mecanismos de glicosilación

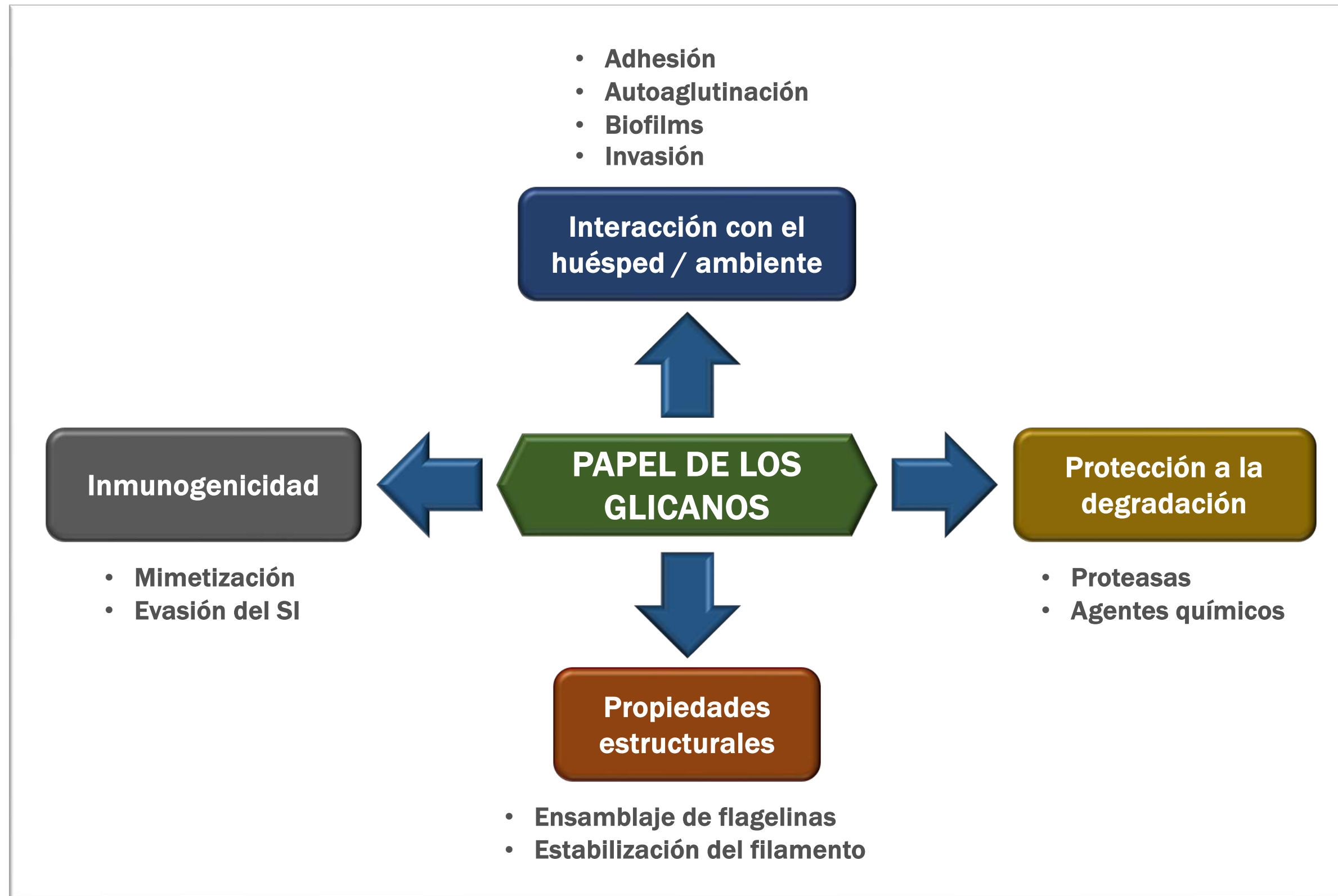


(Merino and Tomás, 2014)

Factores de patogenicidad en *Aeromonas* mesófilas



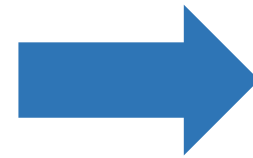
Papel de los glicanos del flagelo procariota



Glicosilación del flagelo de *Aeromonas*

Análisis de motilidad

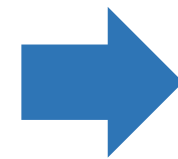
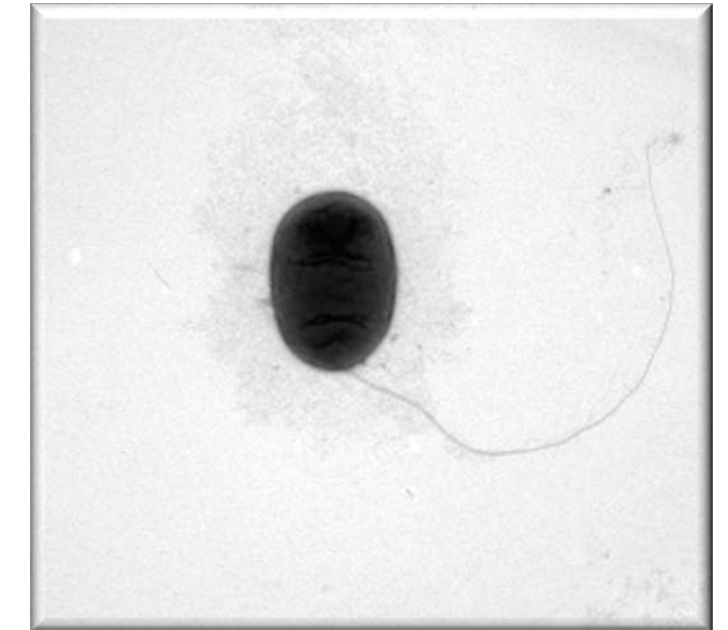
Medio líquido



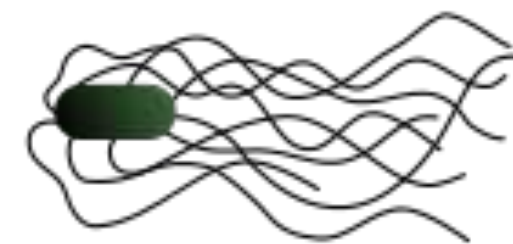
Flagelo polar



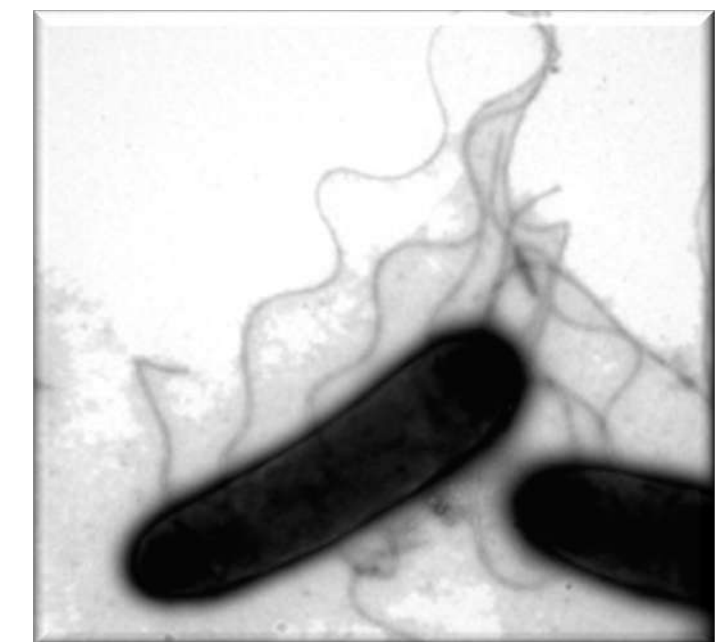
TEM



Flagelos laterales



TEM



Medio semi-sólido

Glicosilación del flagelo de *Aeromonas* mesófilas

Extracción y purificación del flagelo

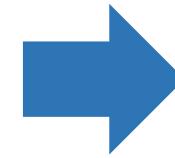
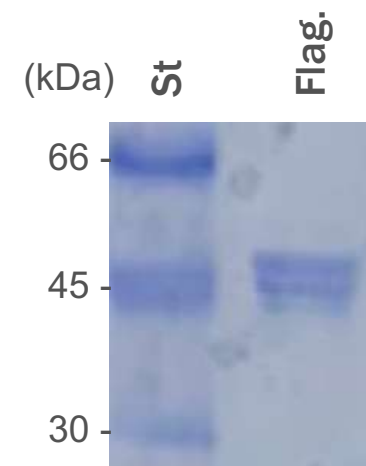


SDS-PAGE

Flagelinas polares de *A. piscicola*

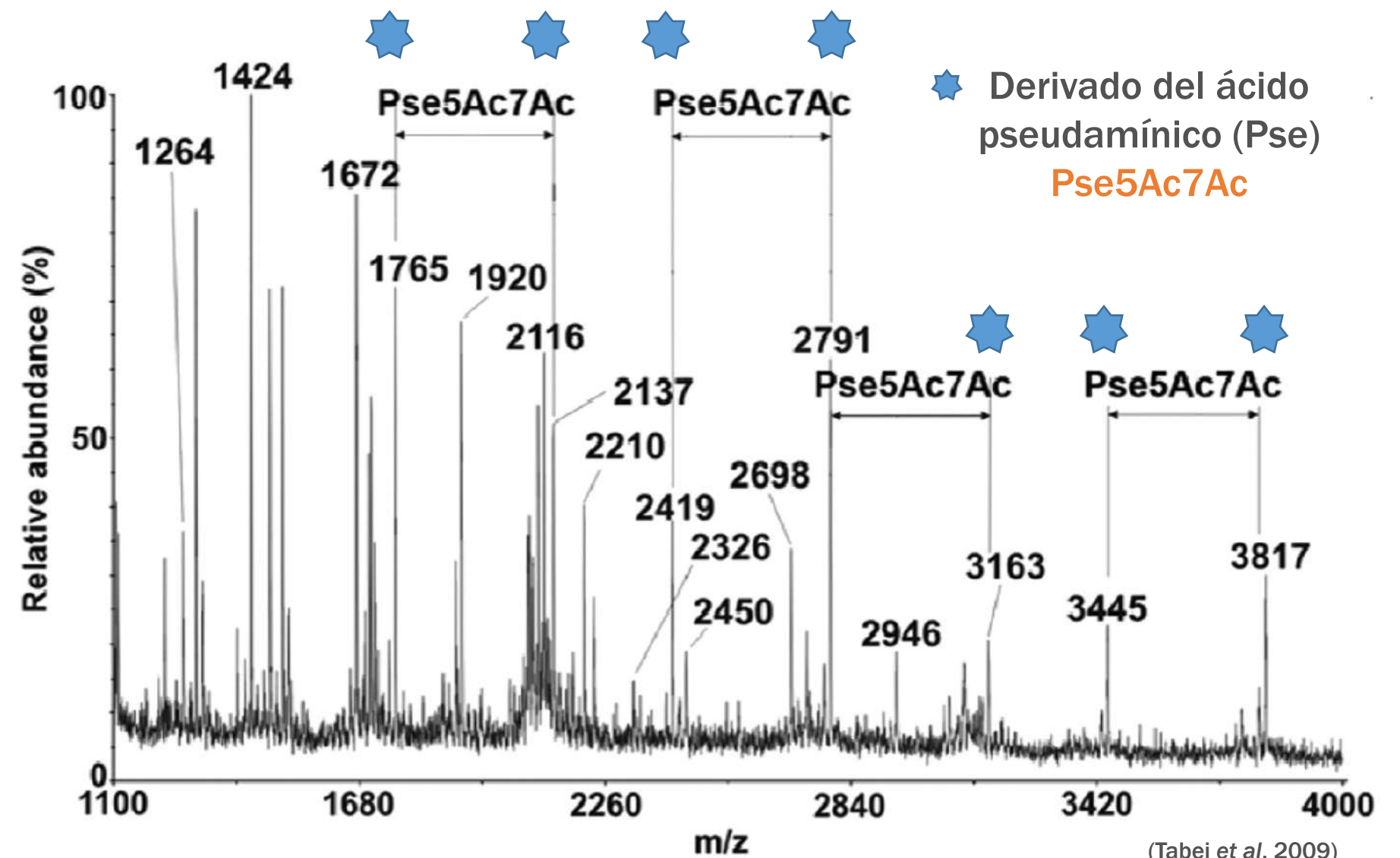
| MW | MW obs. |
|----------|----------|
| ~ 32 kDa | ≥ 45 kDa |

¿Glicosilada?



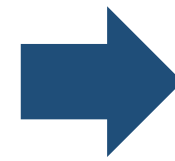
Espectrometría de masas

CID (Flagelinas polares de *Aeromonas caviae*)



Glicosilación del flagelo de *Aeromonas* mesófilas

Flagelinas polares O-glicosiladas



***A. piscicola* AH-3:** Heteroglicano 1679 Da
(Pse 376 Da -Hex-Hex-HexNAc-HexNAc-HexNAc 102 Da)

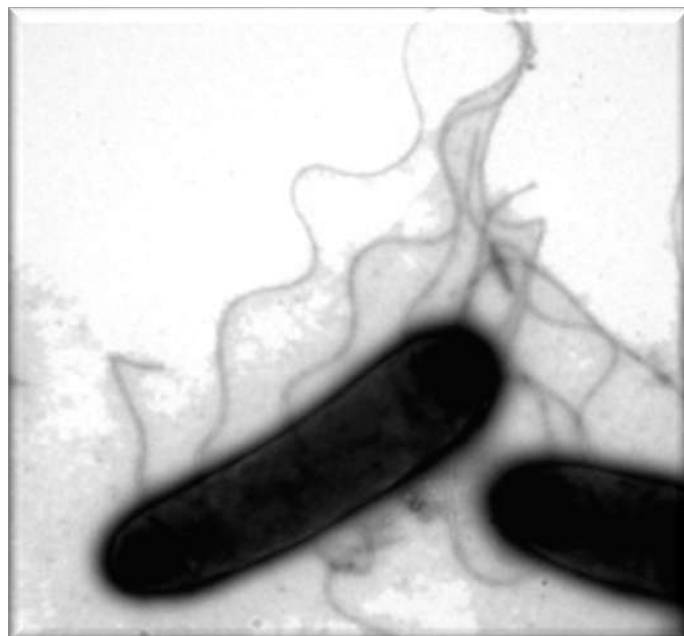
***A. hydrophila* ATCC7966:** Heteroglicano 1285 Da
(Pse 422 Da -Hex-Hex- 1/3 monosacáridos)

***A. hydrophila* AH-1:** Derivado de Pse
(Pse5Ac7Ac9Ac)

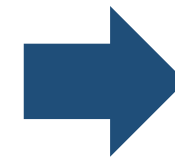
***A. caviae* Sch3N:** Derivado de Pse
(Pse5Ac7Ac)

***A. caviae* UU51:** Derivado de Pse
(Pse5Ac7Ac8Ac)

Flagelinas laterales



O-glicosiladas



[***A. piscicola* AH-3:** Derivado de Pse
(Pse5Ac7Ac)

No glicosiladas



[***A. hydrophila* AH-1**
***A. caviae* Sch3N**

Glicosilación del flagelo de *Aeromonas* mesófilas



¿Cómo se produce la glicosilación?

¿Es necesaria para la correcta formación del flagelo?

¿Qué importancia tiene para las bacterias?

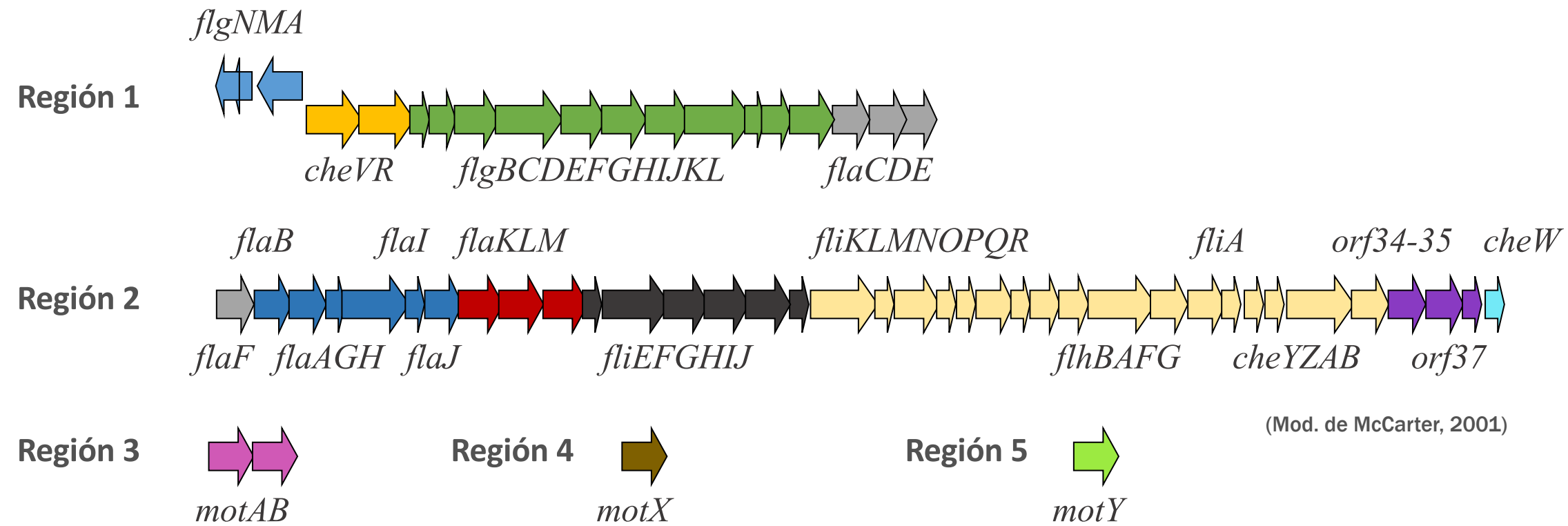
¿Qué genes están implicados en el proceso?

**Análisis de la regulación genética
del flagelo de *Aeromonas* spp.**

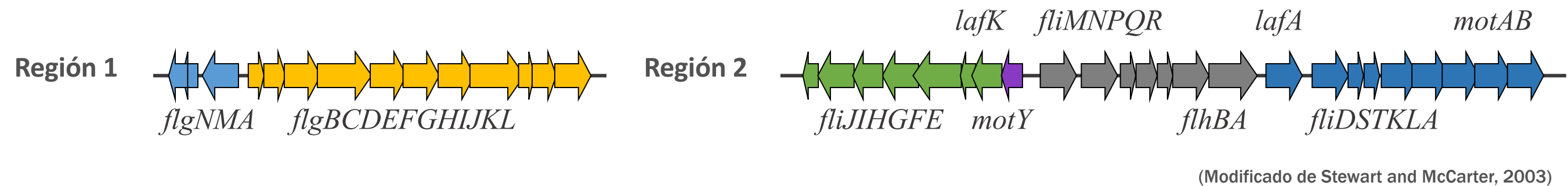
del flagelo de *Aeromonas* spp.

Regulación genética del flagelo procariota

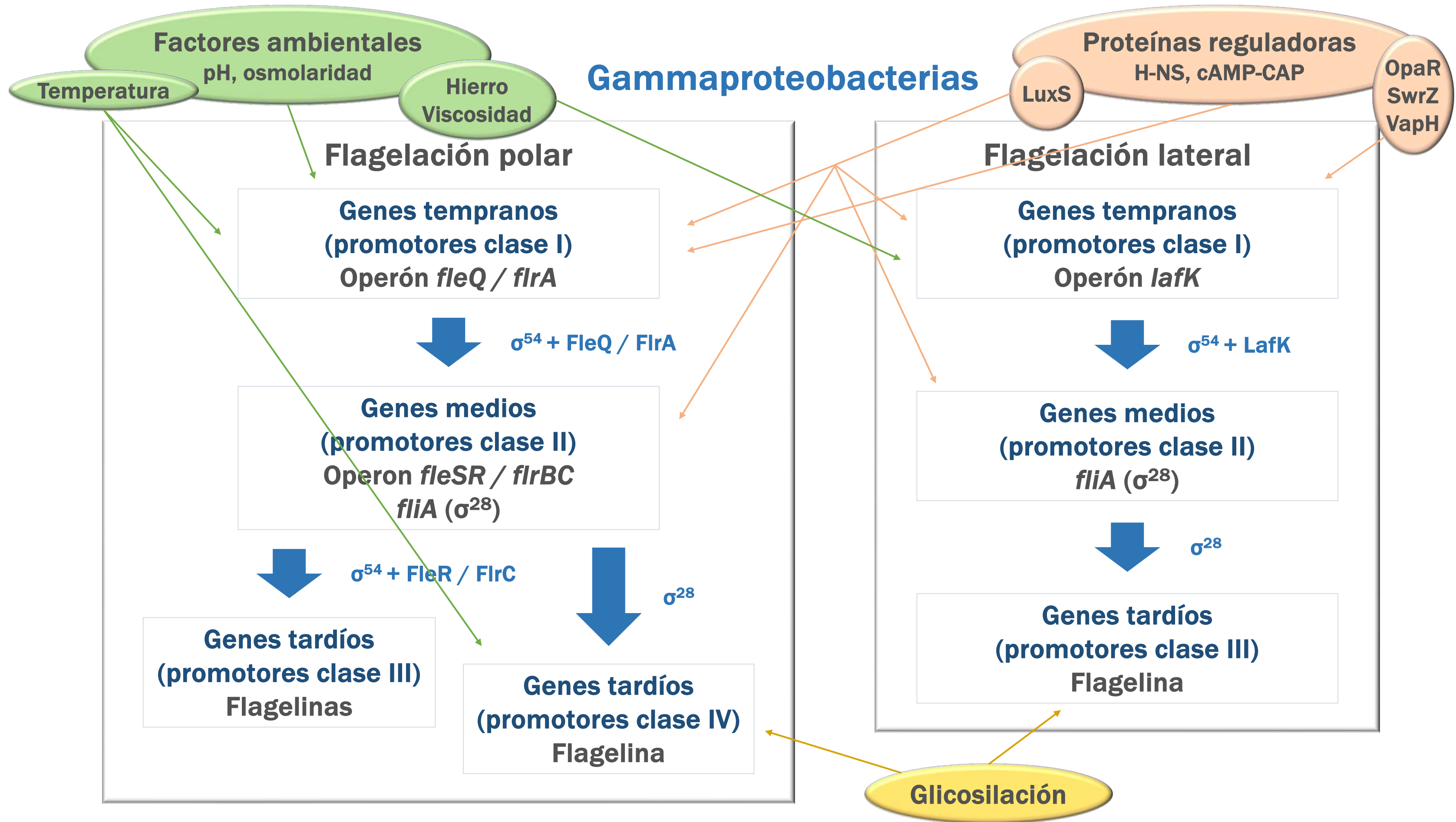
Flagelación polar en *Vibrio parahaemolyticus*



Flagelación lateral en *Vibrio parahaemolyticus*

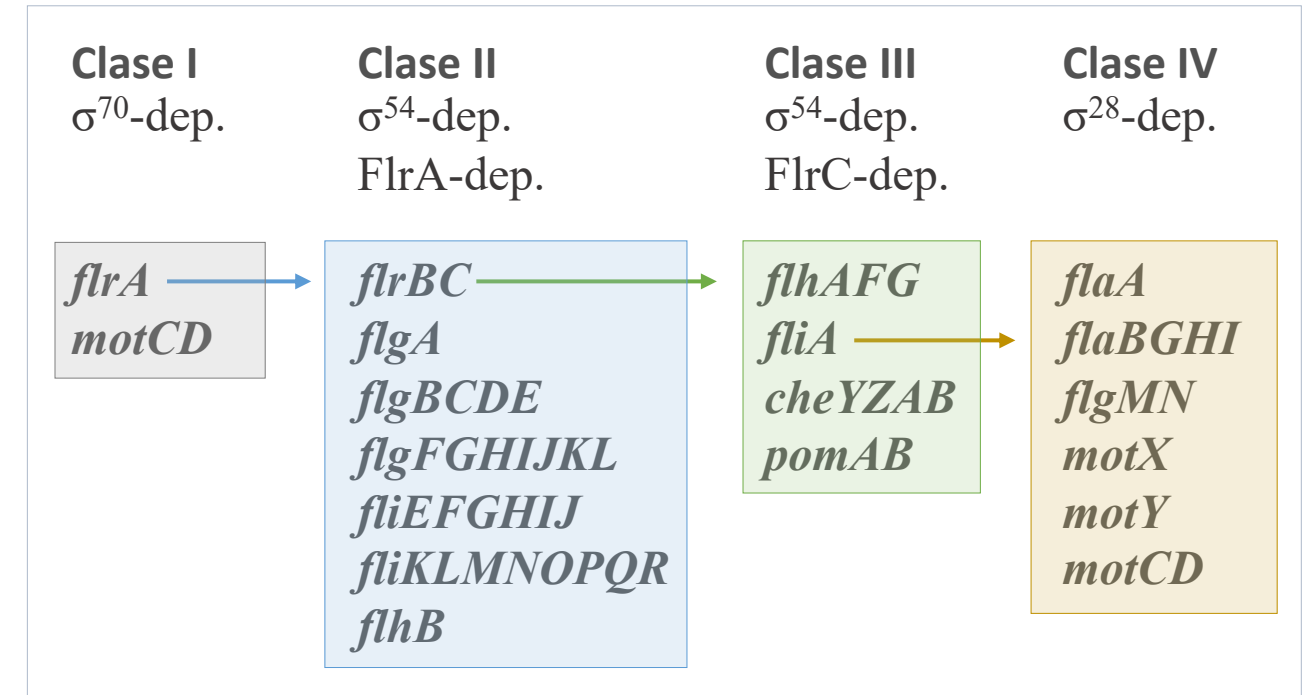
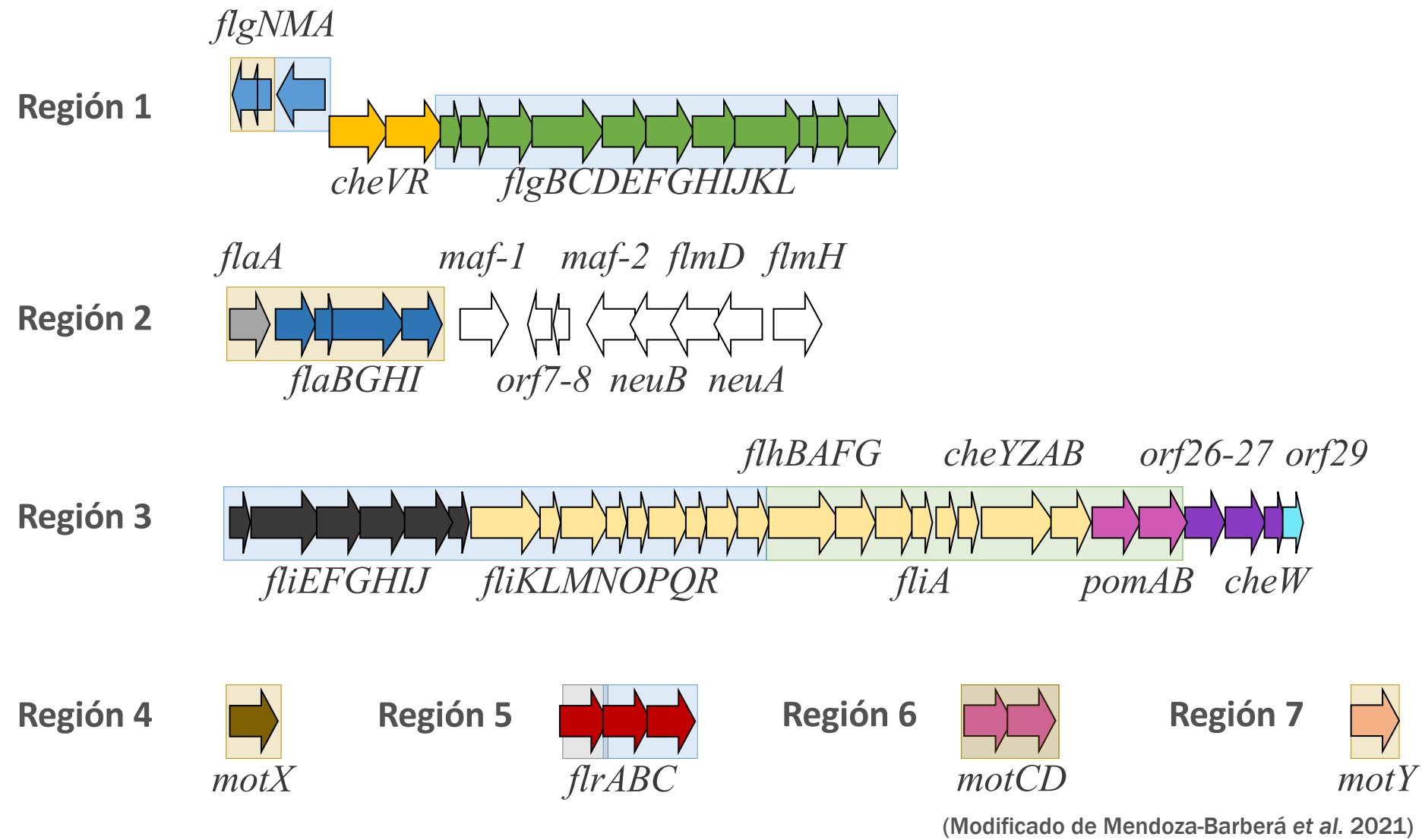


Regulación genética del flagelo procariota

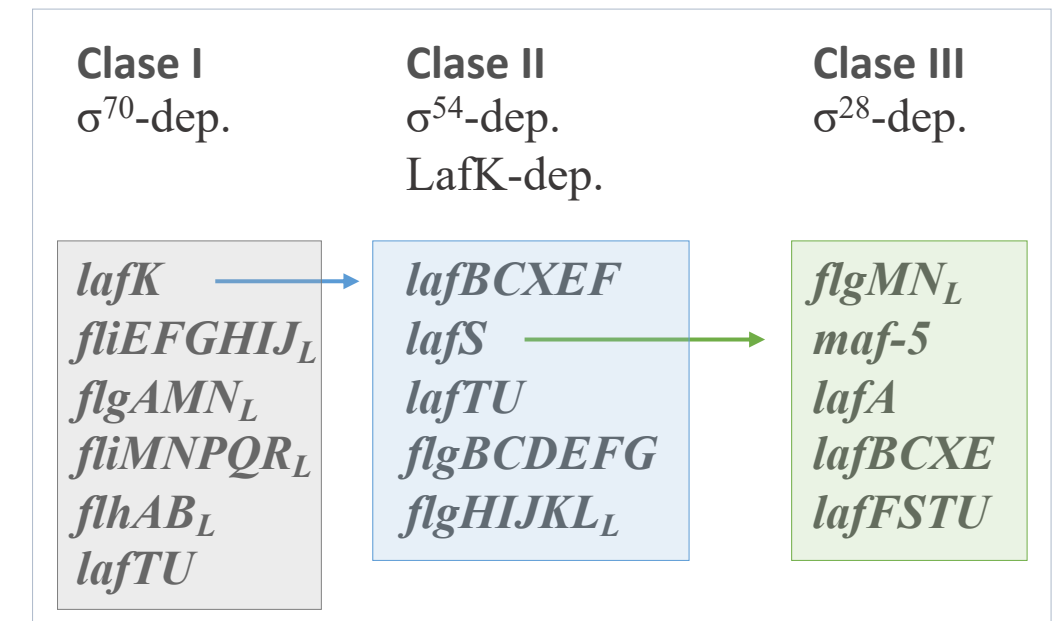
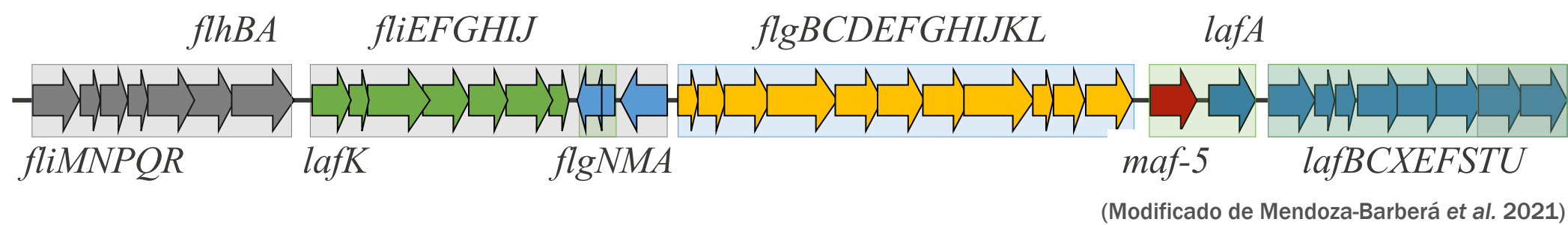


Regulación genética del flagelo de *Aeromonas*

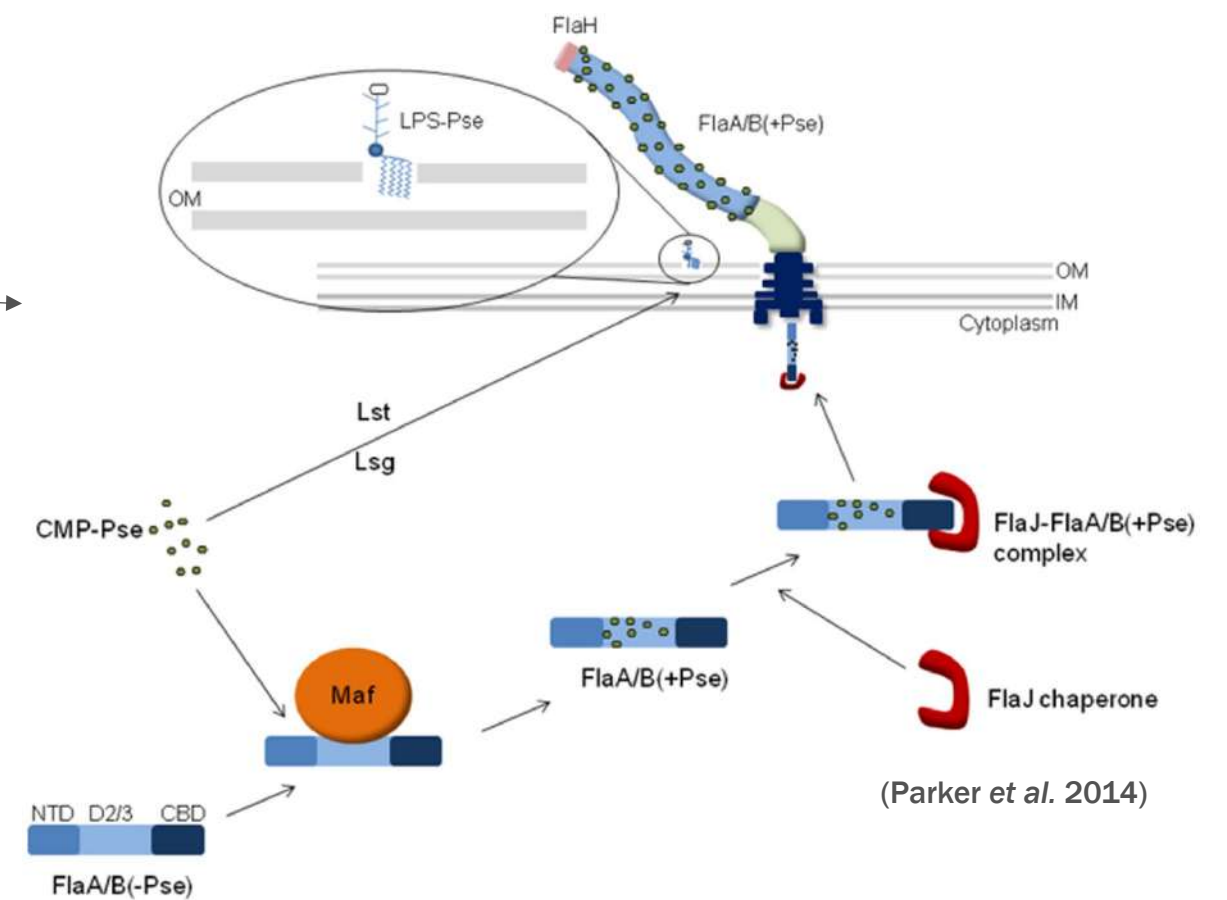
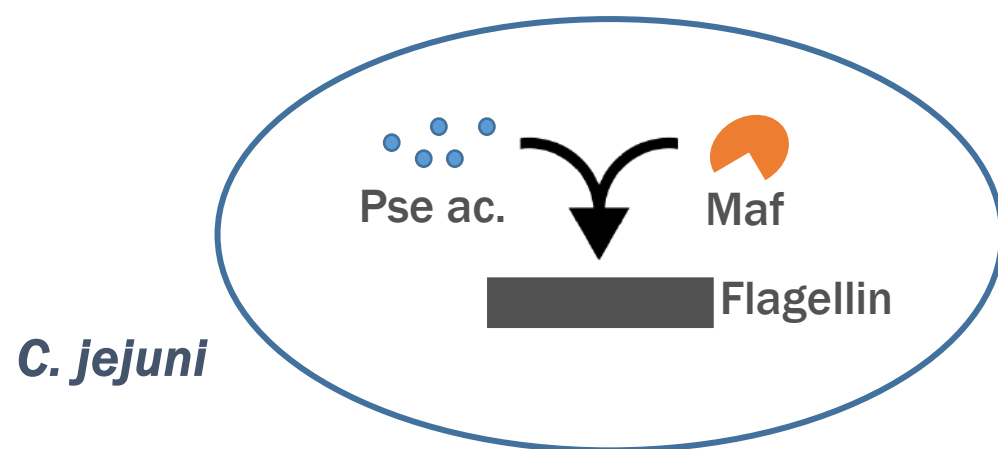
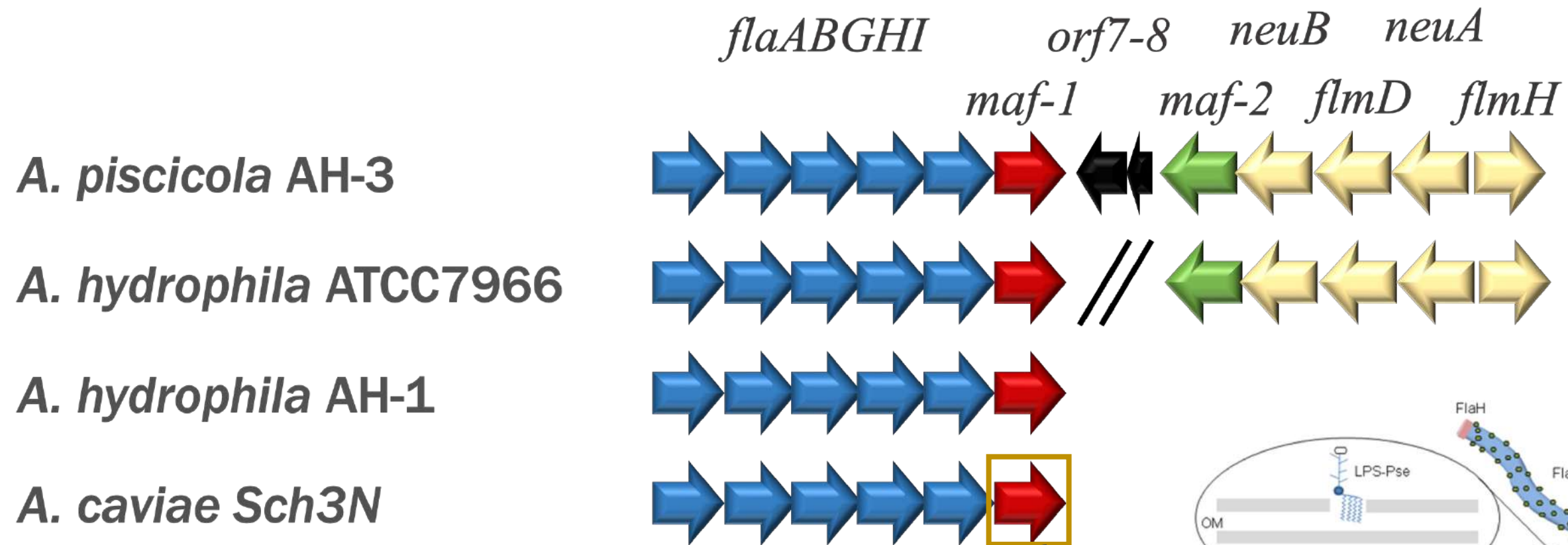
Flagelación polar en *A. piscicola* AH-3



Flagelación lateral en *A. piscicola* AH-3

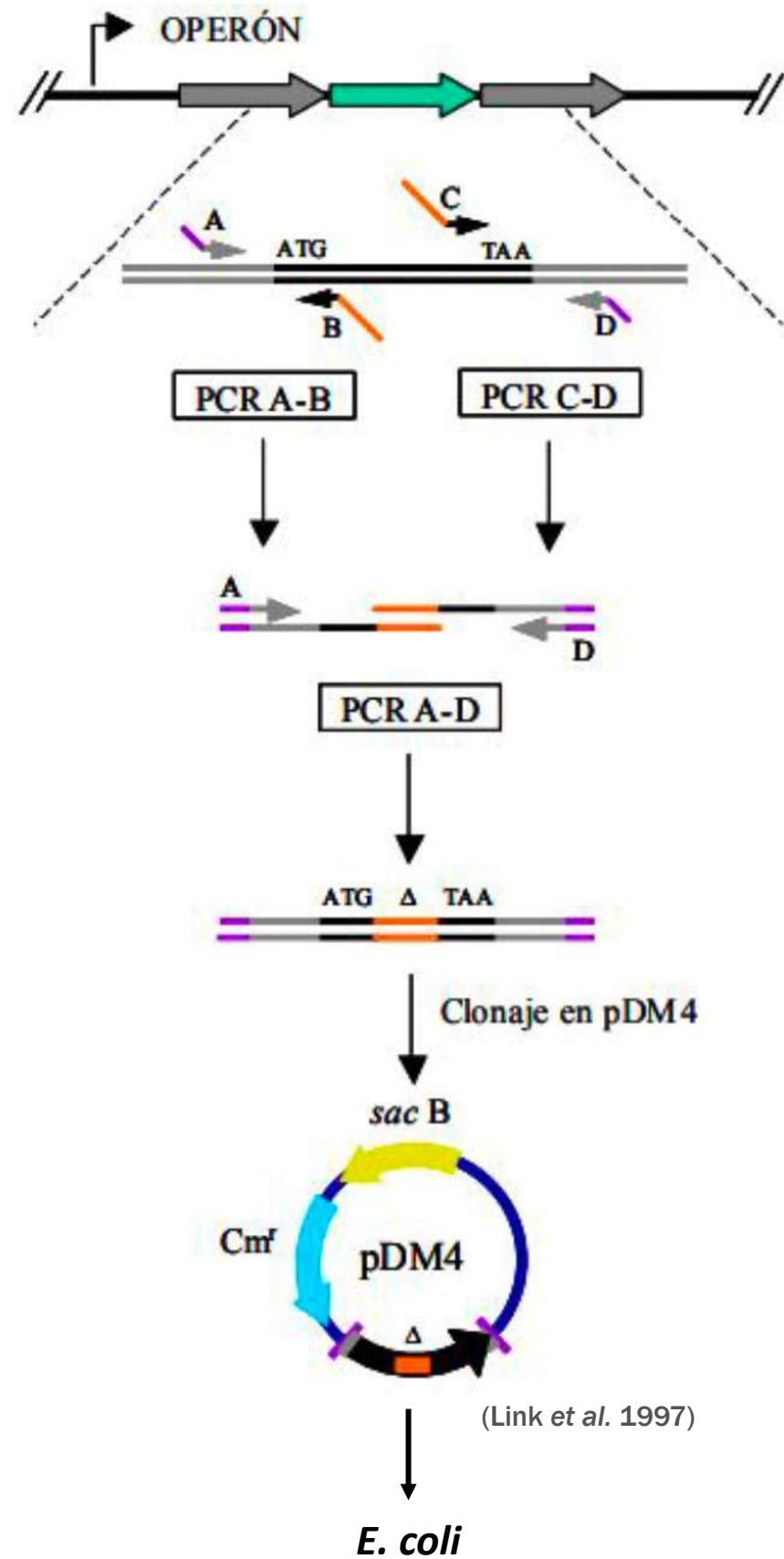


Análisis de las islas de glicosilación

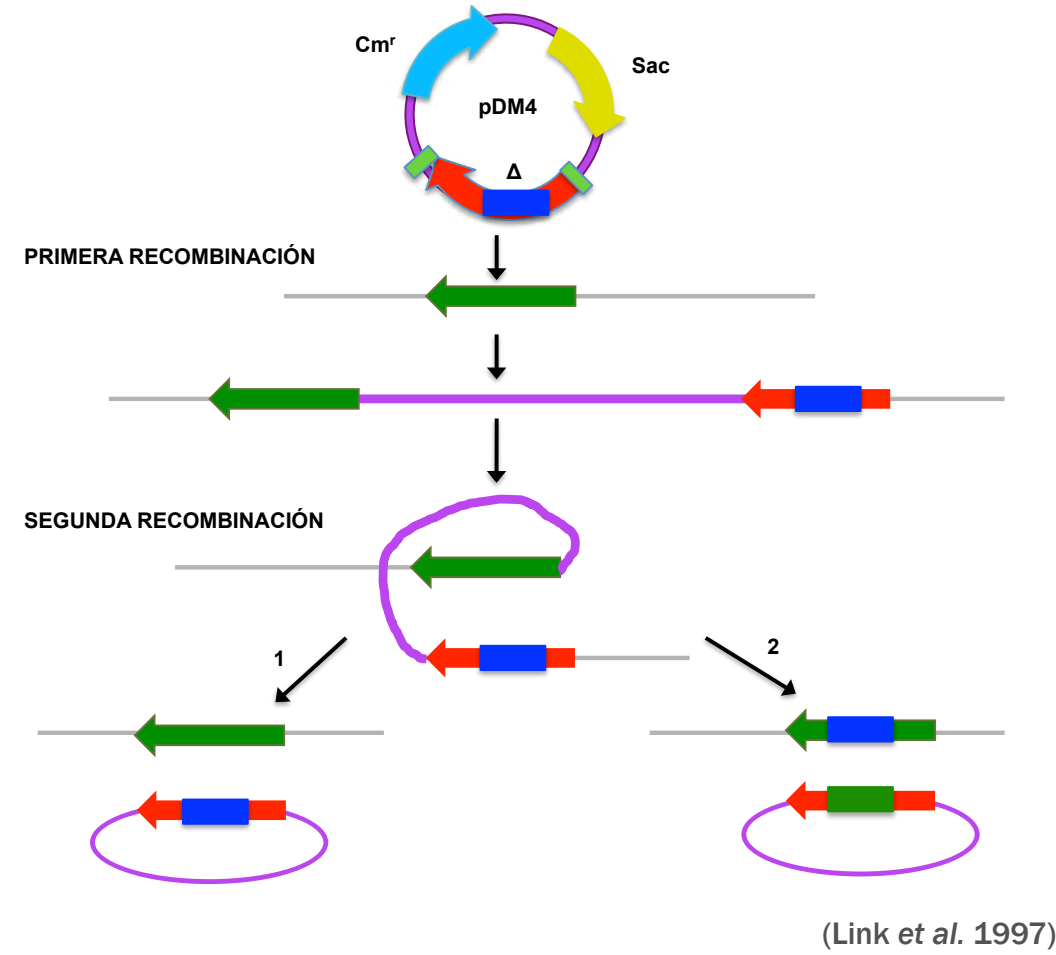


Generación de mutantes en pauta de lectura

1. Generación y clonación del fragmento mutante



2. Apareamiento triparental



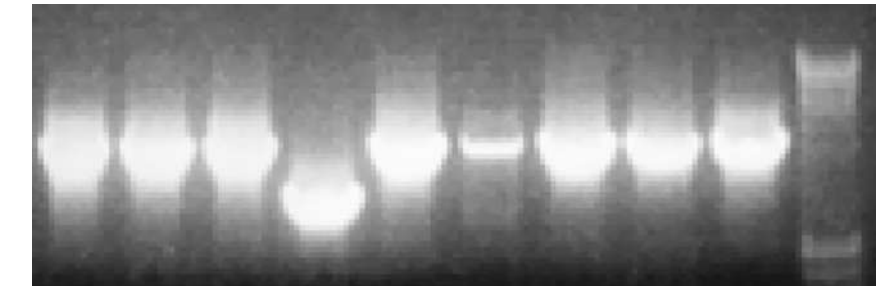
3. Crecimiento con sacarosa



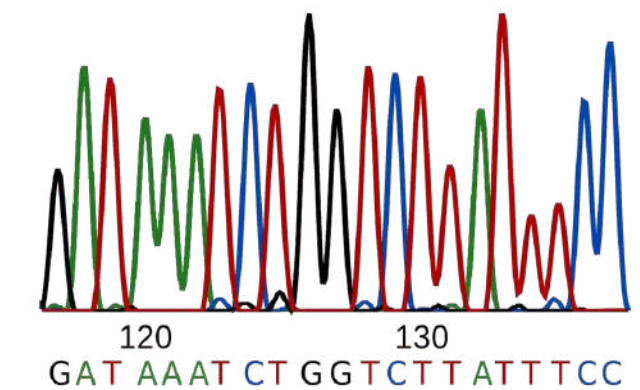
4. Selección de colonias Cm^s



5. Comprobación por PCR

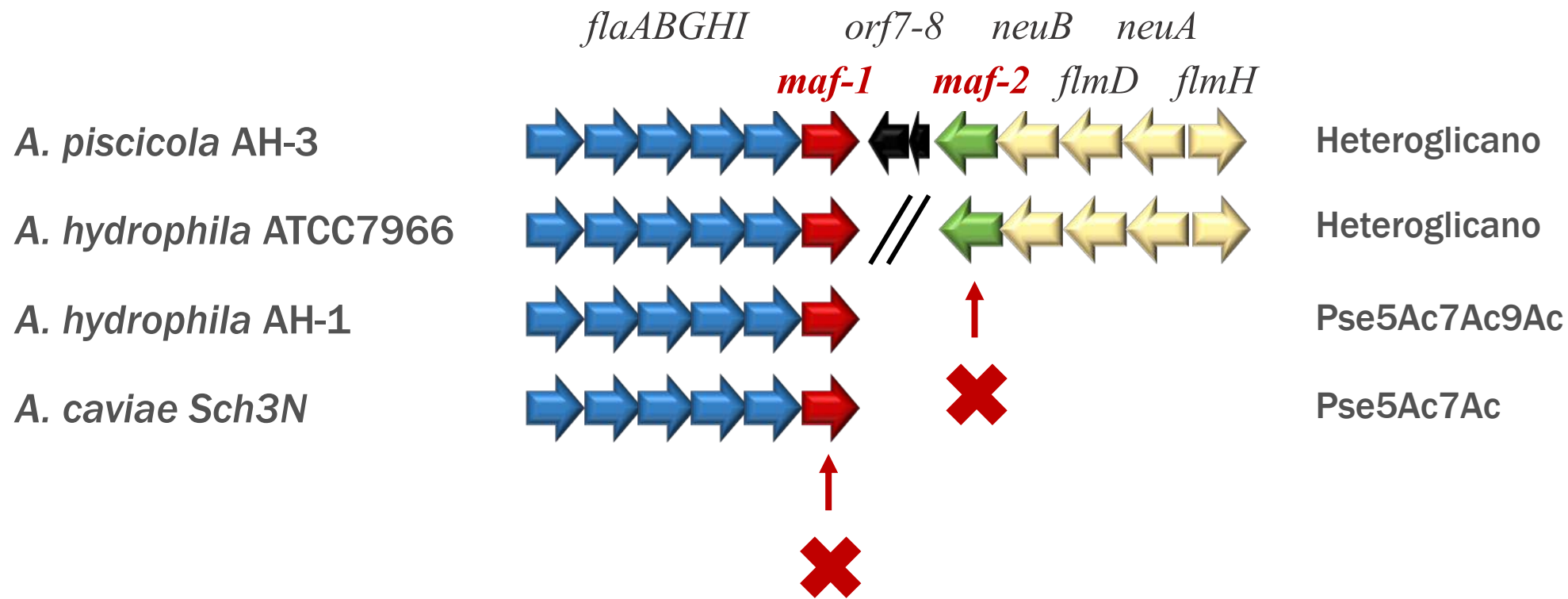


6. Secuenciación

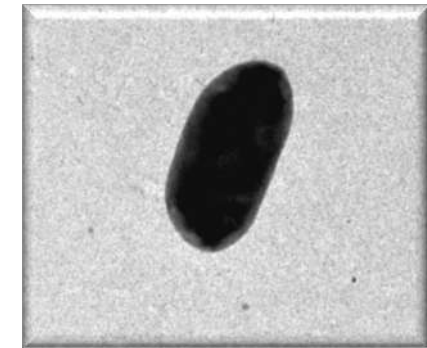


Análisis de las islas de glicosilación

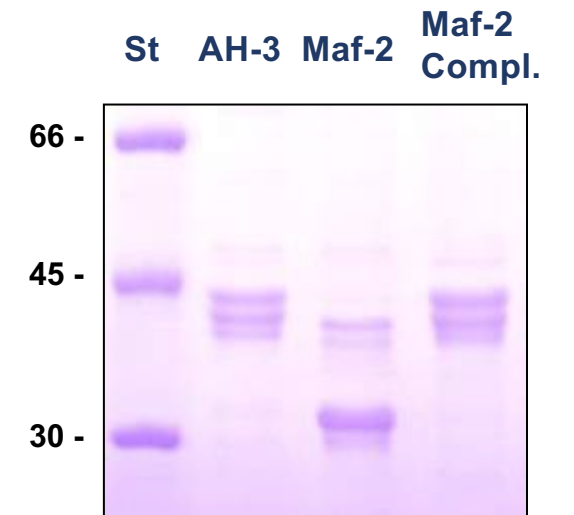
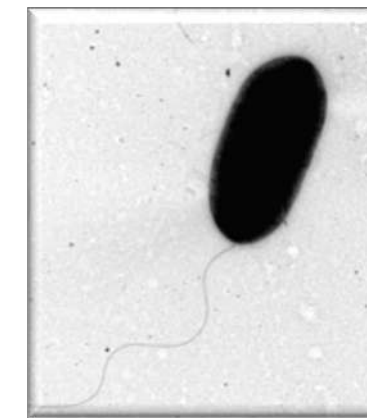
Región de flagelación polar



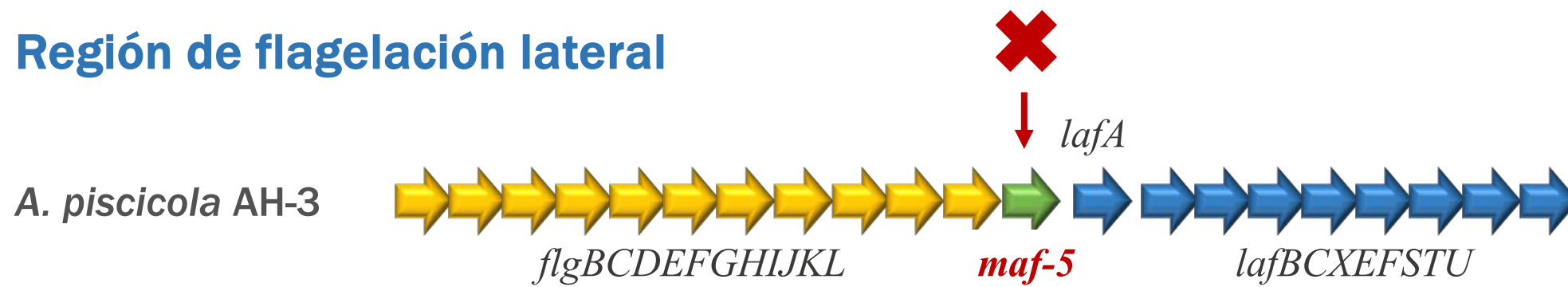
$\Delta maf-1$



$\Delta maf-2$



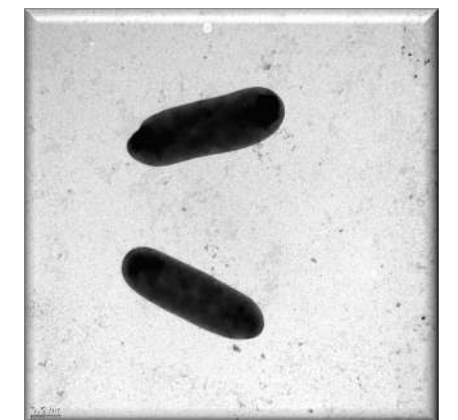
Región de flagelación lateral



$\Delta maf-5$

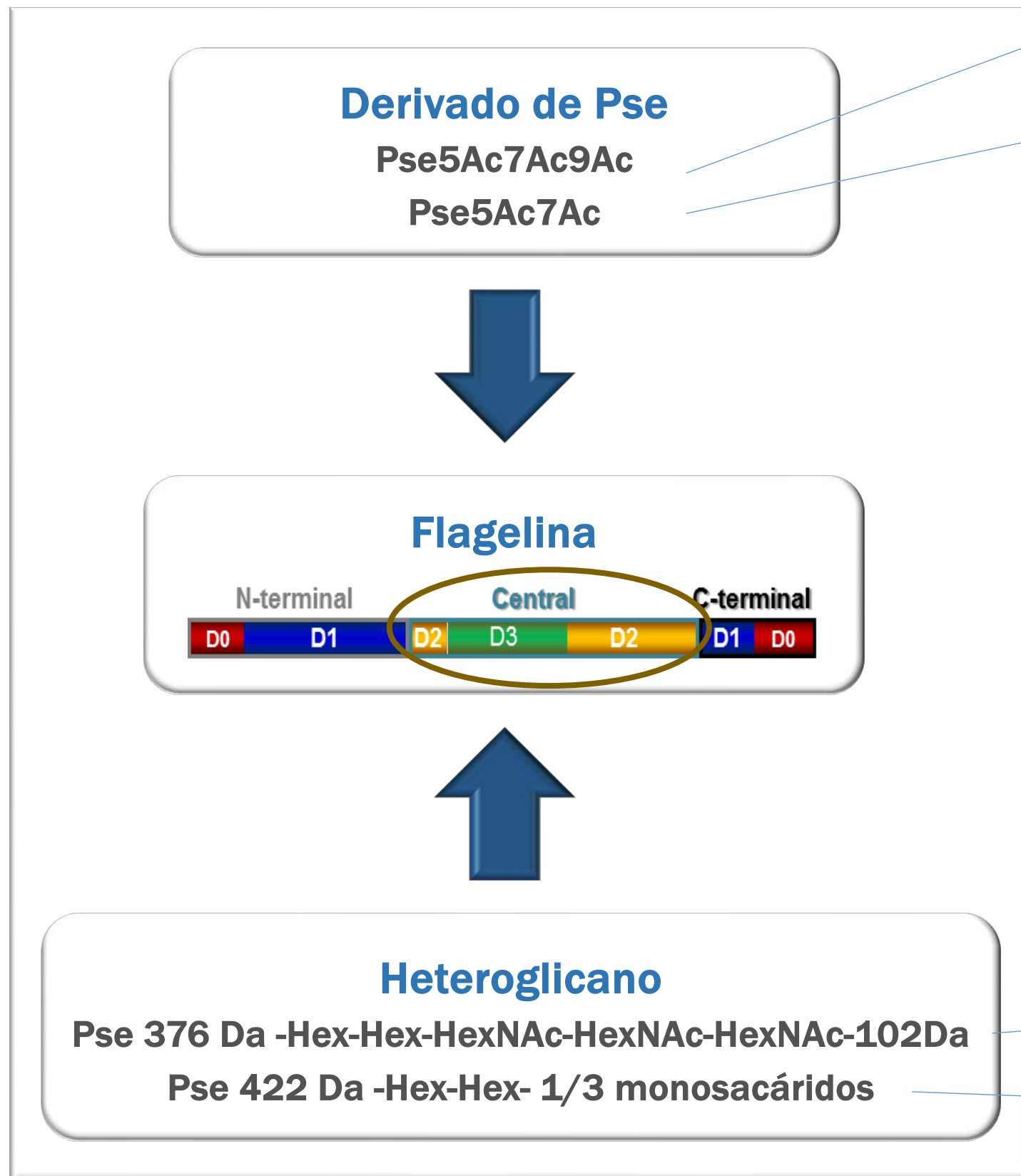


Medio líquido



Medio semi-sólido

Proteínas Maf



A. hydrophila AH-1

A. caviae Sch3N

Similitud entre secuencias de Maf-1

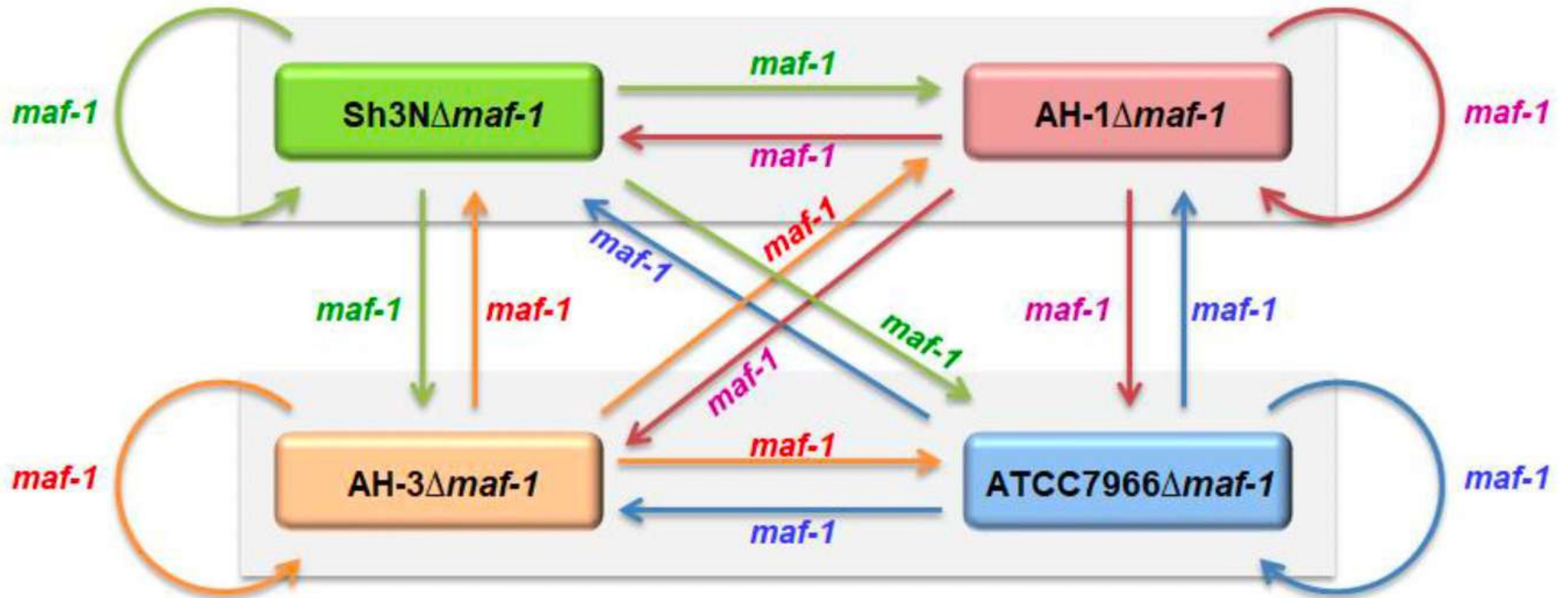
| % Similitud | AH-1 | Sch3N | AH-3 | ATTC7966 |
|-------------|------|-------|------|----------|
| AH-1 | 100% | 68% | 39% | 40% |
| Sch3N | | 100% | 40% | 41% |
| AH-3 | | | 100% | 72% |
| ATTC7966 | | | | 100% |

A. piscicola AH-3

A. hydrophila ATCC7966

Análisis de especificidad sustrato-receptor de Maf-1

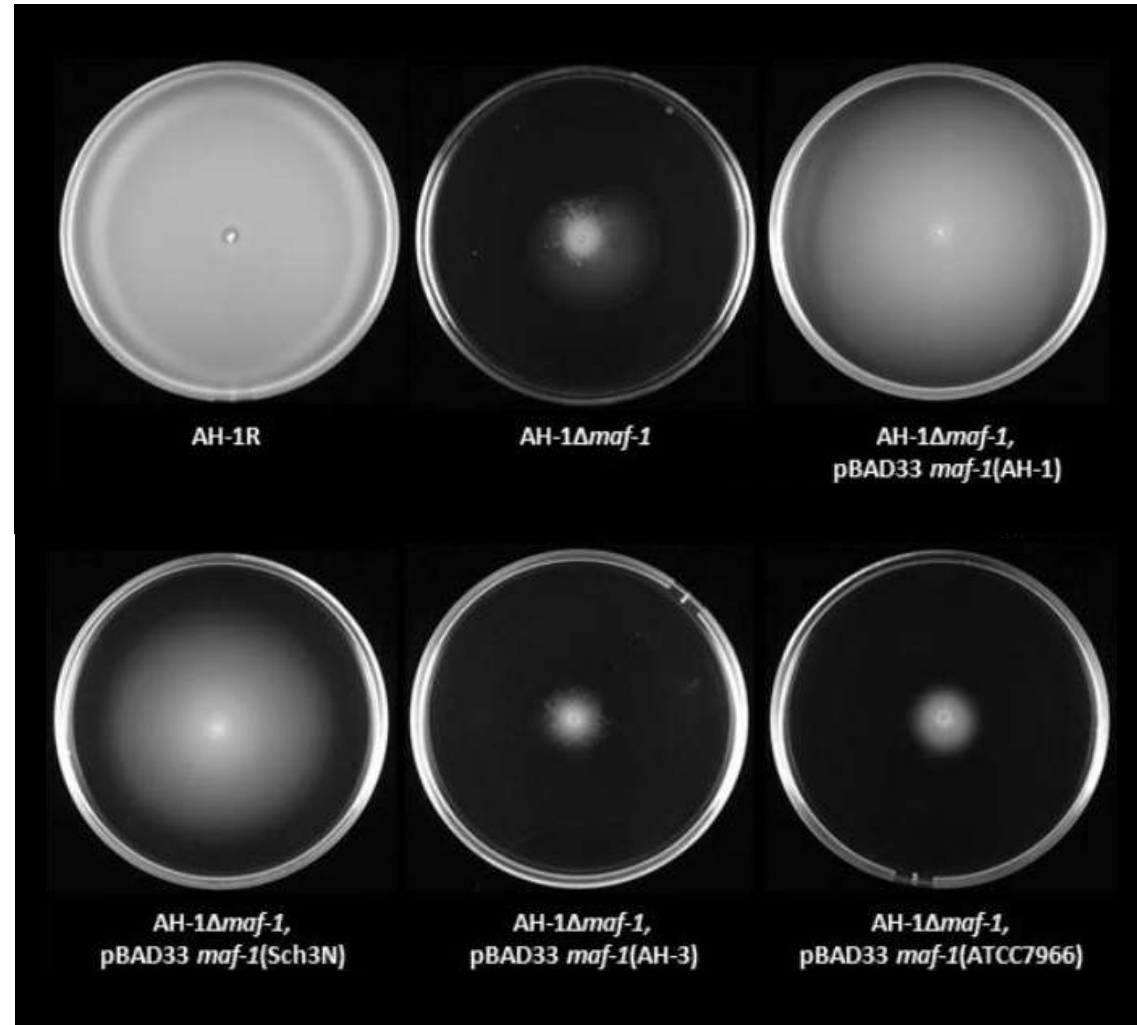
Ensayos de complementación cruzada entre las proteínas Maf-1 de diferentes especies



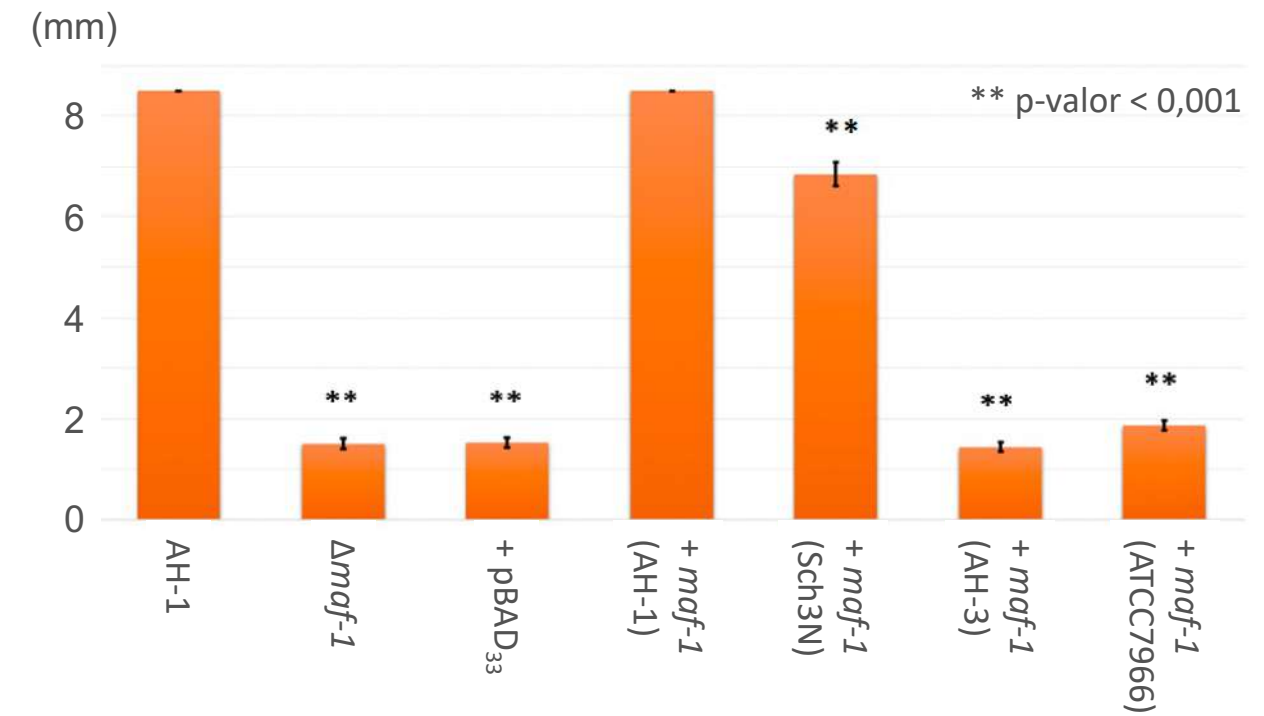
Estudios de complementación cruzada de Maf-1

A. hydrophila AH-1

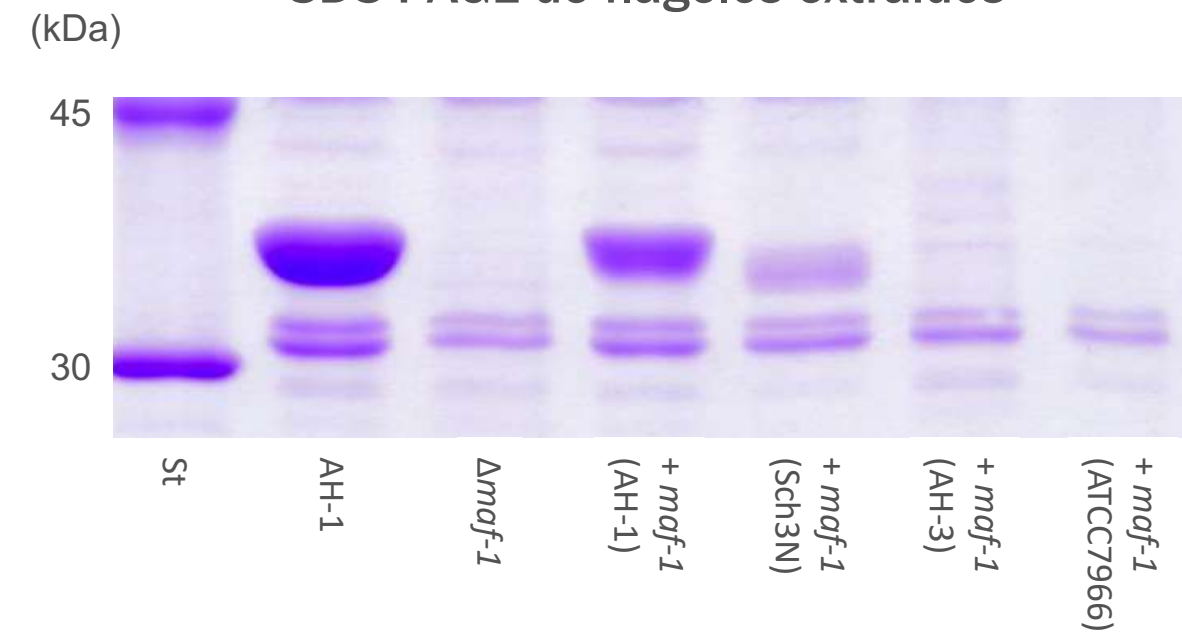
Motilidad en LB agar (0,27%)



Radio de migración en LB agar (0,27%)



SDS-PAGE de flagelos extraídos



A. piscicola AH-3: Heteroglicano 1679 Da

A. hydrophila ATCC7966: Heteroglicano 1285 Da

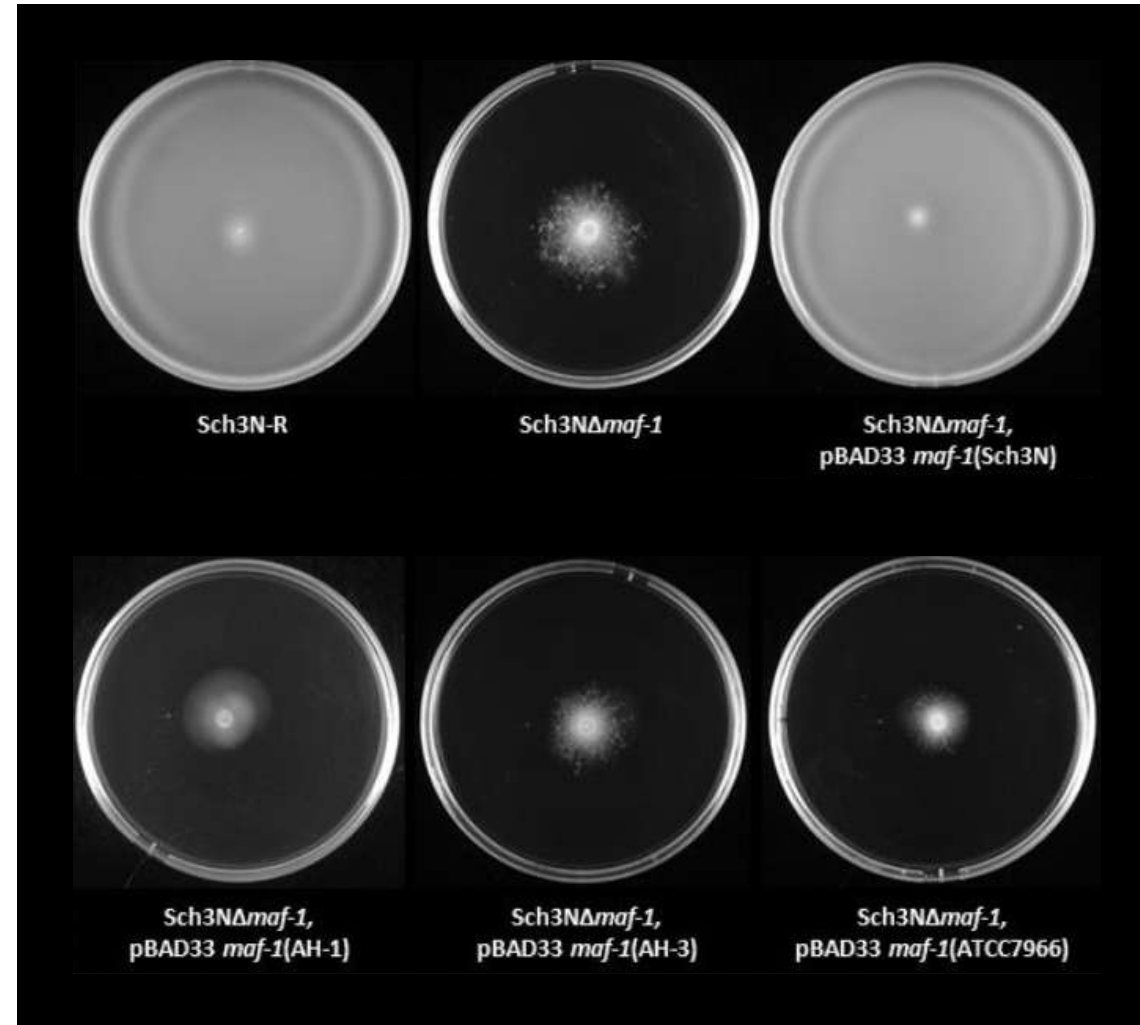
A. hydrophila AH-1: Pse5Ac7Ac9Ac

A. caviae Sch3N: Pse5Ac7Ac

Estudios de complementación cruzada de Maf-1

A. caviae Sch3N

Motilidad en LB agar (0,27%)



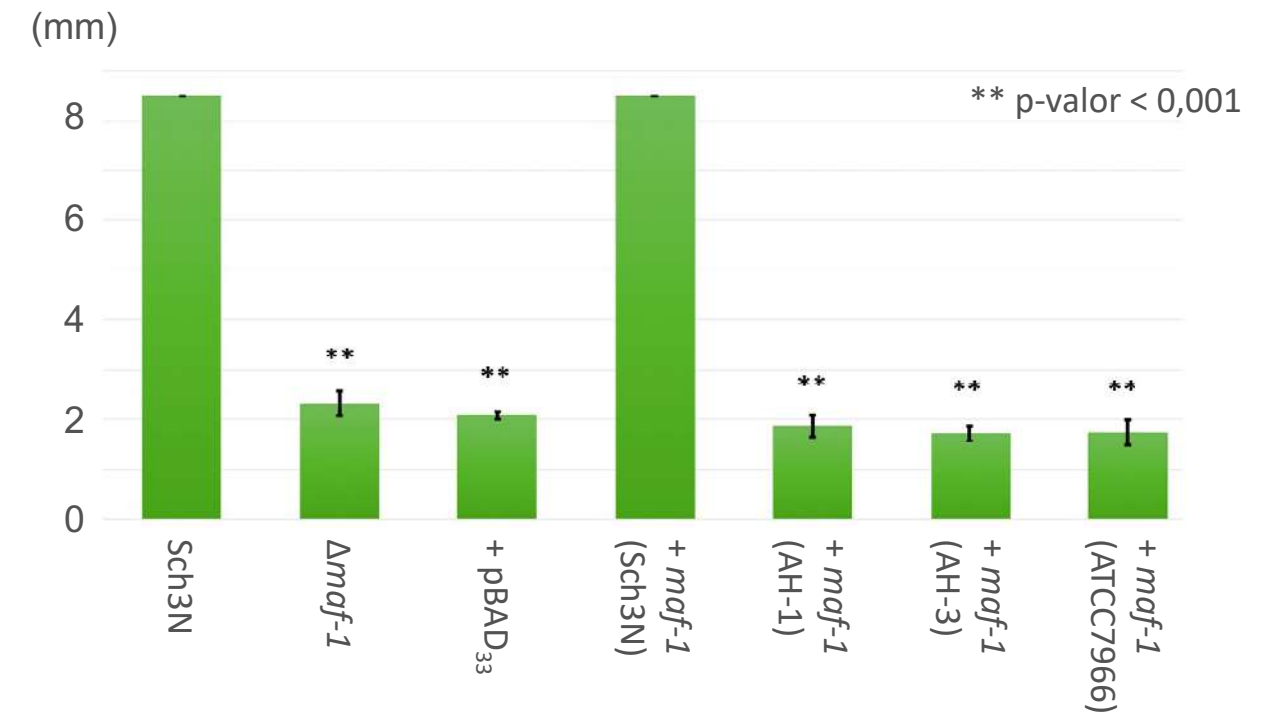
A. piscicola AH-3: Heteroglicano 1679 Da

A. hydrophila ATCC7966: Heteroglicano 1285 Da

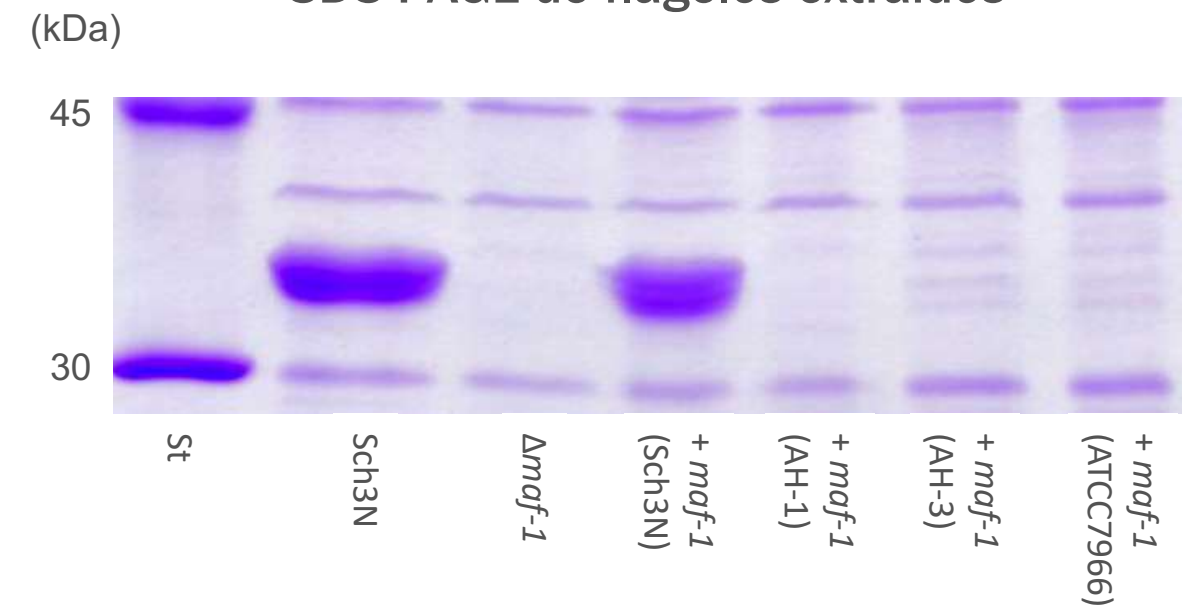
A. hydrophila AH-1: Pse5Ac7Ac9Ac

A. caviae Sch3N: Pse5Ac7Ac

Radio de migración en LB agar (0,27%)

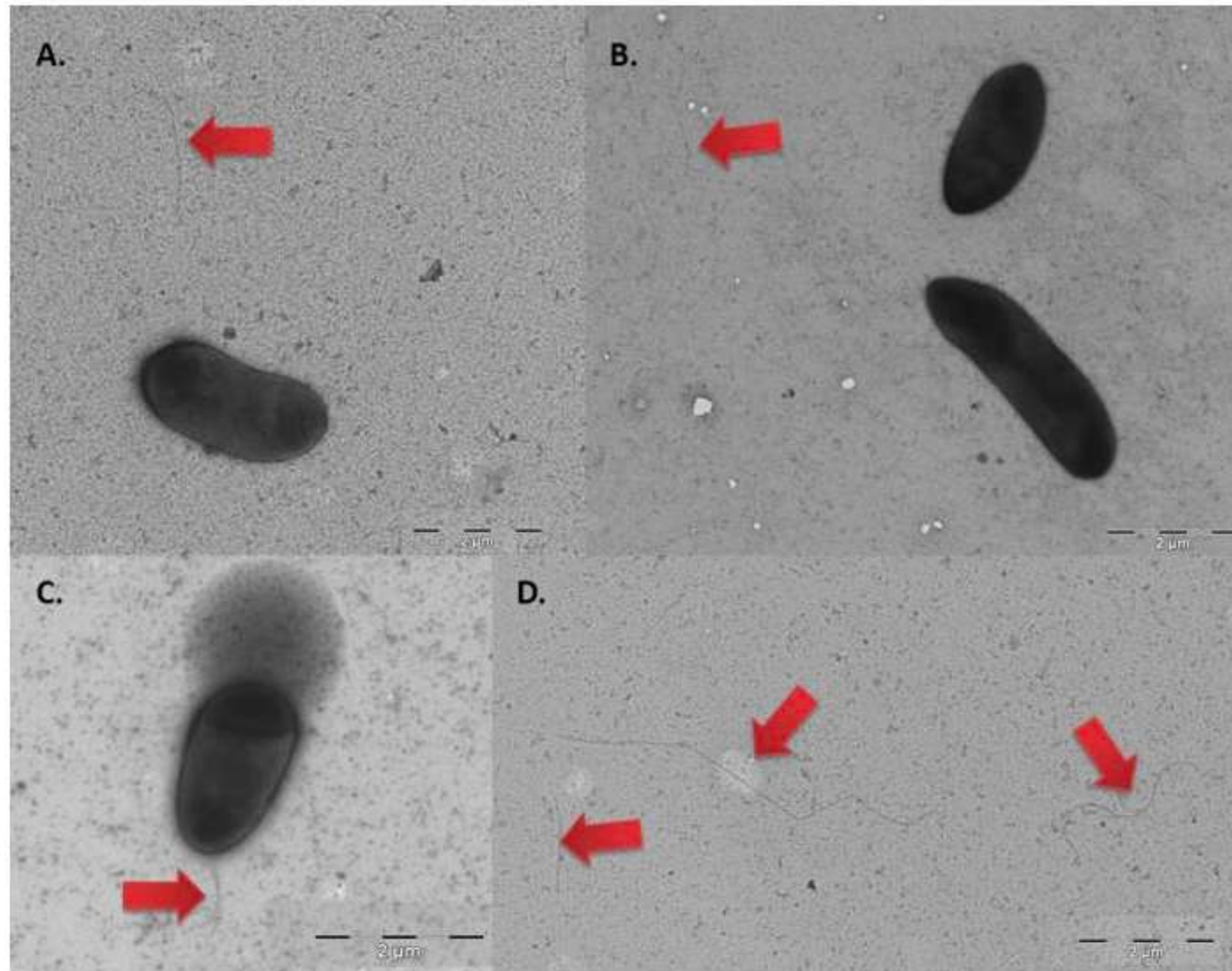


SDS-PAGE de flagelos extraídos



Estudios de complementación cruzada de Maf-1

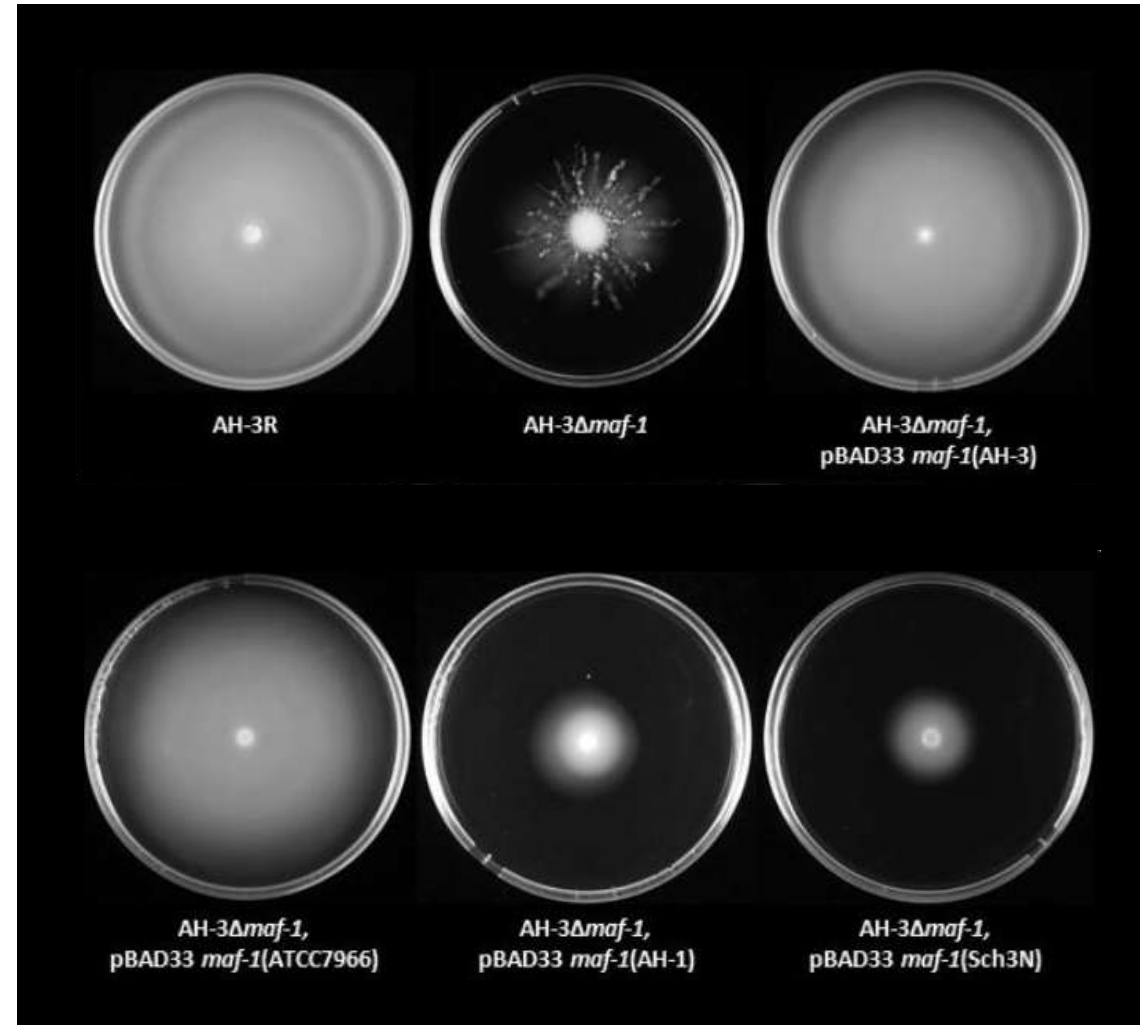
TEM de *A. caviae* Sch3N Δ *maf-1* + *maf-1* de *A. hydrophila* AH-1



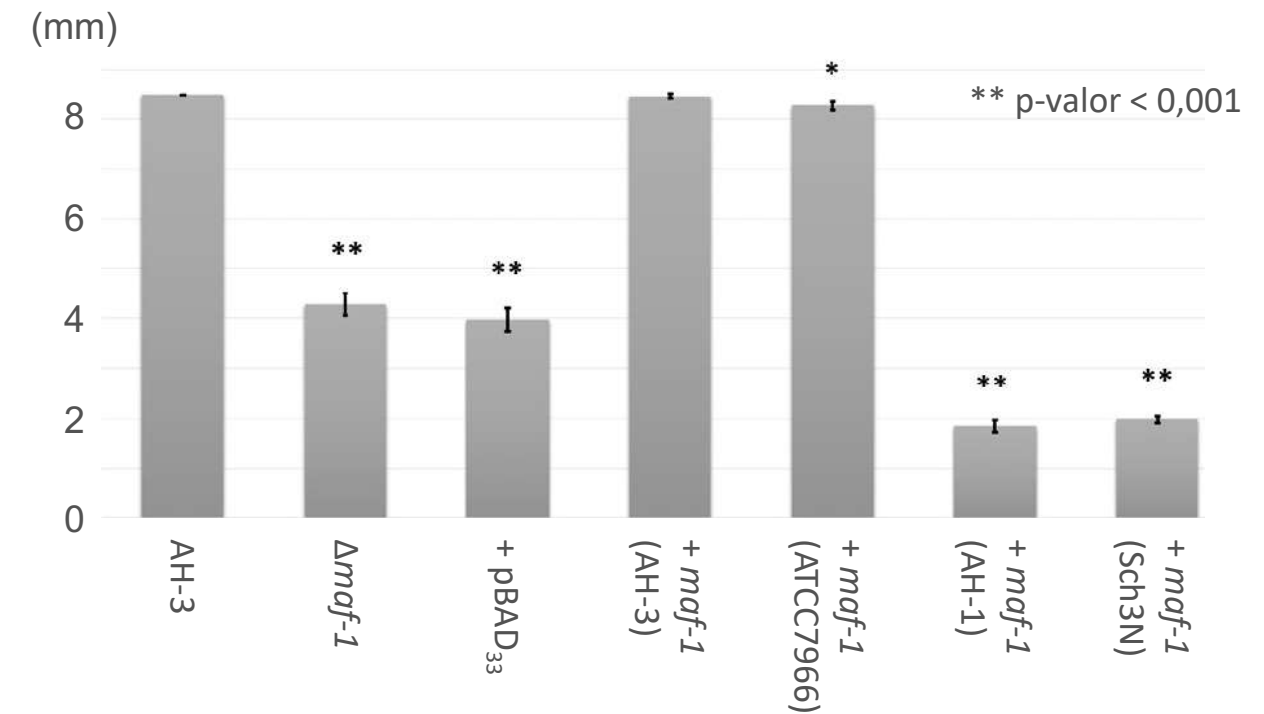
Estudios de complementación cruzada de Maf-1

A. piscicola AH-3

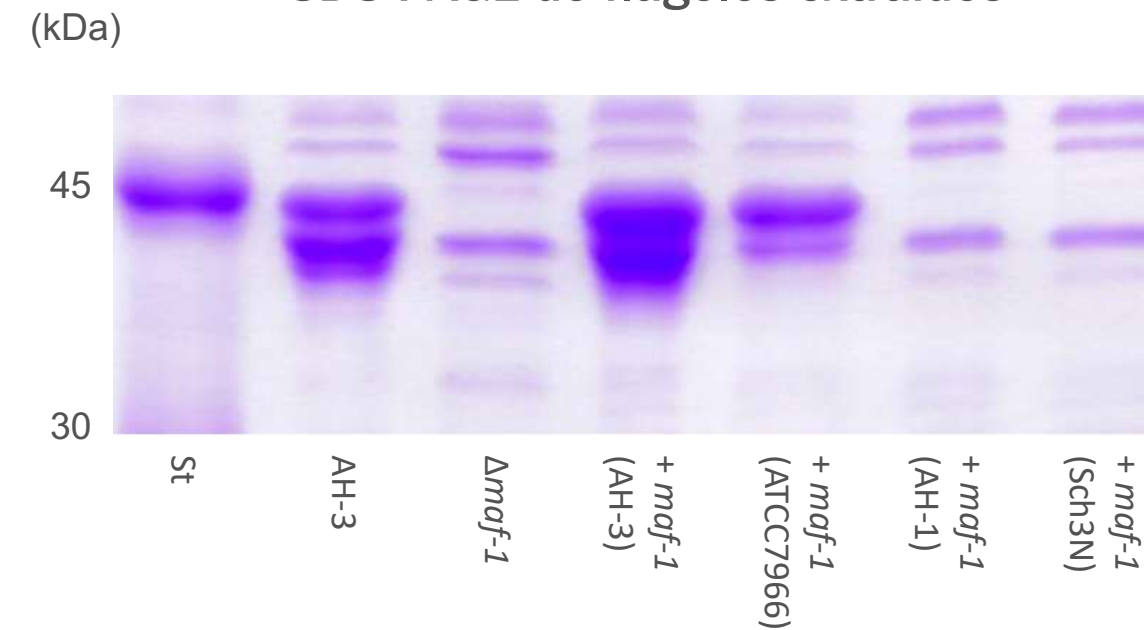
Motilidad en LB agar (0,27%)



Radio de migración en LB agar (0,27%)



SDS-PAGE de flagelos extraídos



A. piscicola AH-3: Heteroglicano 1679 Da

A. hydrophila ATCC7966: Heteroglicano 1285 Da

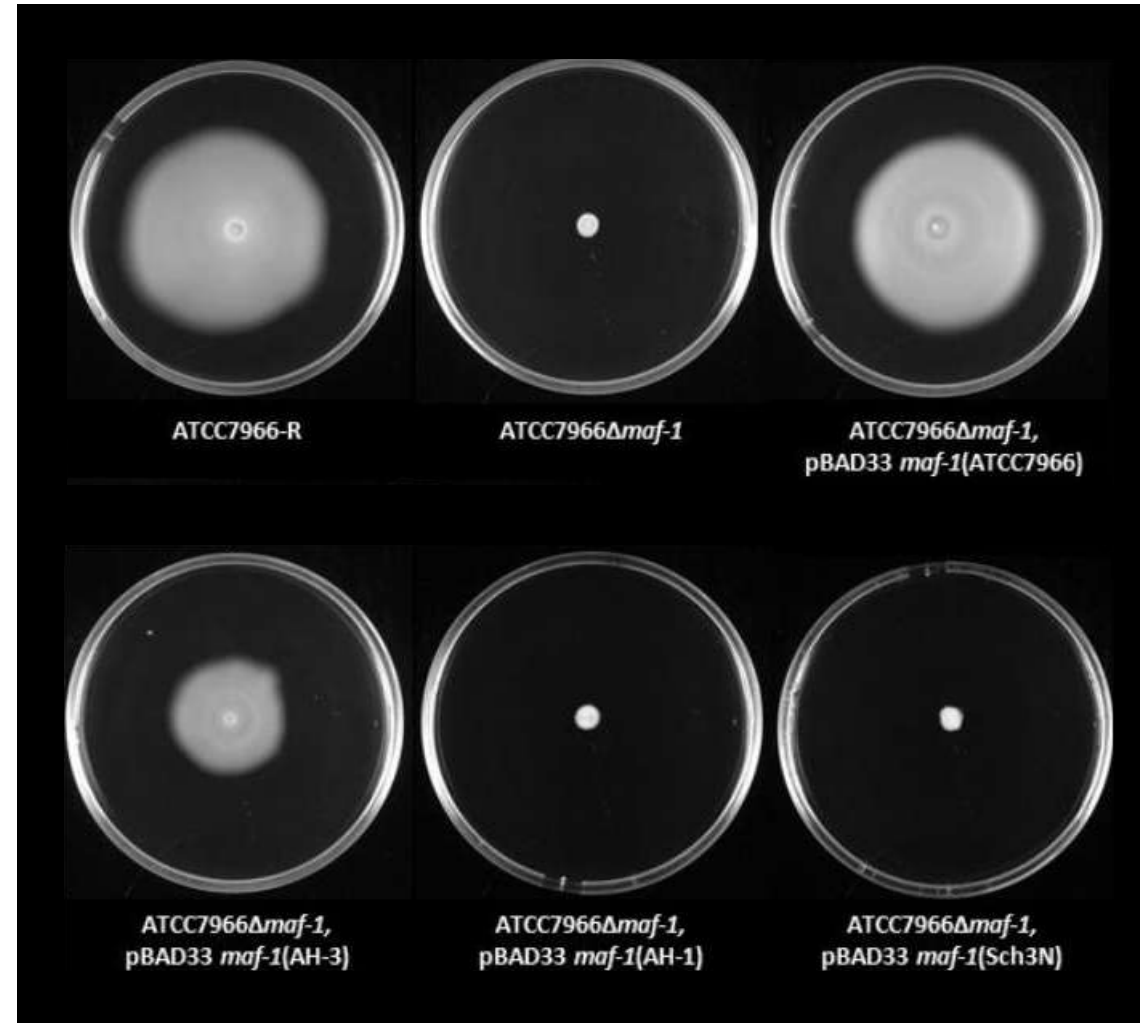
A. hydrophila AH-1: Pse5Ac7Ac9Ac

A. caviae Sch3N: Pse5Ac7Ac

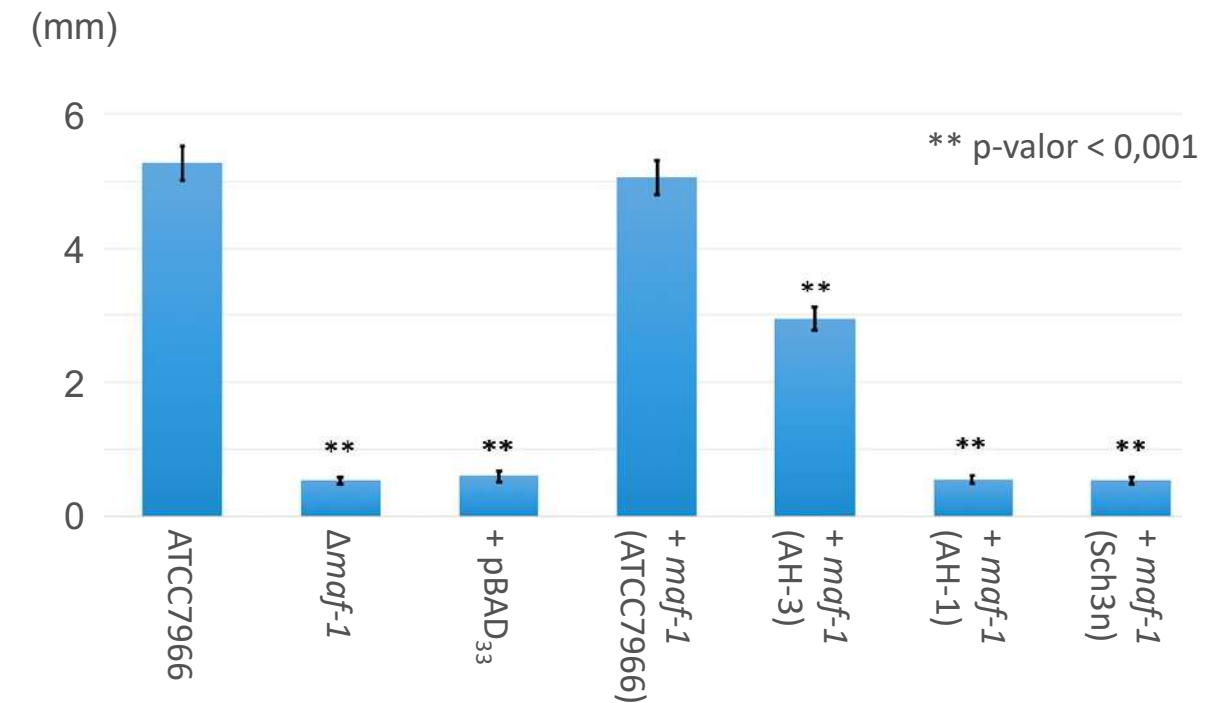
Estudios de complementación cruzada de Maf-1

A. hydrophila ATCC7966

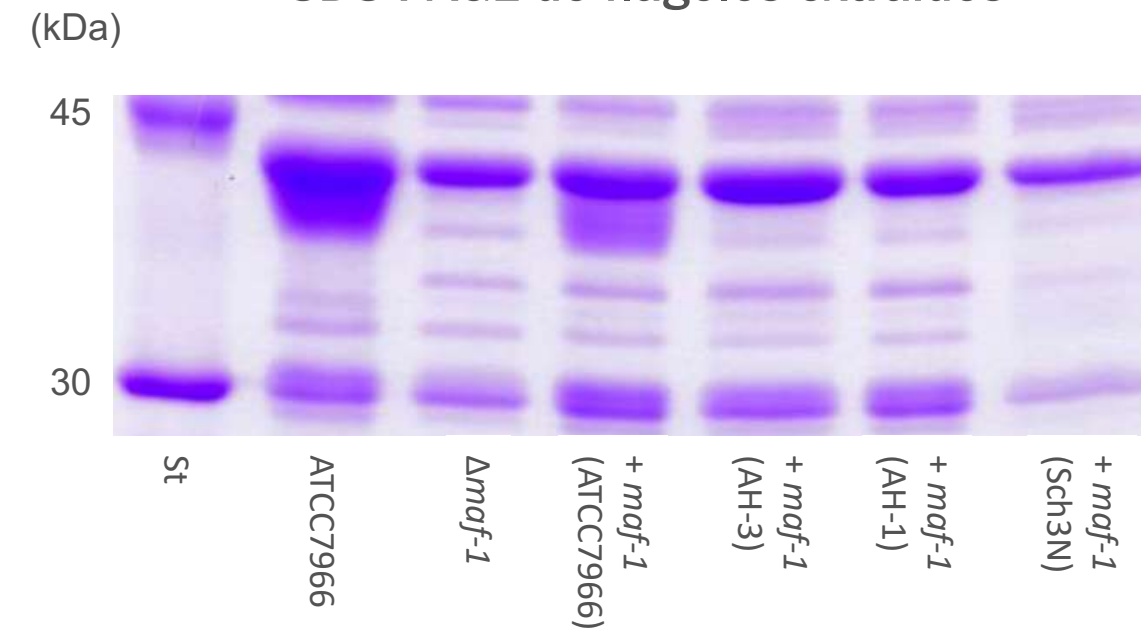
Motilidad en LB agar (0,27%)



Radio de migración en LB agar (0,27%)



SDS-PAGE de flagelos extraídos



A. piscicola AH-3: Heteroglicano 1679 Da

A. hydrophila ATCC7966: Heteroglicano 1285 Da

A. hydrophila AH-1: Pse5Ac7Ac9Ac

A. caviae Sch3N: Pse5Ac7Ac

Análisis de especificidad sustrato-receptor de Maf-1

Similitud entre secuencias de FlaA o FlaB

| % Similitud | FlaA (AH-1) | FlaB (AH-1) | FlaA (Sch3N) | FlaB (Sch3N) | FlaA (AH-3) | FlaB (AH-3) | FlaA (ATCC7966) | FlaB (ATCC7666) |
|-----------------|-------------|-------------|--------------|--------------|-------------|-------------|-----------------|-----------------|
| FlaA (AH-1) | 100% | 94% | 87% | 84% | 71% | 72% | 71% | 71% |
| FlaB (AH-1) | | 100% | 86% | 86% | 71% | 75% | 73% | 75% |
| FlaA (Sch3N) | | | 100% | 92% | 72% | 72% | 72% | 73% |
| FlaB (Sch3N) | | | | 100% | 71% | 73% | 71% | 73% |
| FlaA (AH-3) | | | | | 100% | 86% | 86% | 82% |
| FlaB (AH-3) | | | | | | 100% | 85% | 91% |
| FlaA (ATCC7966) | | | | | | | 100% | 87% |
| FlaB (ATCC7666) | | | | | | | | 100% |

A. piscicola AH-3: Heteroglicano 1679 Da

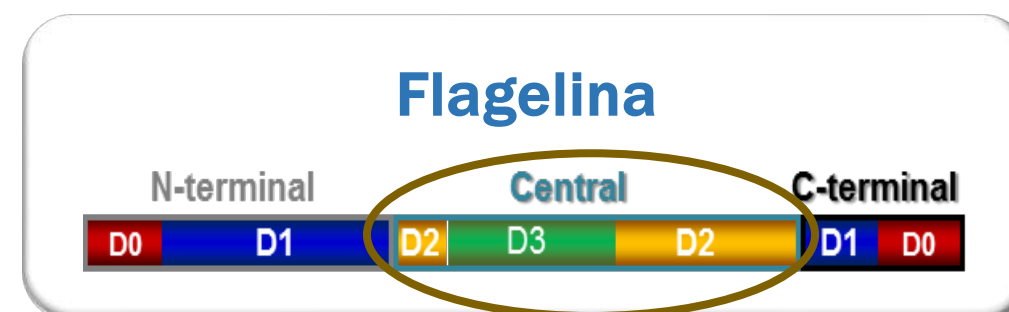
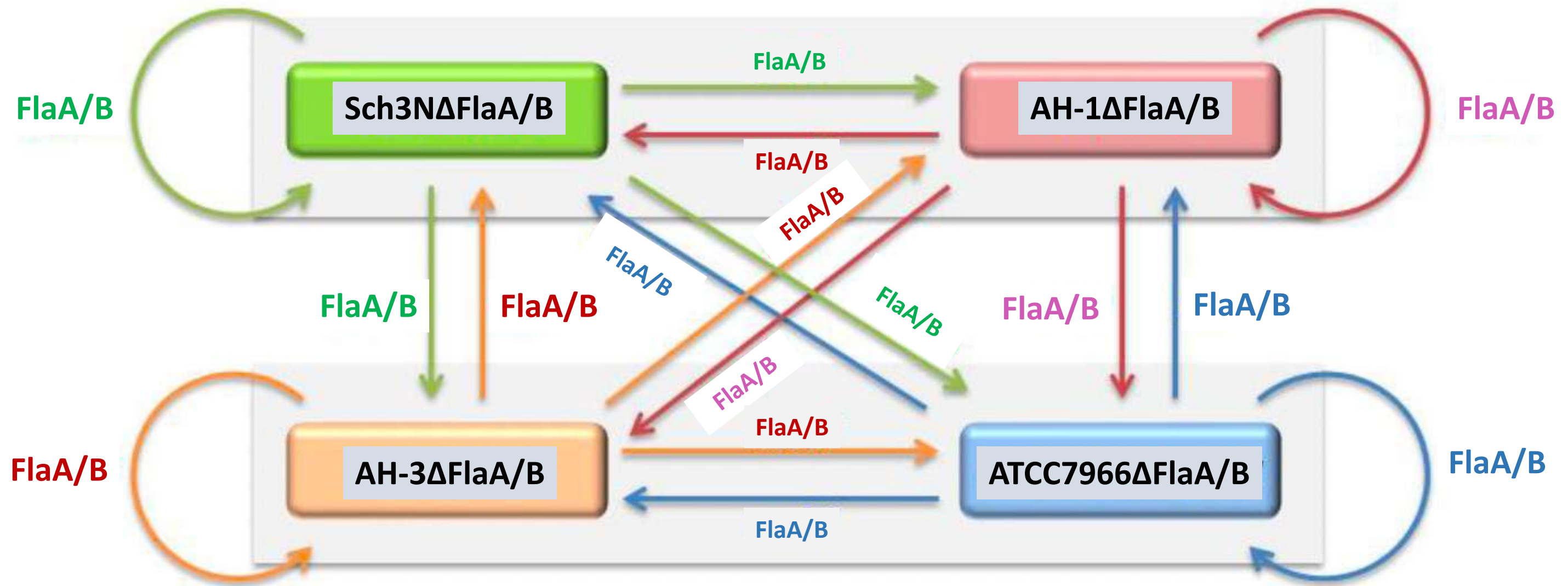
A. hydrophila ATCC7966: Heteroglicano 1285 Da

A. hydrophila AH-1: Pse5Ac7Ac9Ac

A. caviae Sch3N: Pse5Ac7Ac

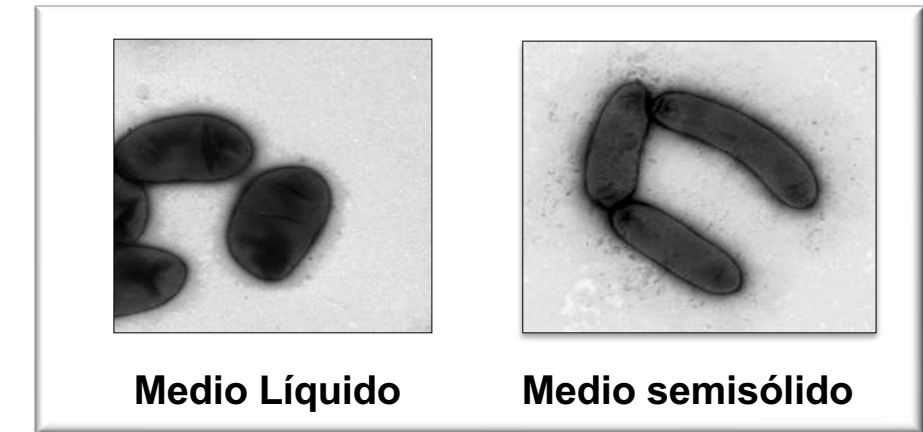
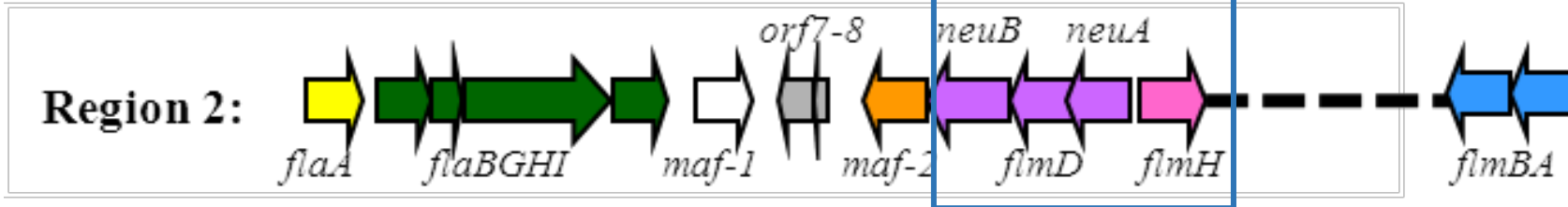
Análisis de especificidad sustrato-receptor de Maf-1

Ensayos de complementación cruzada entre las flagelinas polares de diferentes especies

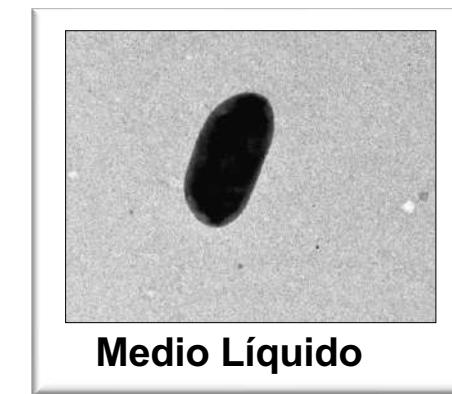
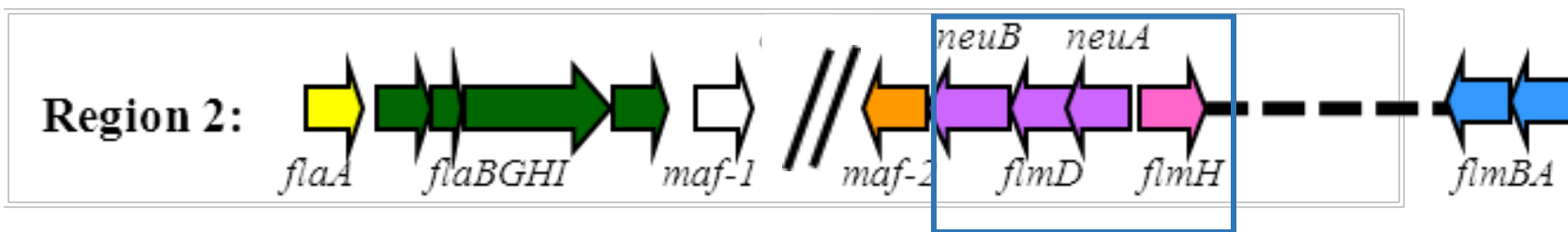


Análisis de genes implicados en glicosilación

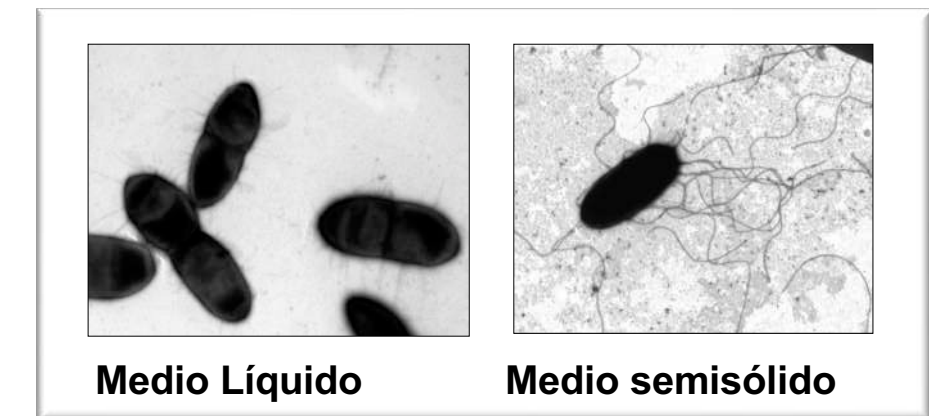
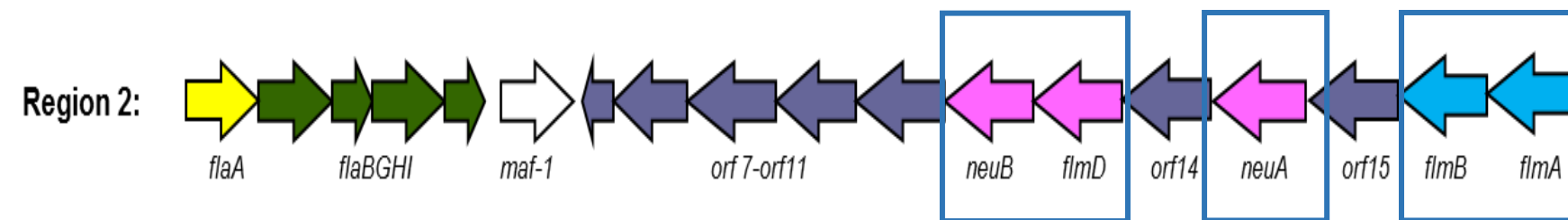
A. piscicola AH-3



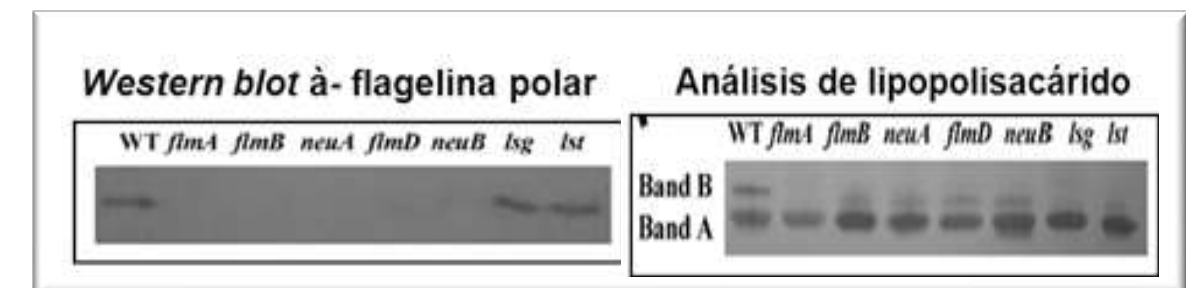
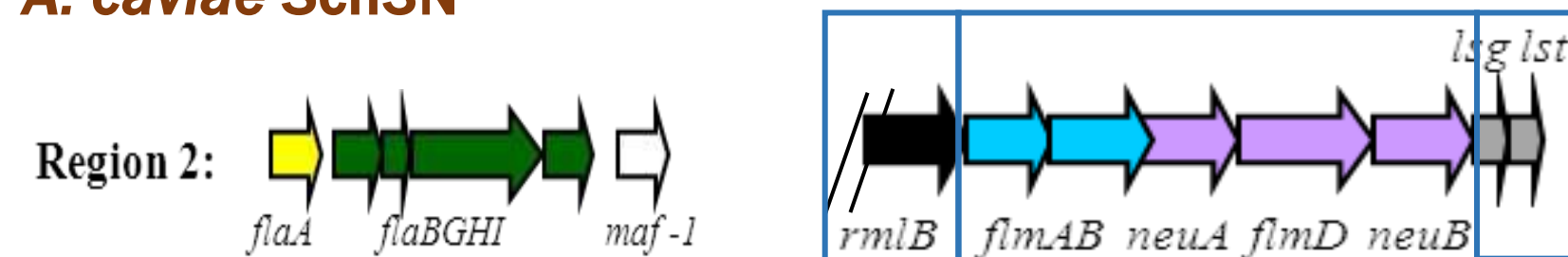
A. hydrophila ATCC 7966^T



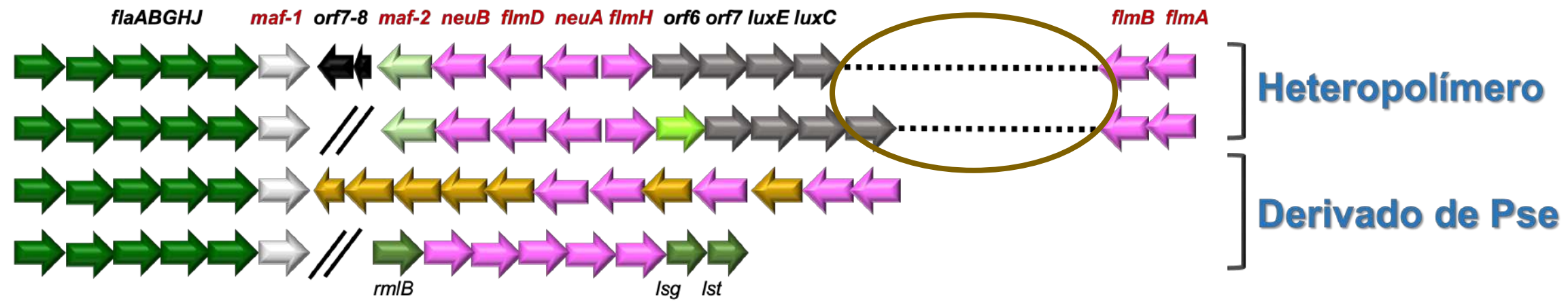
A. hydrophila AH-1



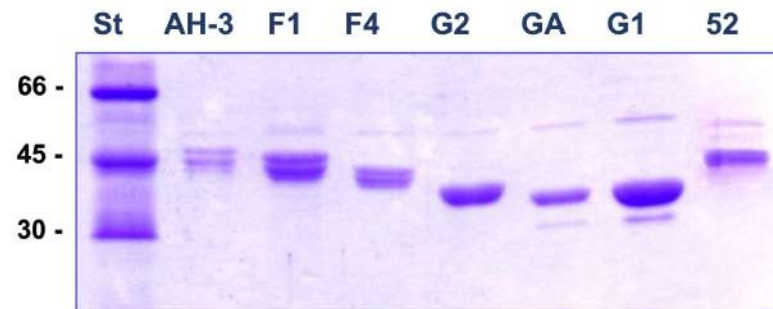
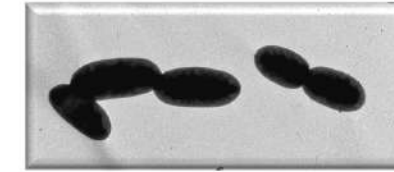
A. caviae Sch3N



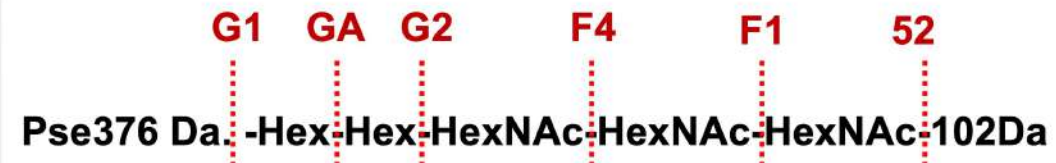
Análisis de genes implicados en glicosilación



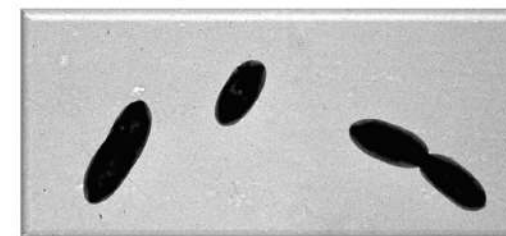
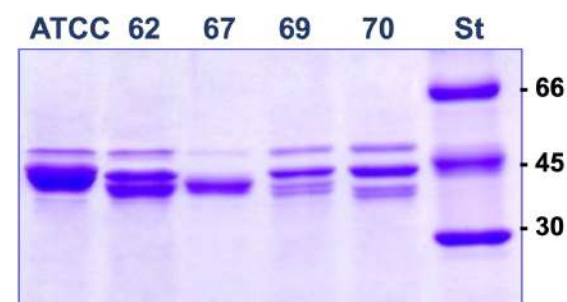
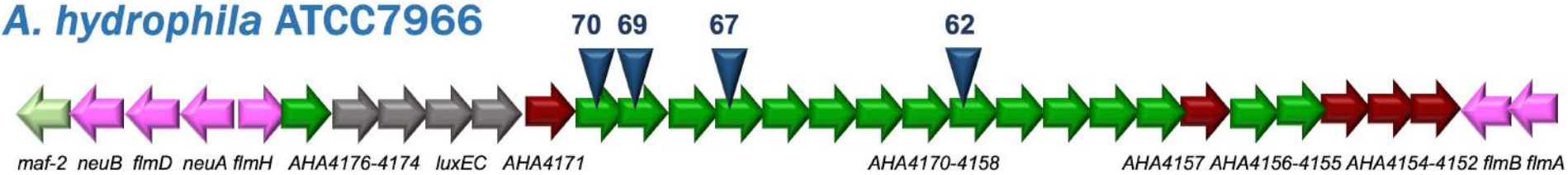
A. piscicola AH-3



Posible acción de las GTs



A. hydrophila ATCC7966

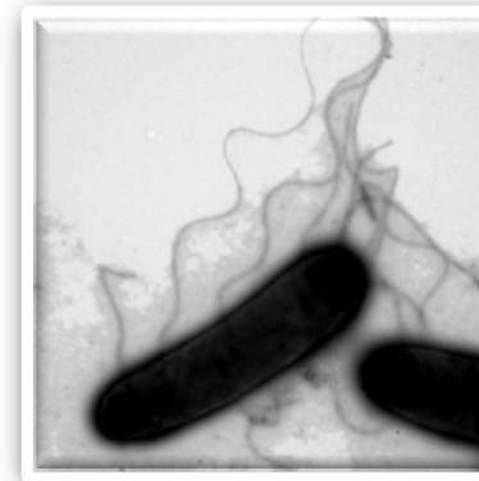


Regulación por temperatura de la expresión del flagelo

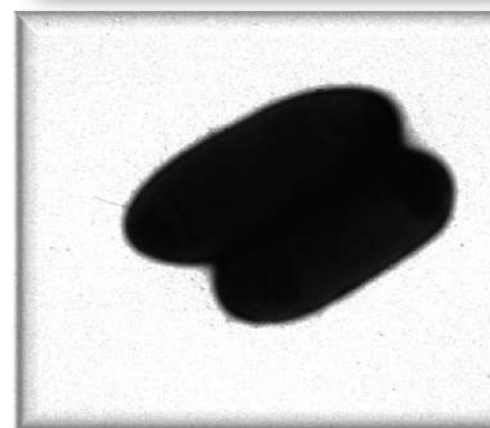
Aeromonas piscicola AH-3



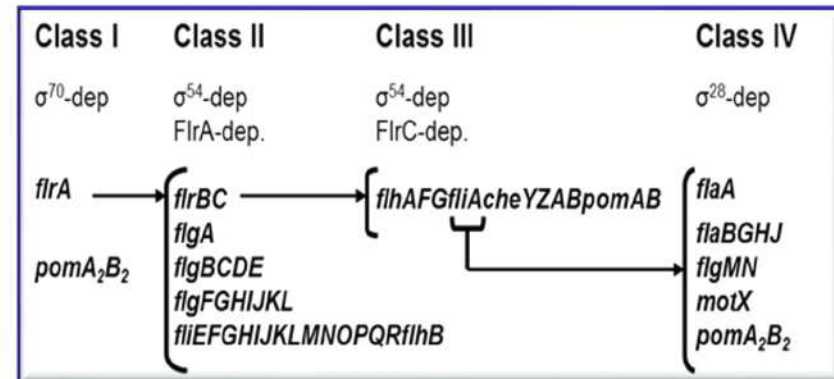
25°C



37°C

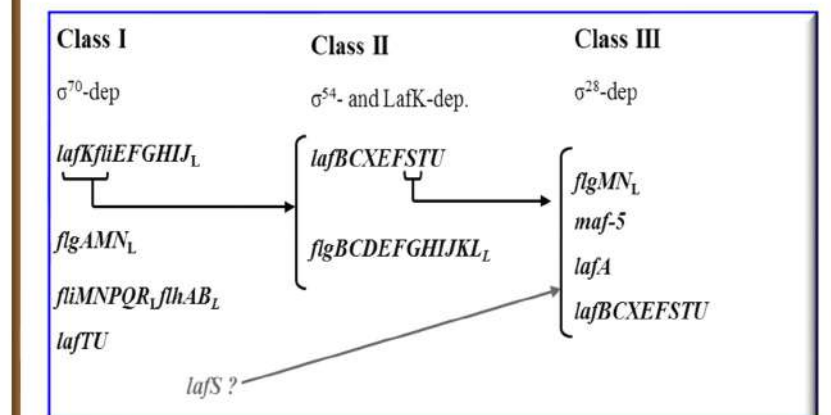


Análisis de la transcripción



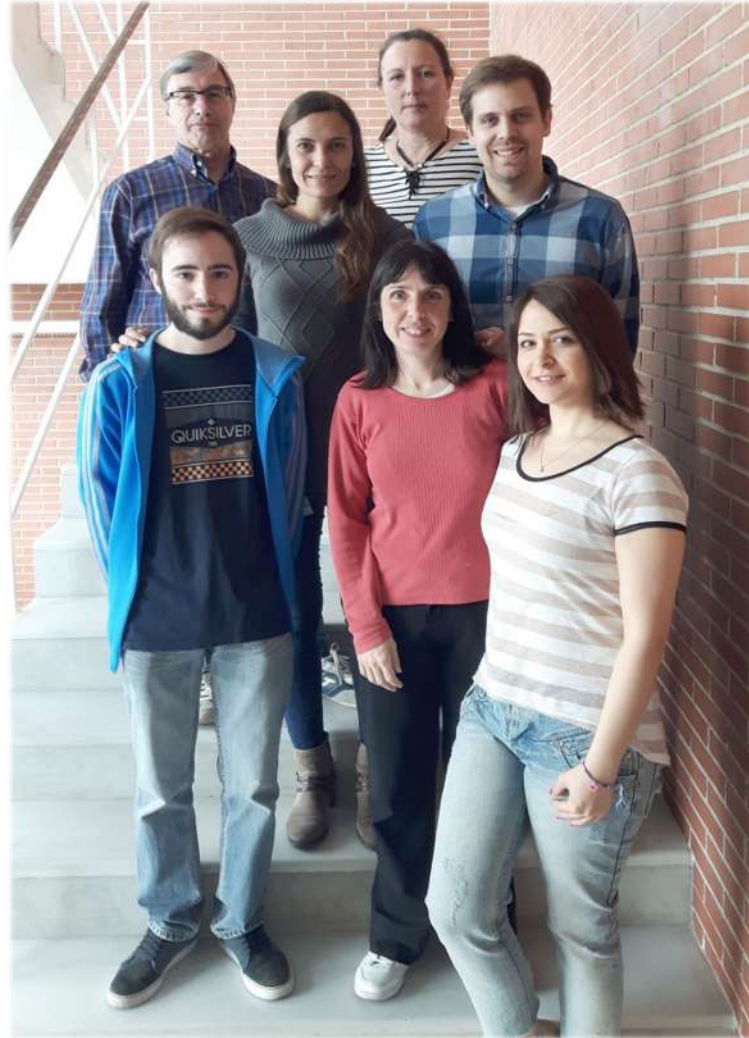
flmAB, *neuA flmD neuB y flmH*
maf-1

Análisis de la transcripción



maf-5

Agradecimientos



Miembros del grupo

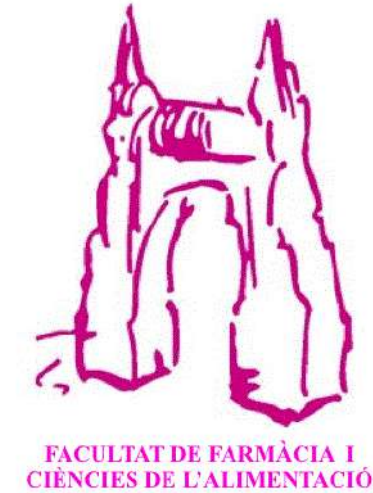
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Susana Merino Montero
(Profesora catedrática)

María Teresa Polo Blasco
(Técnico de laboratorio)



Microbiologia d'Aigües
Relacionada amb la Salut



Seminaris de Recerca i Seminaris Tecnològics



Yolanda Cajal Visa
(Coordinadora)



UNIVERSITAT DE
BARCELONA

Facultat de Farmàcia i Ciències de l'Alimentació

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Elena de Mendoza Barberá

Professora lectora

Departament de Biologia, Sanitat i Medi Ambient
Secció de Microbiologia

21 MARÇ 2024

GRACIAS