



UNIVERSIDAD DE
COSTA RICA

Introducción a la genómica bacteriana

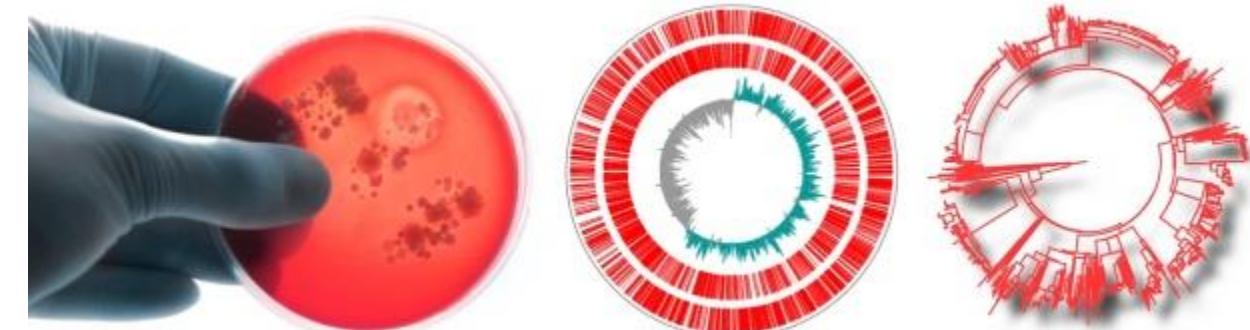
Bradd Mendoza Guido



Introducción a la genómica bacteriana

Agenda

- Estructura genómica en bacterias
- Mutaciones y cambios genómicos
- Elementos genómicos extracromosomales
- Transferencia horizontal
- Aplicaciones de la genómica bacteriana



Algunos conceptos importantes

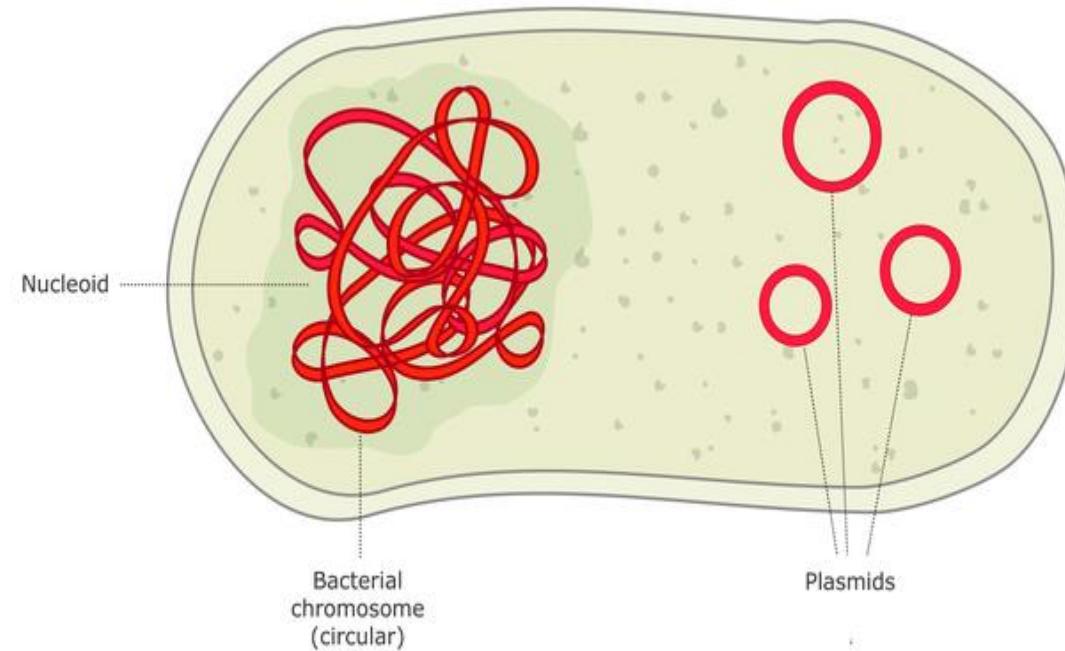
- **GEN:** UNIDAD DE INFORMACIÓN dentro del genoma que contiene todos los elementos necesarios para su EXPRESIÓN controlada. Usualmente es una secuencia de nucleótidos (ARN o ADN) cuya información puede ser utilizada para la SÍNTESIS DE MACROMOLECULAS (ARNr, ARNt, ARNr, otros tipos de ARN, ARNm/proteínas).
- **REPLICÓN:** moléculas de ADN con la capacidad de replicarse (generalmente posee un sitio ori).
- **CROMOSOMA BACTERIANO:** molécula estructural de ADN generalmente en forma circular que contiene la información necesaria para la célula.

Algunos conceptos importantes

- **COREGENOME**: genes compartidos entre TODAS las cepas de una misma especie.
- **PANGENOME**: genes presentes en al menos un genoma de una especie, generalmente accesorios.
- **PLÁSMIDO**: molécula de ADN, usualmente circular, de REPLICACIÓN INDEPENDIENTE al cromosoma bacteriano, que contiene INFORMACIÓN accesoria.



Estructura genómica en bacterias



Estructura cromosómica en bacterias

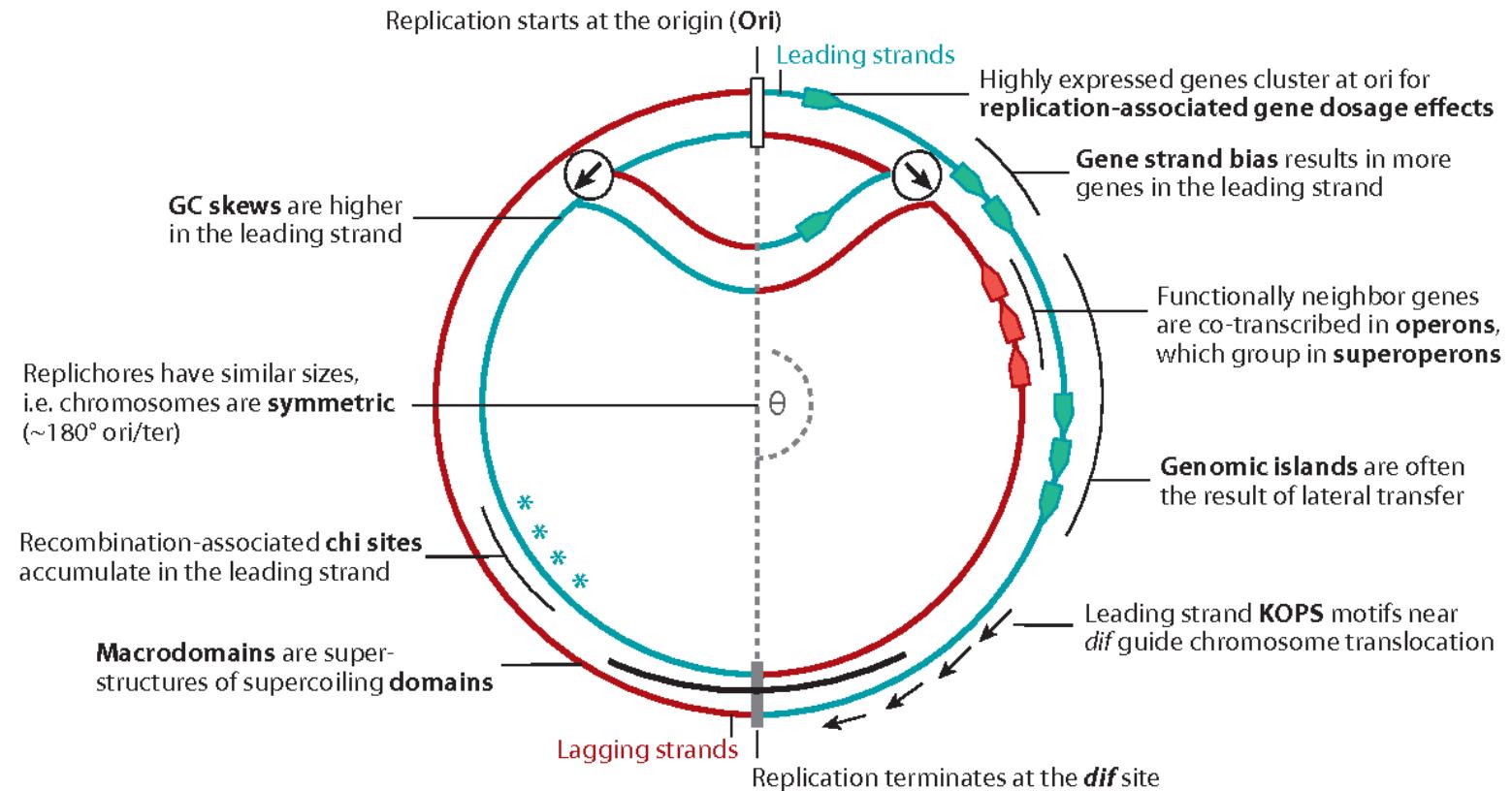
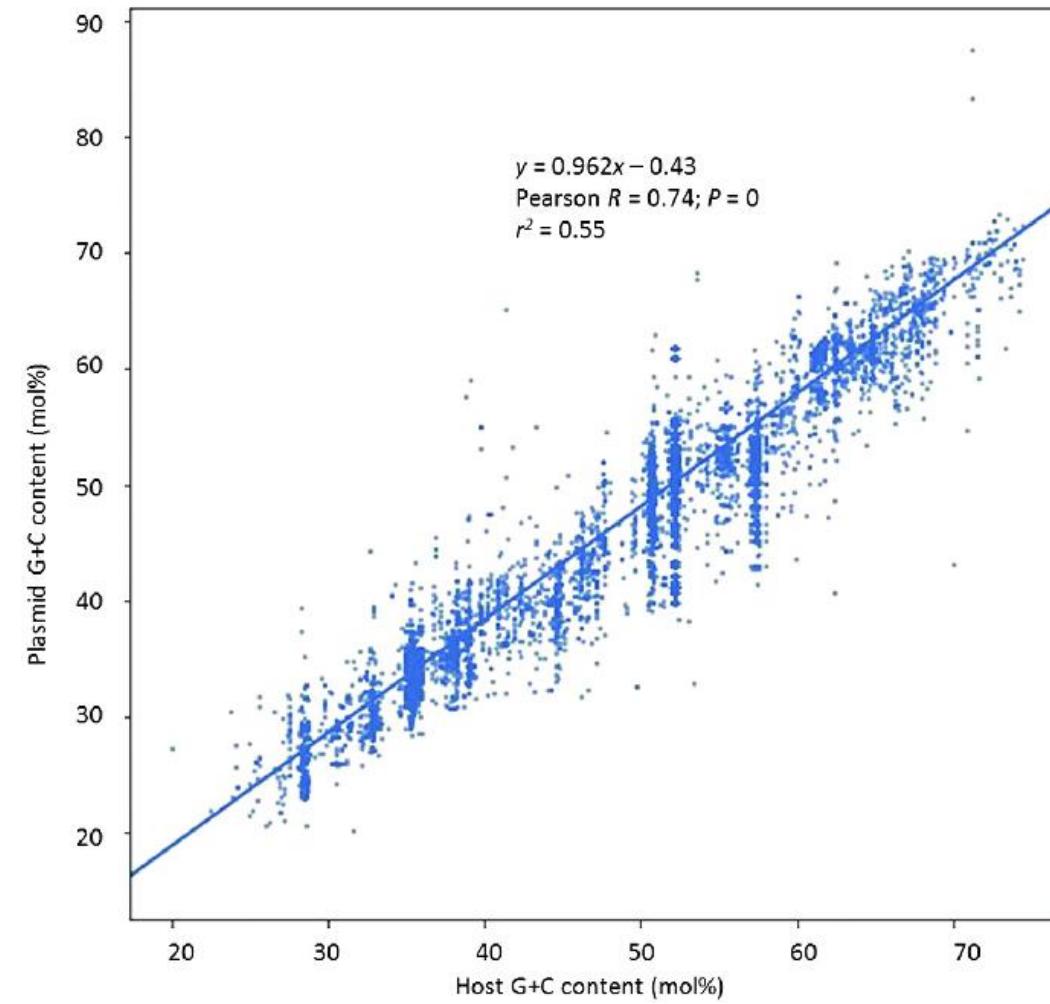
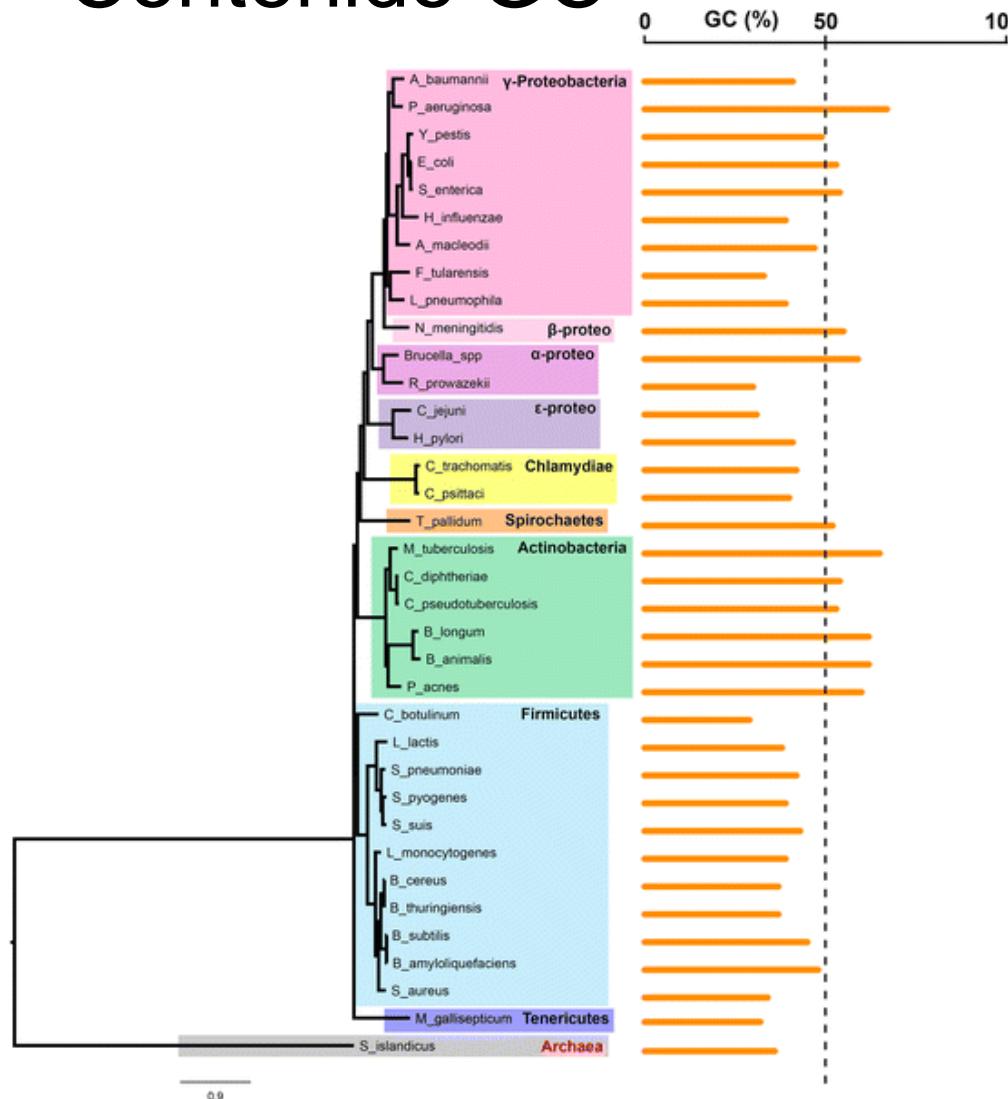


Figure 1

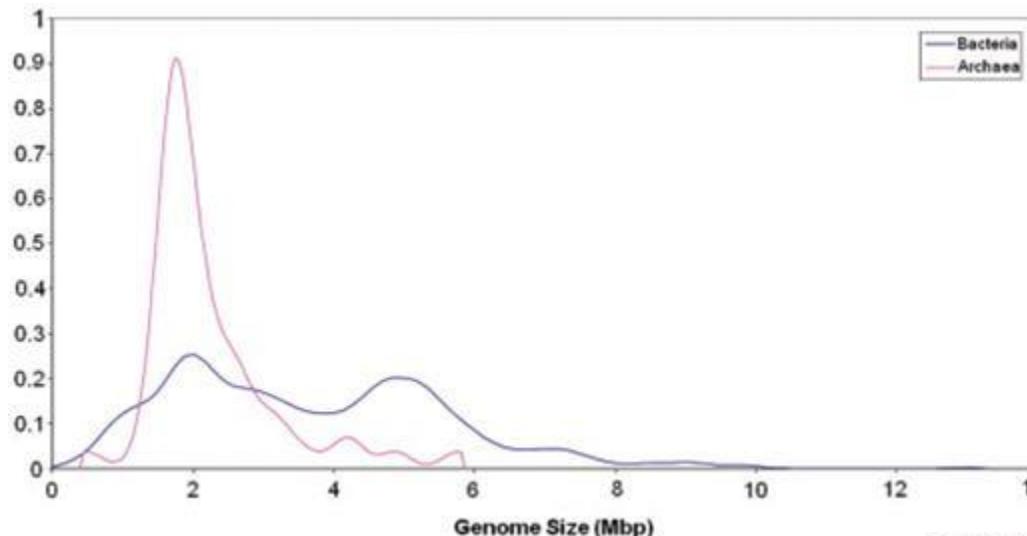
Elements of genome organization.

DOI: 10.1146/annurev.genet.42.110807.091653

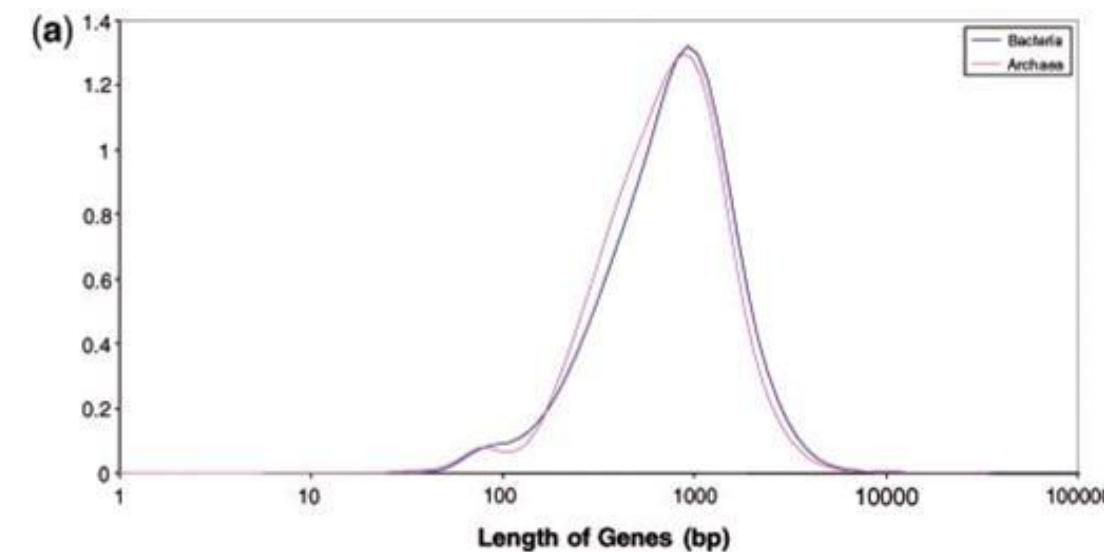
Contenido GC



Tamaño del genoma

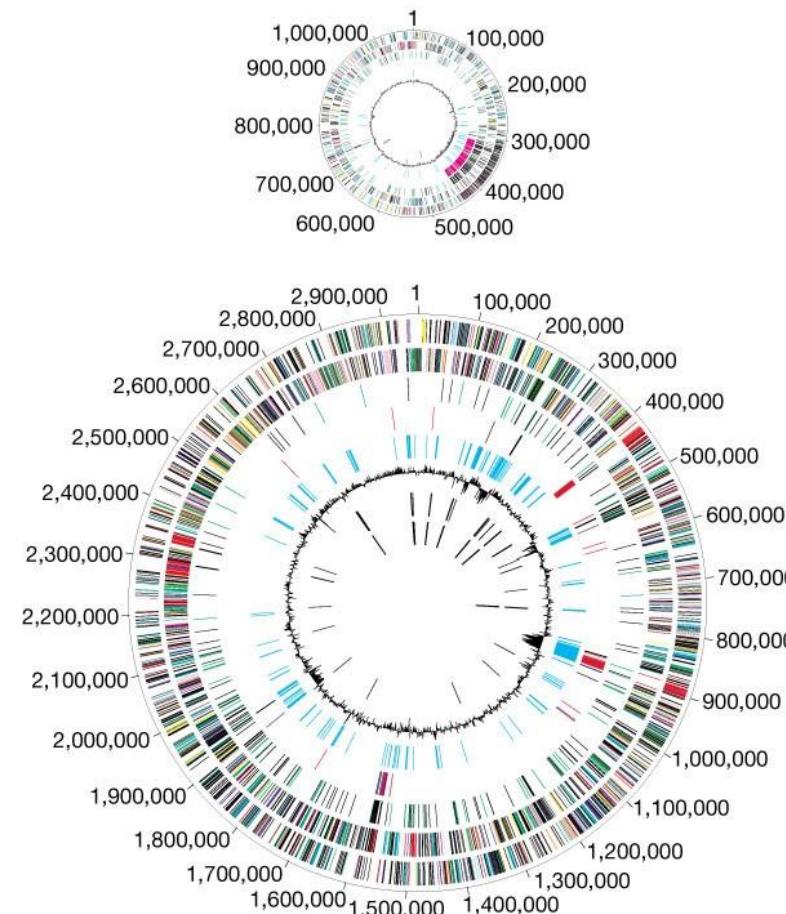


<https://doi.org/10.1093/nar/gkn668>



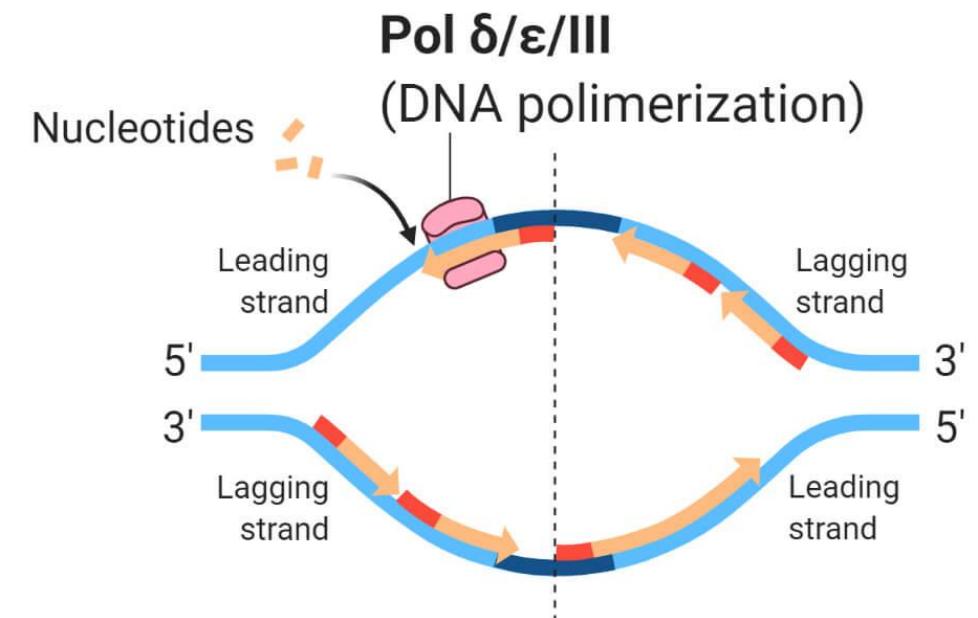


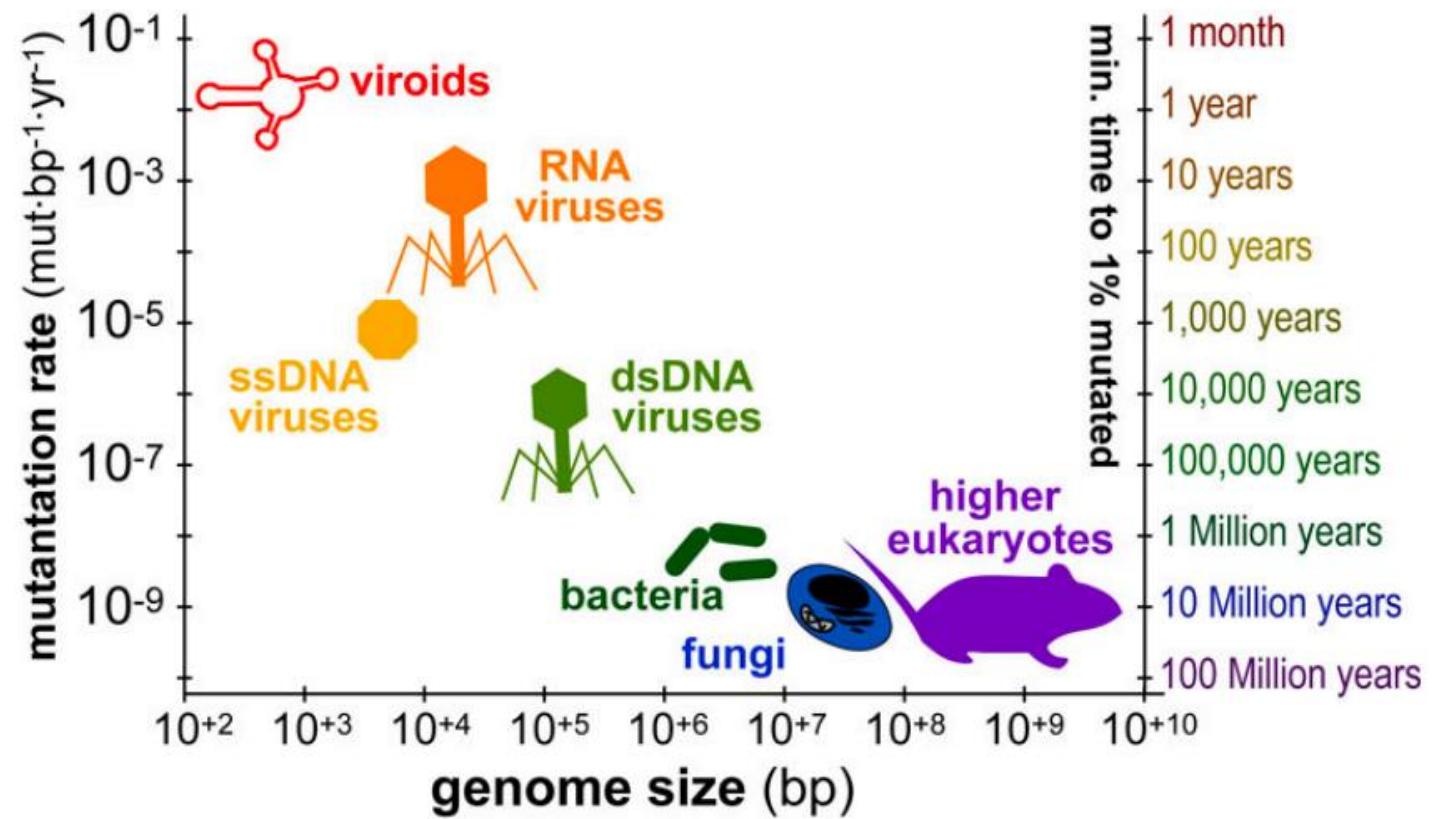
Cromosomas *V. cholerae*



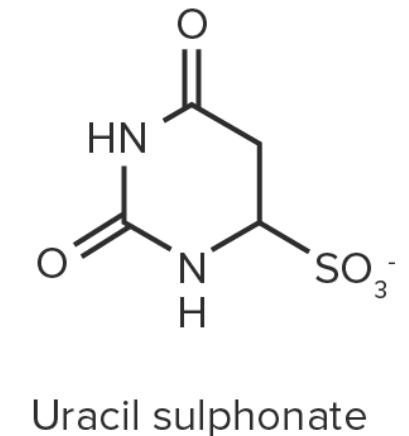
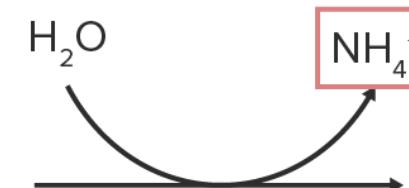
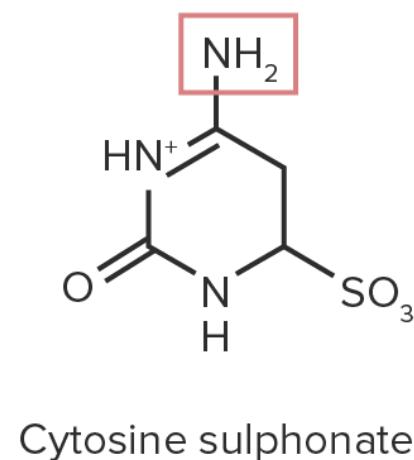
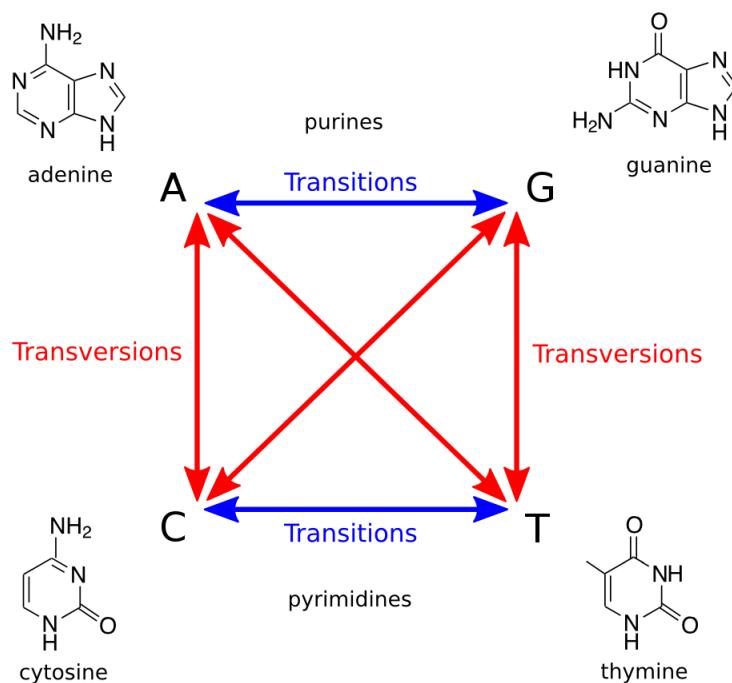
Mutagenesis y cambios genómicos

- En general las tasas de error de la polimerasa son bajas ($\sim 10^{10}$ por base replicada) debido a:
 - Alta fidelidad
 - Sistema 5 exonucleasa
 - Sistemas de reparación del ADN





Mutaciones puntuales

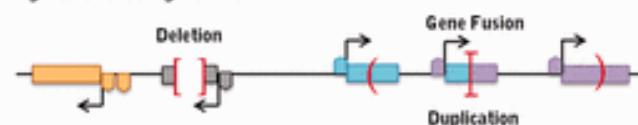




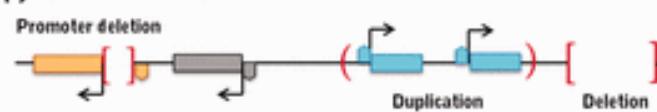
A. Reference genome structure



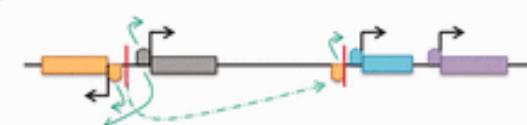
B. Intra-gene rearrangements



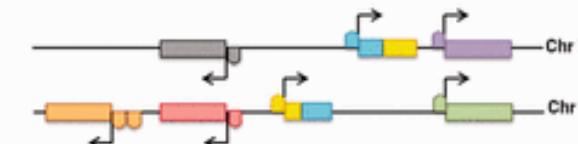
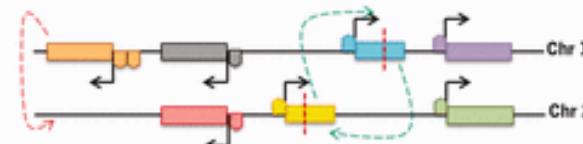
C. Copy number variants



D. Inversion

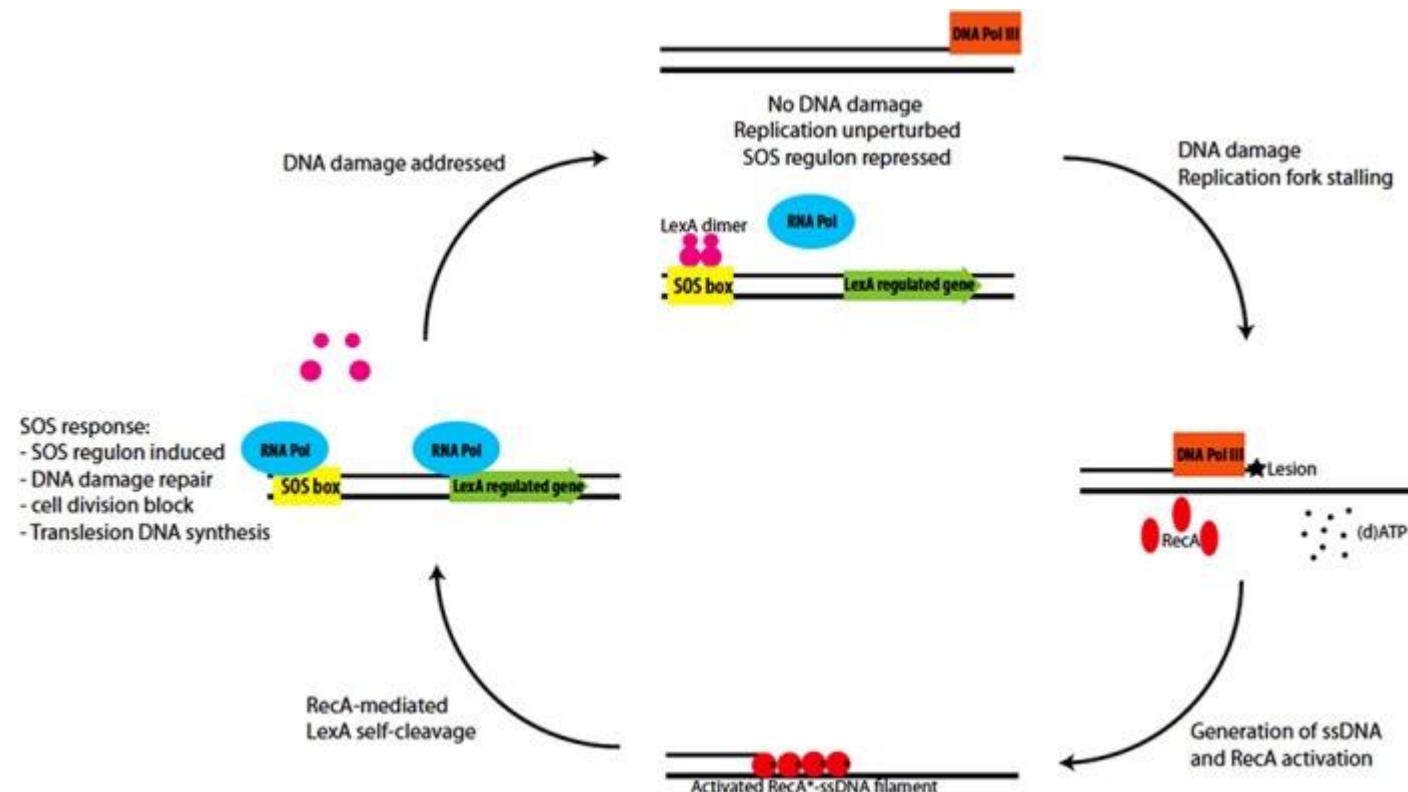


E. Translocation



Legend:
Promoter activity [] Deletion () Duplication || Inverted region | Breakpoints

Respuesta SOS

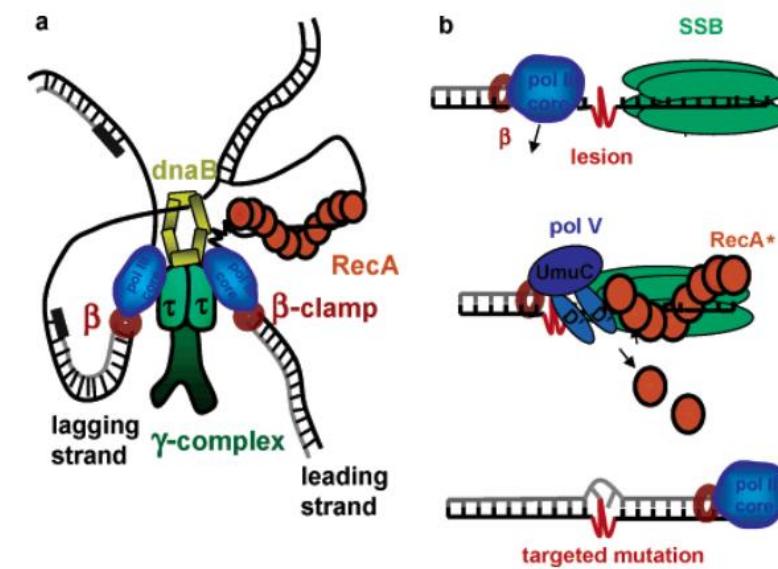
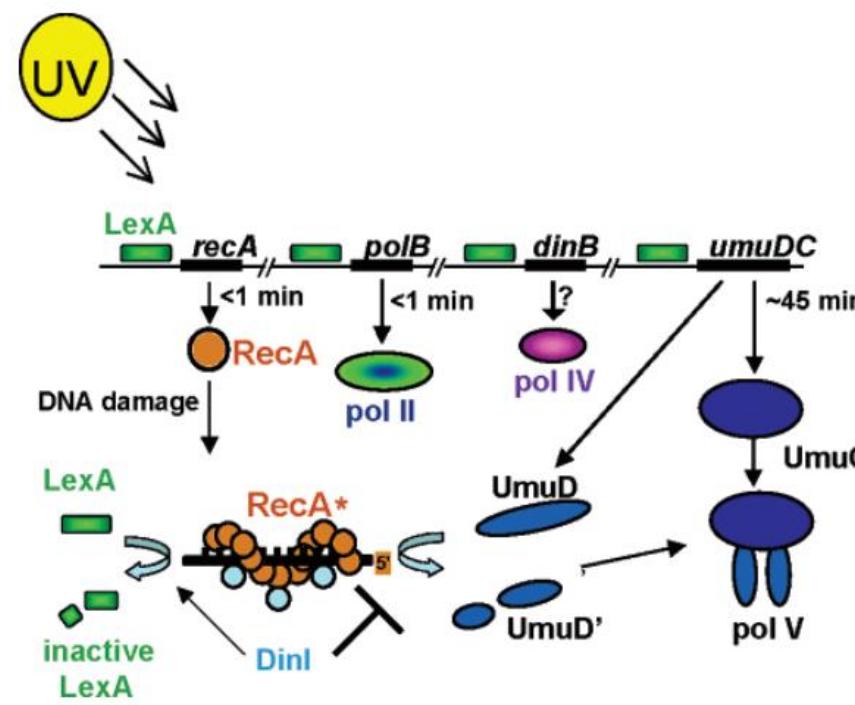


Gene	Function
<i>Known SOS box</i>	
<i>cho</i> (<i>ydjQ</i>)	endonuclease of nucleotide excision repair
<i>dinB</i>	DNA polymerase IV
<i>dinD</i> (<i>pcsA</i>)	DNA-damage-inducible protein
<i>dinF*</i>	DinF MATE Transporter
<i>dinG</i>	ATP-dependent helicase
<i>dinI</i>	DNA-damage-inducible protein
<i>dinQ</i>	toxic inner membrane peptide DinQ
<i>dinS</i>	DNA-damage-inducible protein
<i>ftsK</i>	cell division protein
<i>hokE</i>	toxic polypeptide
<i>lexA</i>	regulator for SOS
<i>molR</i>	molybdate metabolism regulator
<i>polB</i>	DNA polymerase II
<i>recA</i>	DNA strand exchange, LexA coprotease
<i>recN</i>	protein used in recombination and DNA repair
<i>recX*</i>	recA inhibitor
<i>rmuC</i> (<i>yigN</i>)	predicted recombination limiting protein
<i>ruvAB</i>	Holliday junction helicase subunit A and B
<i>sbmC</i>	DNA gyrase inhibitor
<i>ssb</i>	ssDNA-binding protein
<i>sulA</i>	inhibits cell division
<i>umuDC</i>	DNA polymerase V
<i>uvrA</i>	excision nuclease subunit A
<i>uvrB</i>	excision nuclease subunit B
<i>uvrD</i>	DNA-dependent ATPase I and helicase II
<i>ybfE</i>	DNA-damage-inducible protein
<i>ydjM</i>	inner membrane protein
<i>yebG</i>	DNA-damage-inducible protein

<i>symE</i> (<i>yjiW</i>)	toxin-like protein of the SOS response
<i>tisAB</i> (<i>ysdAB</i>)	toxic peptides induced by SOS
<i>rmuC</i> (<i>yigN</i>)	putative recombination limiting protein
<i>Potential SOS box</i>	
<i>ymfE</i>	c14 prophage putative inner membrane protein
<i>ymfI</i>	unknown
<i>ydeO</i>	DNA-binding transcriptional dual regulator
<i>ydeS</i>	putative fimbrial protein YdeS
<i>yoaB</i>	RutC family protein
<i>intE</i>	c14 prophage putative integrase
<i>ogrK</i>	prophage P2 late control protein
<i>yggC</i> (<i>afuC</i>)	CP4-6 prophage; ABC transporter ATP-binding protein
<i>yhiL</i>	unknown
<i>glvB</i>	putative PTS enzyme II component GlvB
<i>ipbA</i>	small heat shock protein

*genes that do not have their own LexA-binding operator sequence, but are co-transcribed with others that do; *dinF* is co-transcribed with *lexA*, whereas *recX* with *recA*.

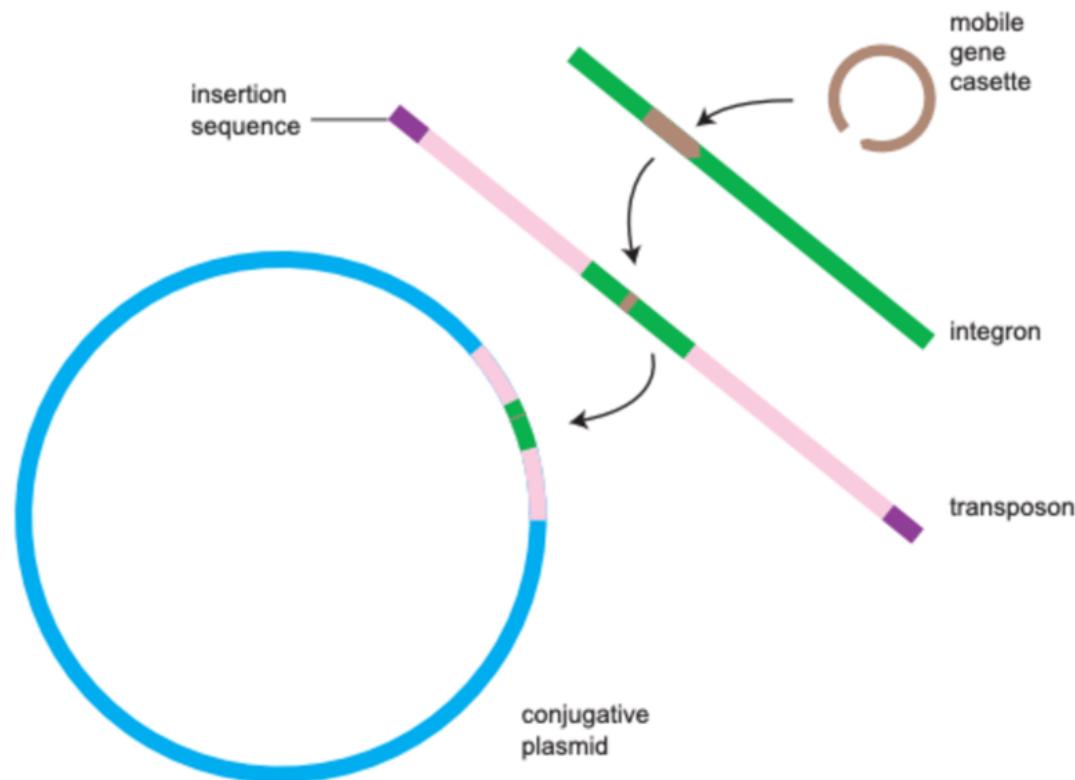
doi:10.1002/em.22267



doi:10.1021/cr0404951

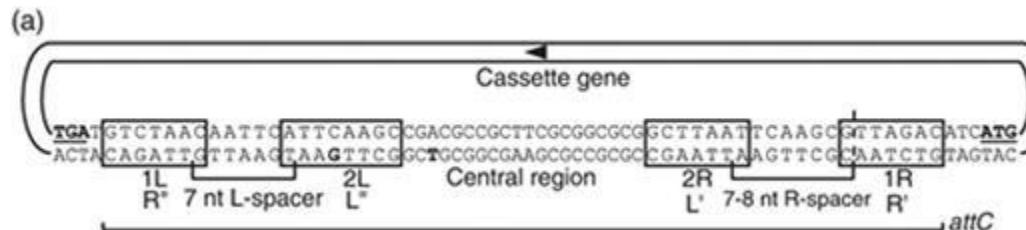


Elementos Genéticos Móviles (EGM)

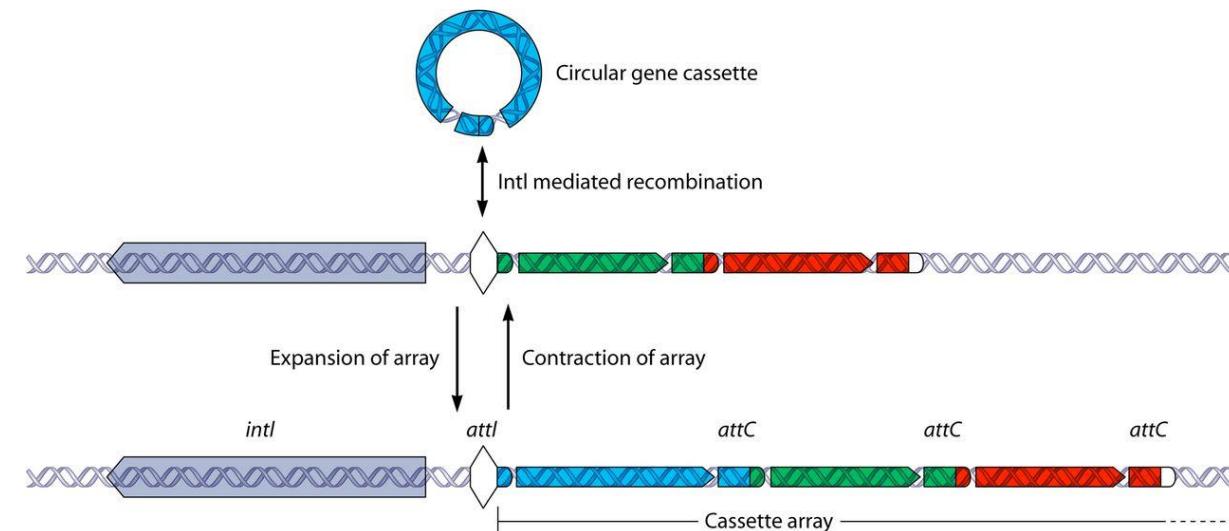




Casete genético e integrones:



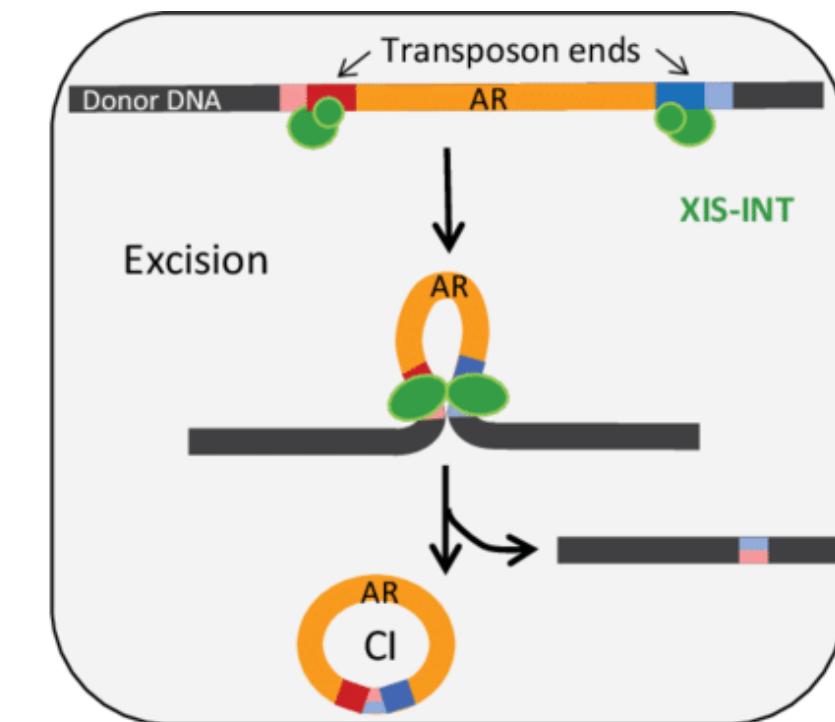
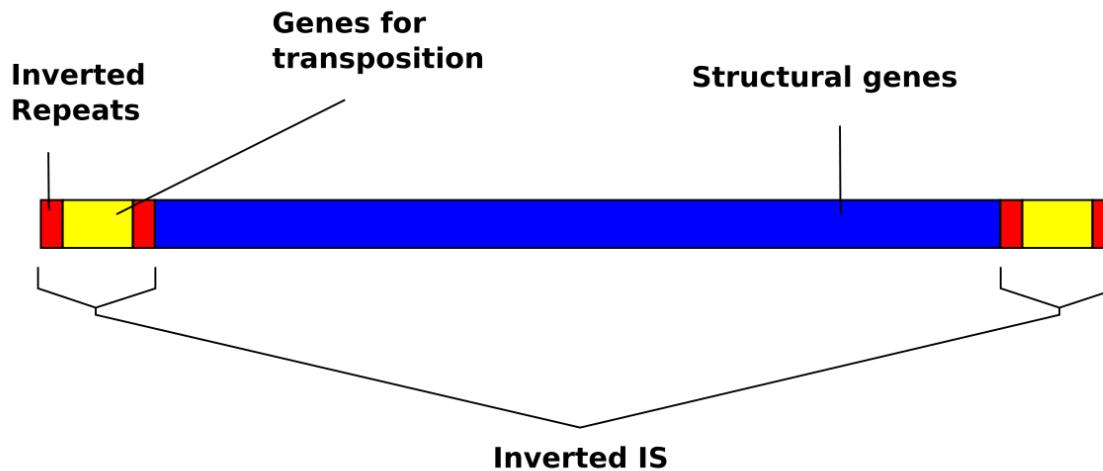
<https://doi.org/10.1111/j.1574-6976.2009.00175.x>



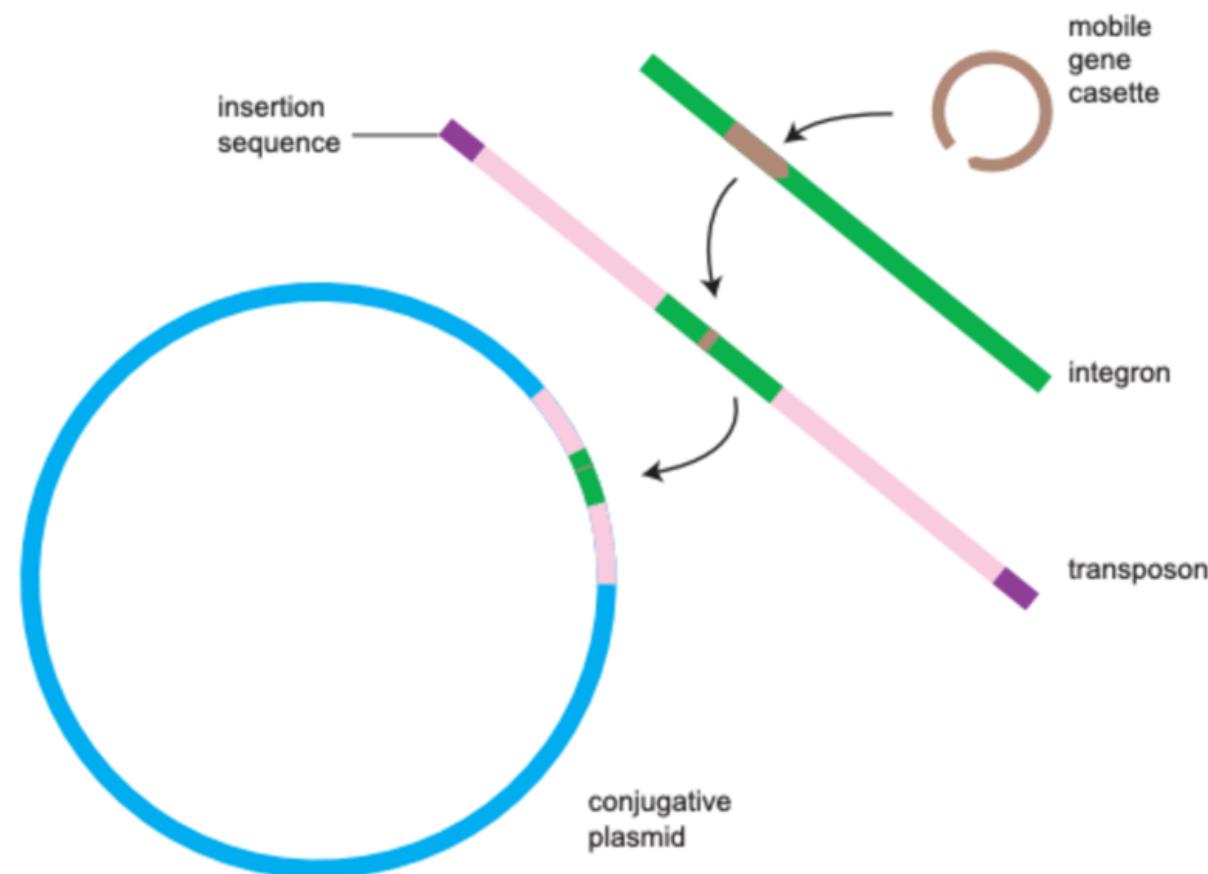
DOI: <https://doi.org/10.1128/mmb.00056-13>

Transposones

Bacterial composite transposon

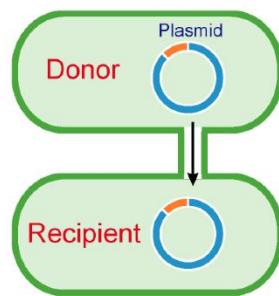


EGMs

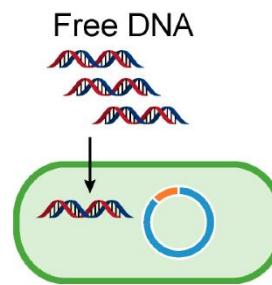


Transferencia Horizontal

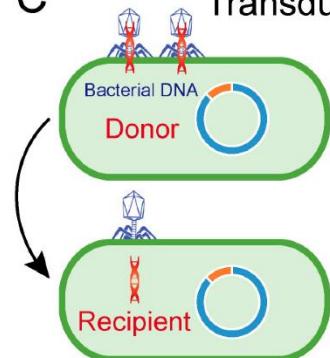
A Conjugation



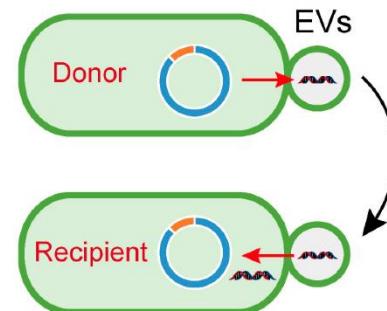
B Transformation



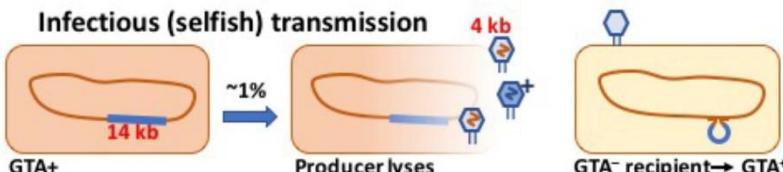
C Transduction



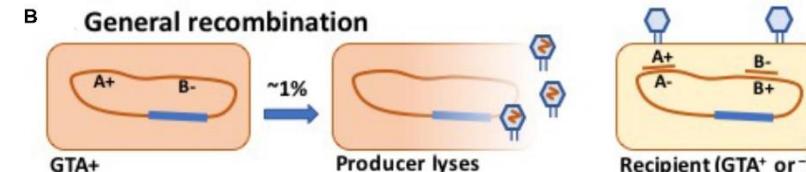
D Vesiculation

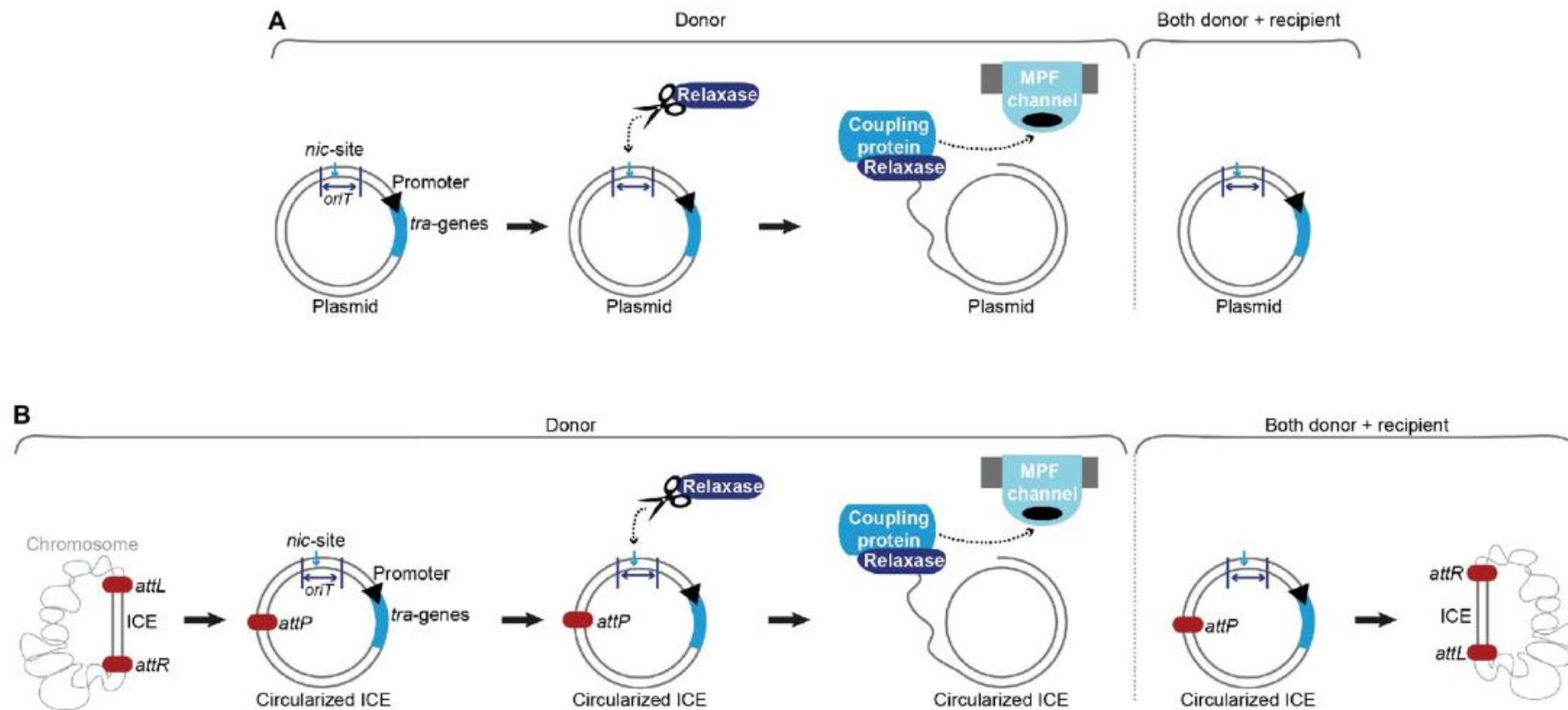


A

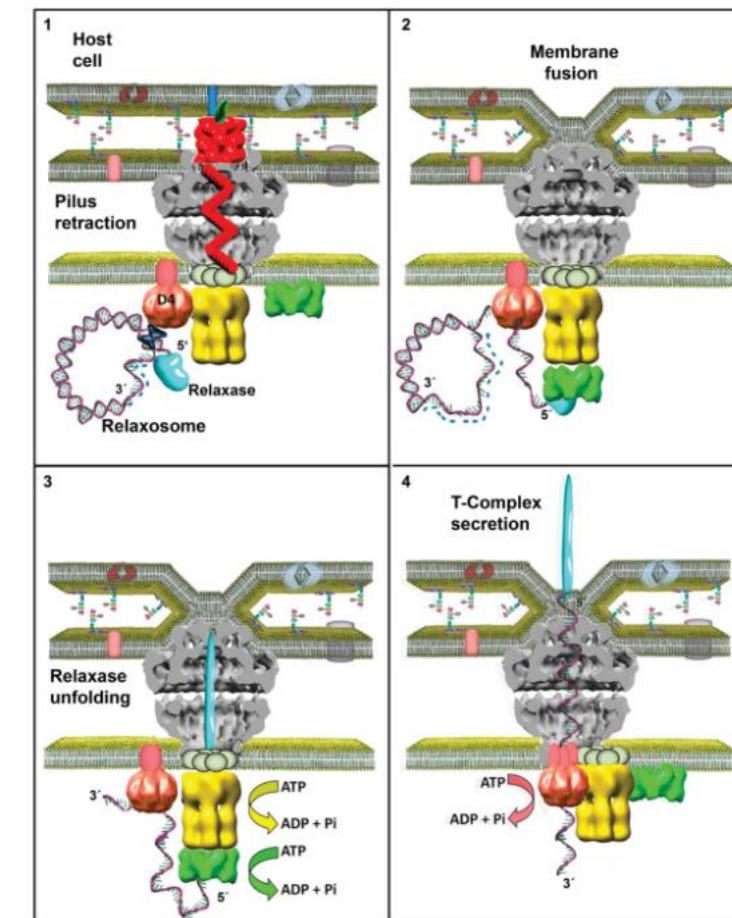
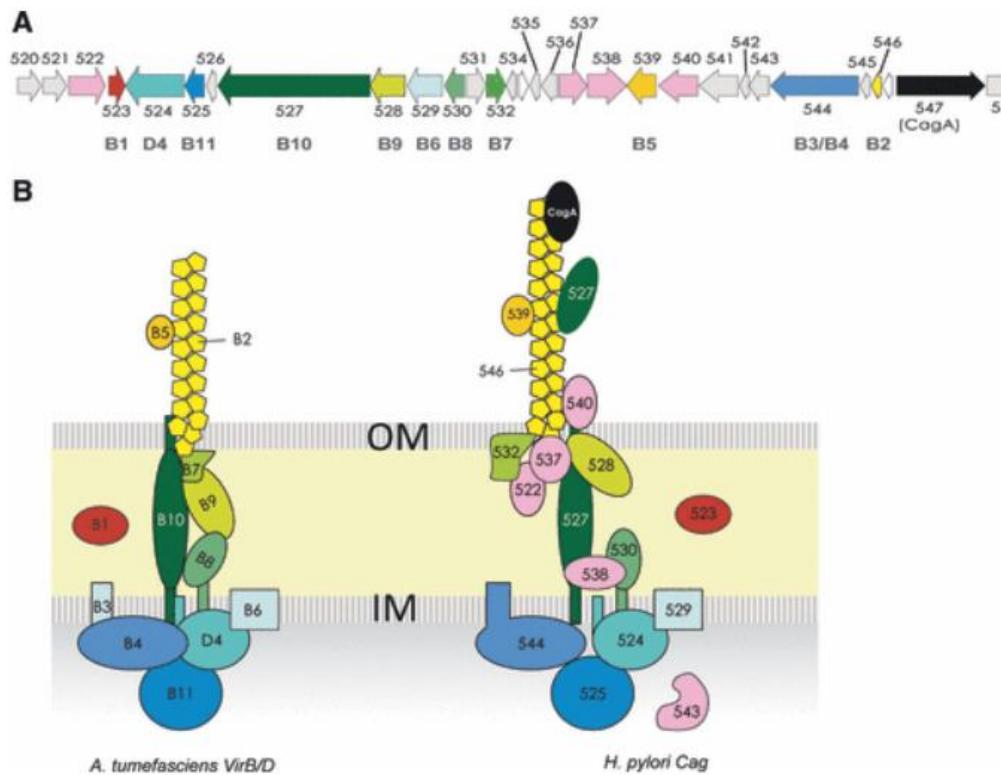


B General recombination

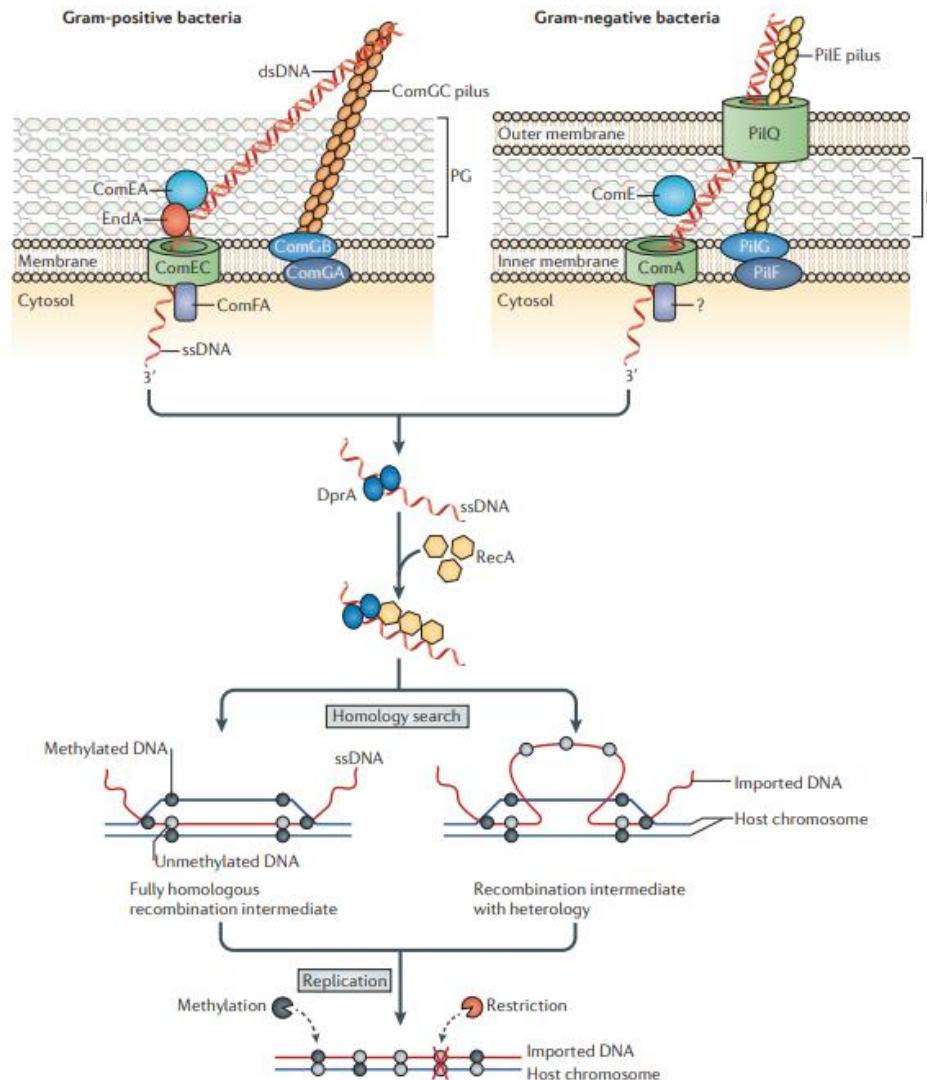




Conjugación

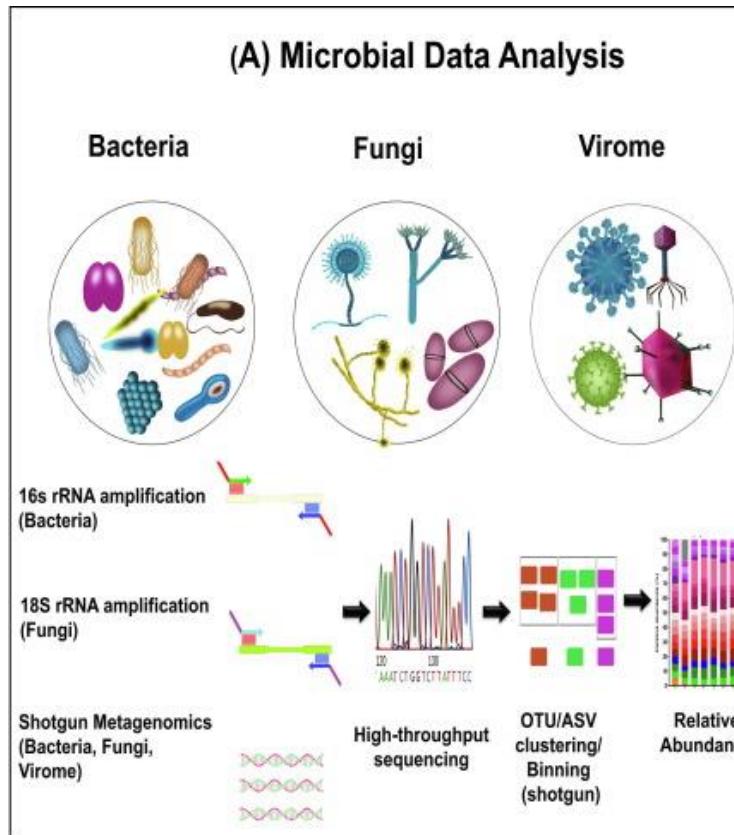


Transformación

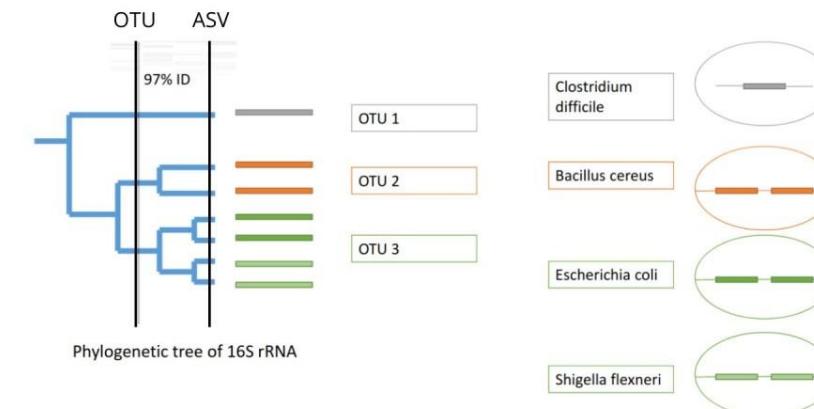


Aplicaciones de la genómica bacteriana

- Análisis de comunidades microbianas

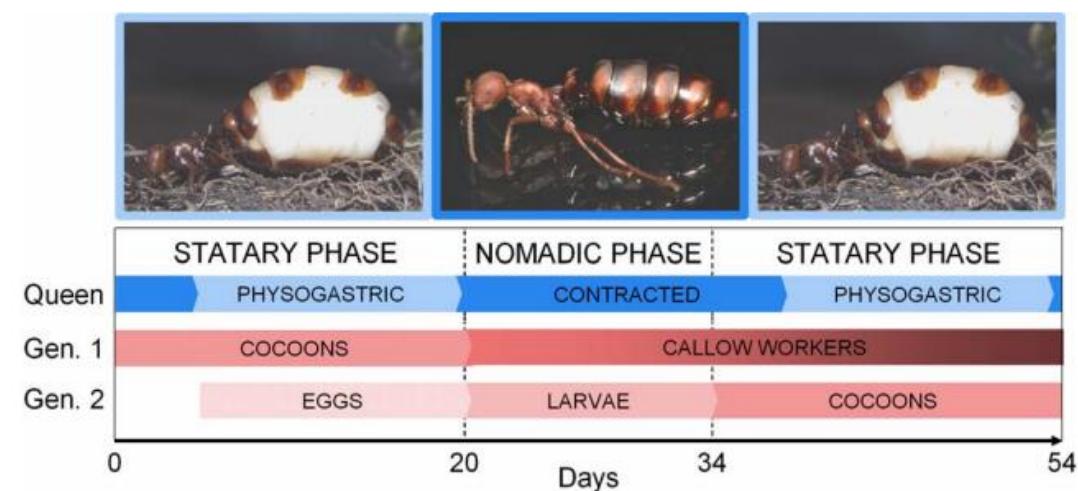


OTU vs. ASV



Low diversity and host specificity in the gut microbiome community of *Eciton* army ants (Hymenoptera: Formicidae: Dorylinae) in a Costa Rican rainforest

Bradd MENDOZA-GUIDO*, Natalia RODRÍGUEZ-HERNÁNDEZ*, Aniek B.F. IVENS, Christoph von BEEREN, Catalina MURILLO-CRUZ, Ibrahim ZUNIGA-CHAVES, Piotr ŁUKASIK, Ethel SANCHEZ, Daniel J.C. KRONAUER & Adrián A. PINTO-TOMÁS



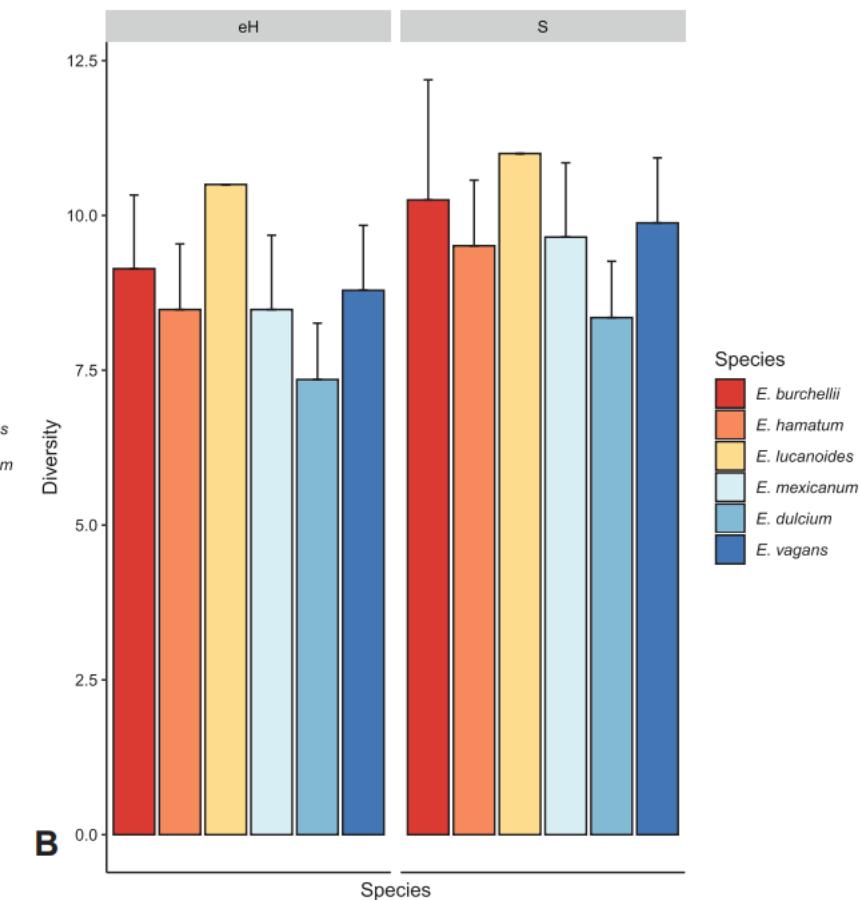
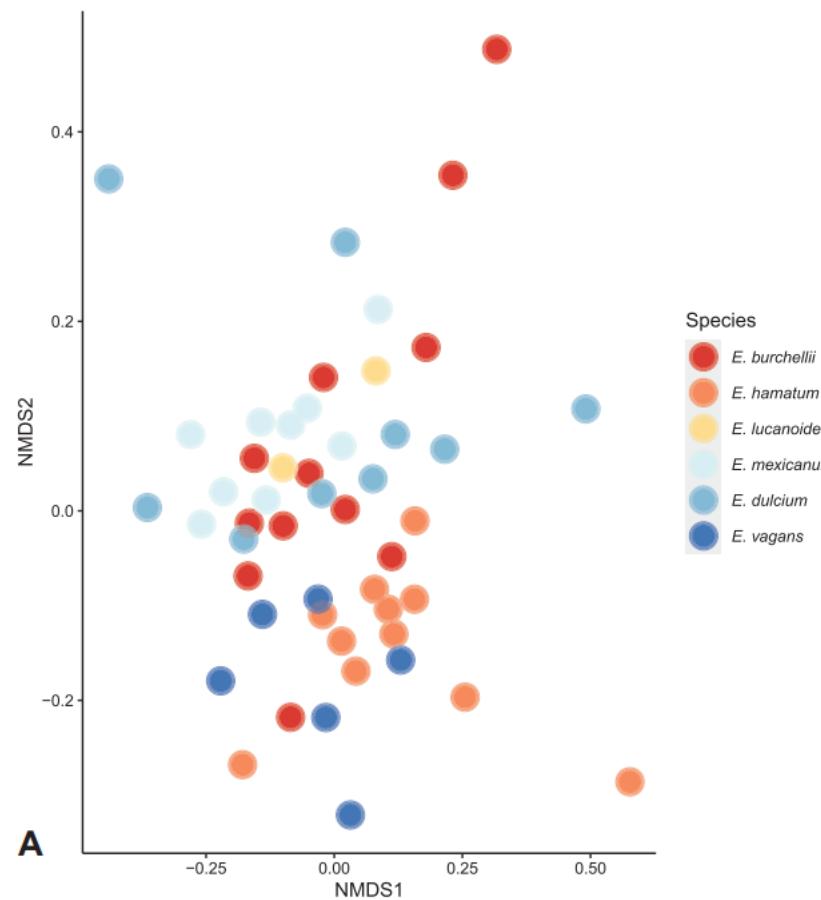
ISSN 1997-3500

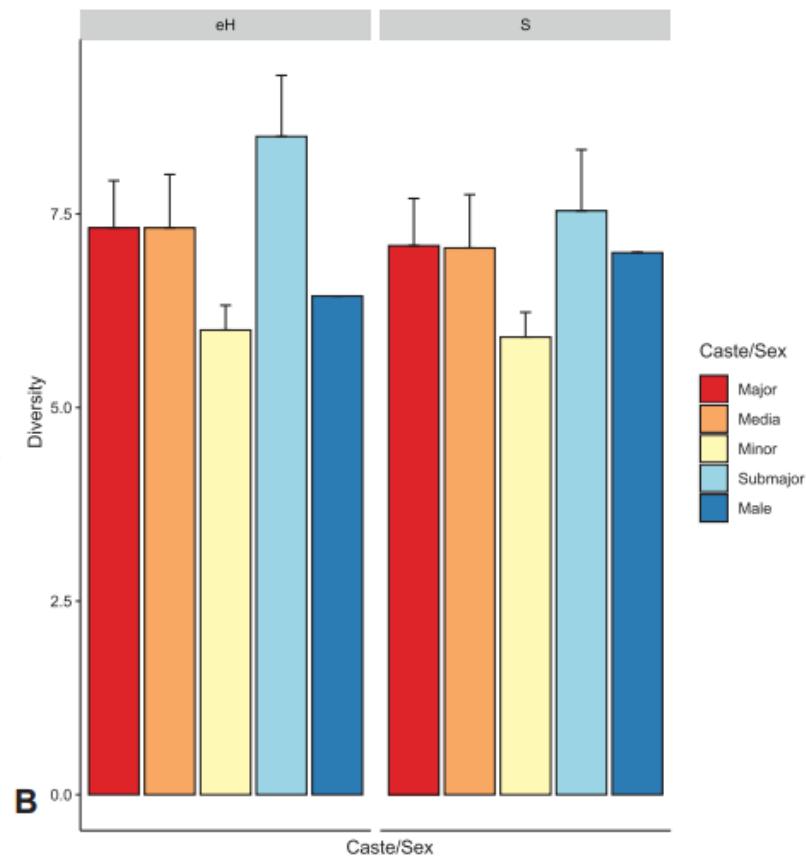
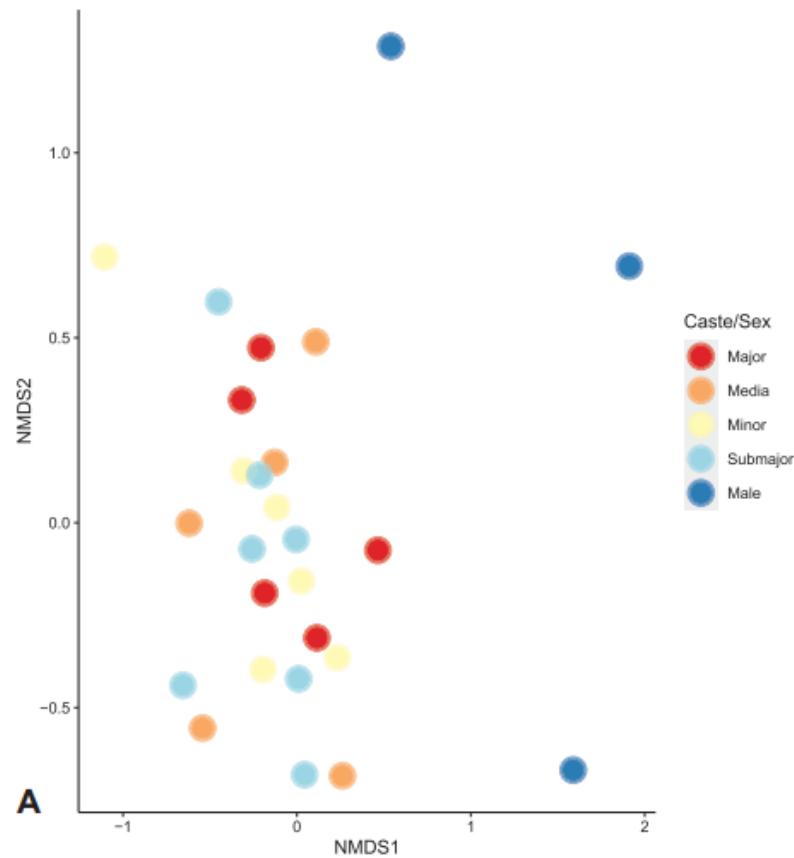


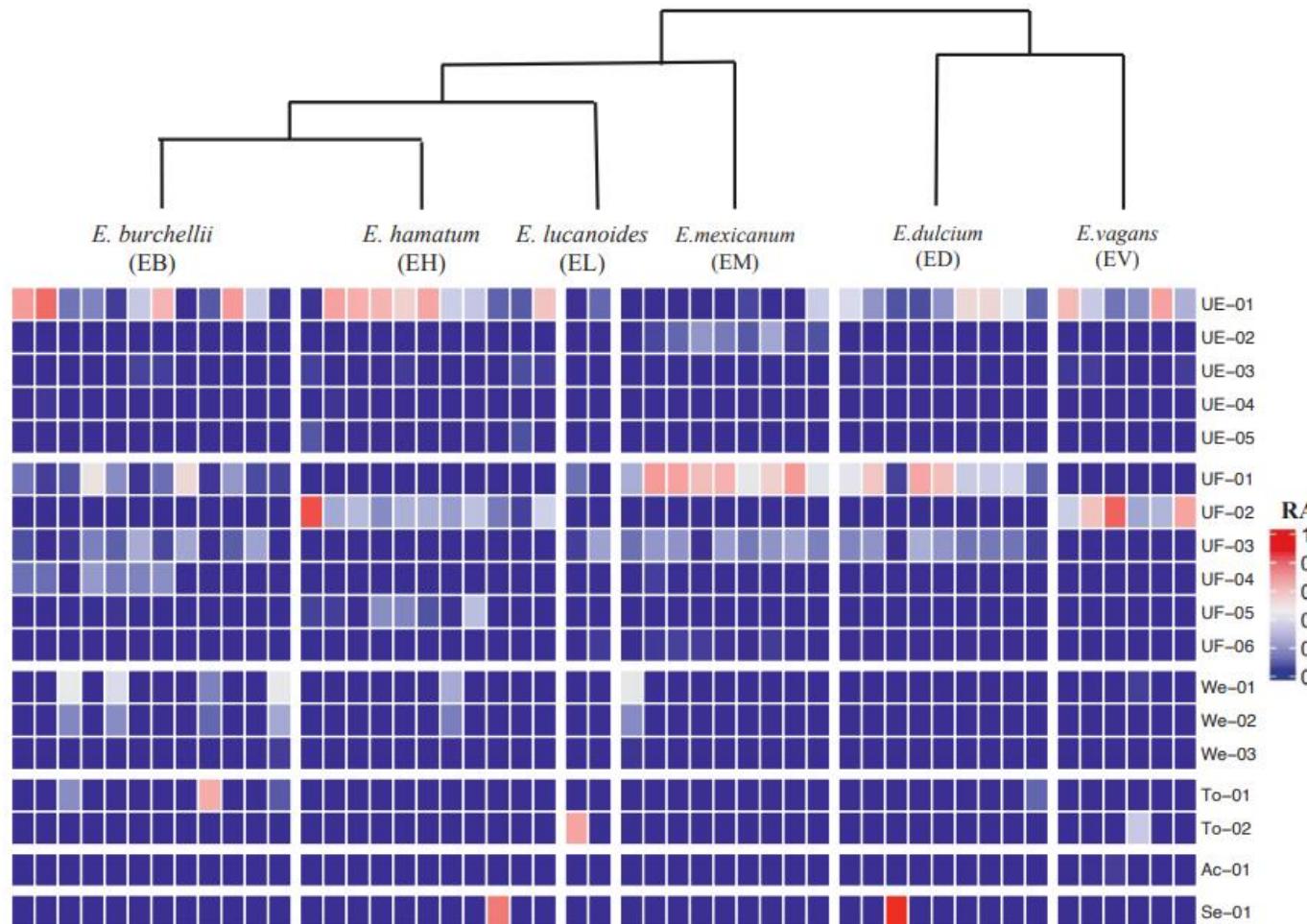
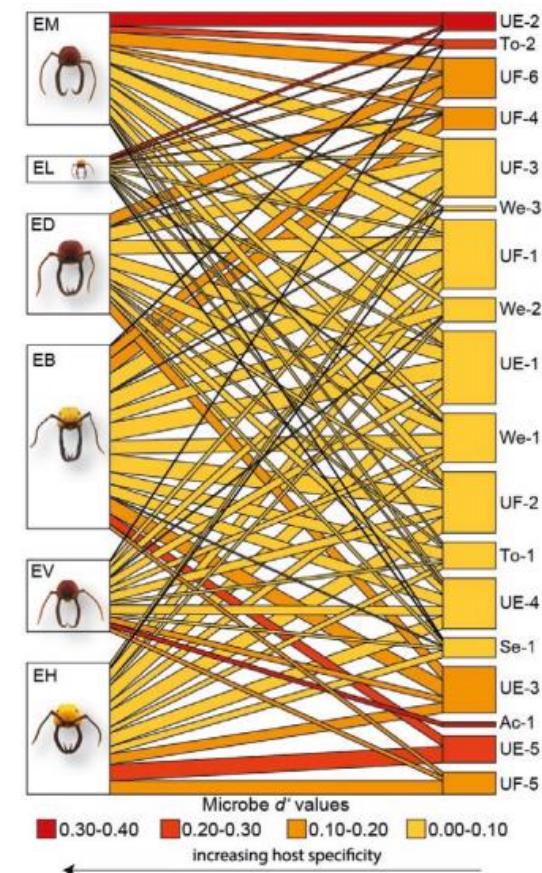
The largest animal association centered on one species: the army ant *Eciton burchellii* and its more than 300 associates

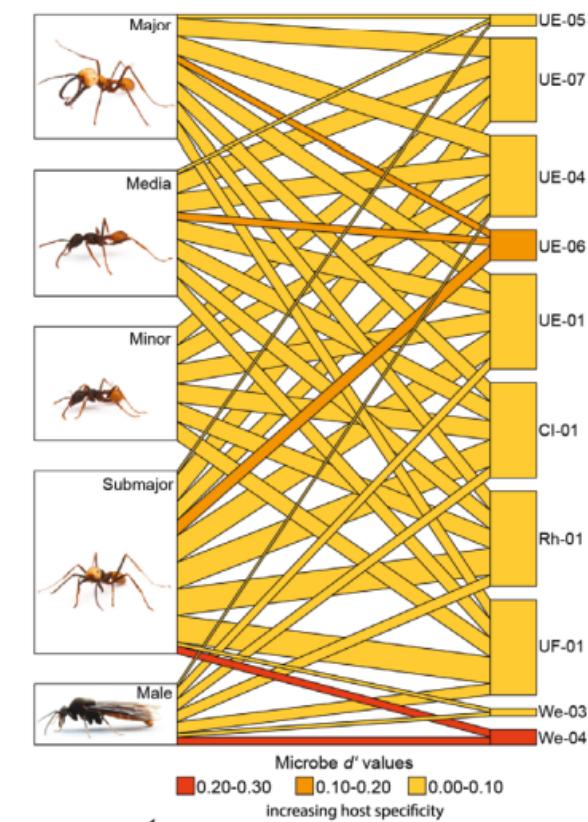
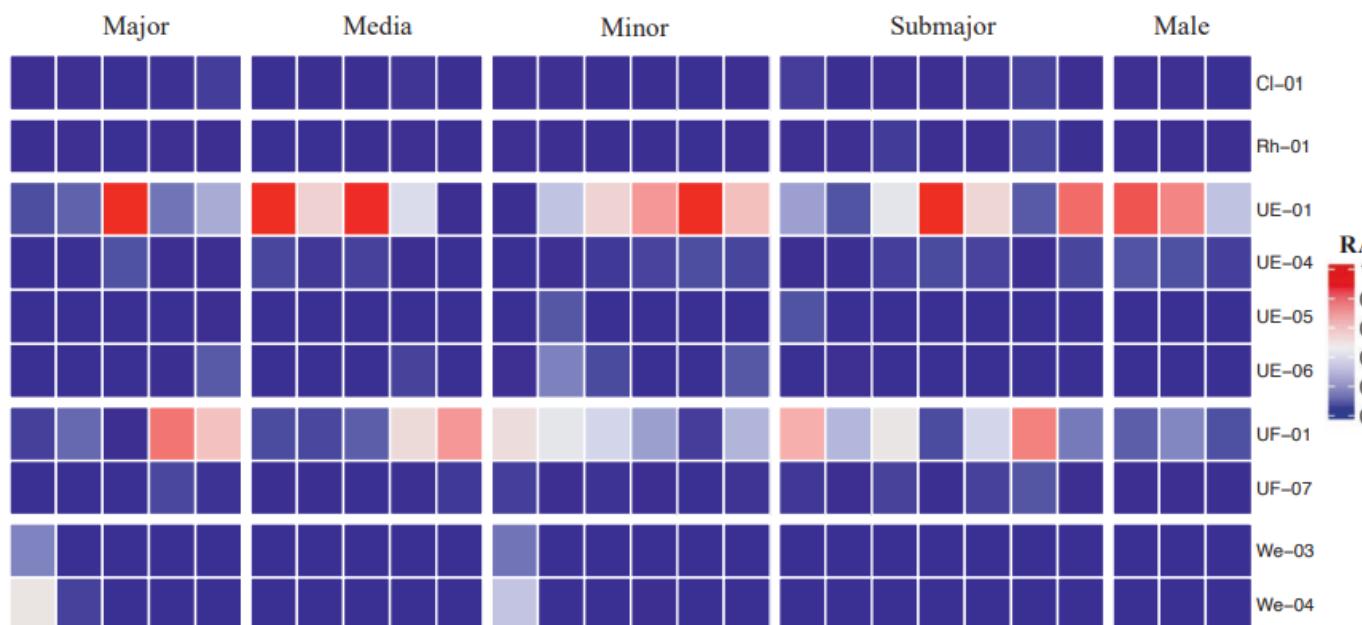
C. W. Rettenmeyer · M. E. Rettenmeyer ·
J. Joseph · S. M. Berghoff

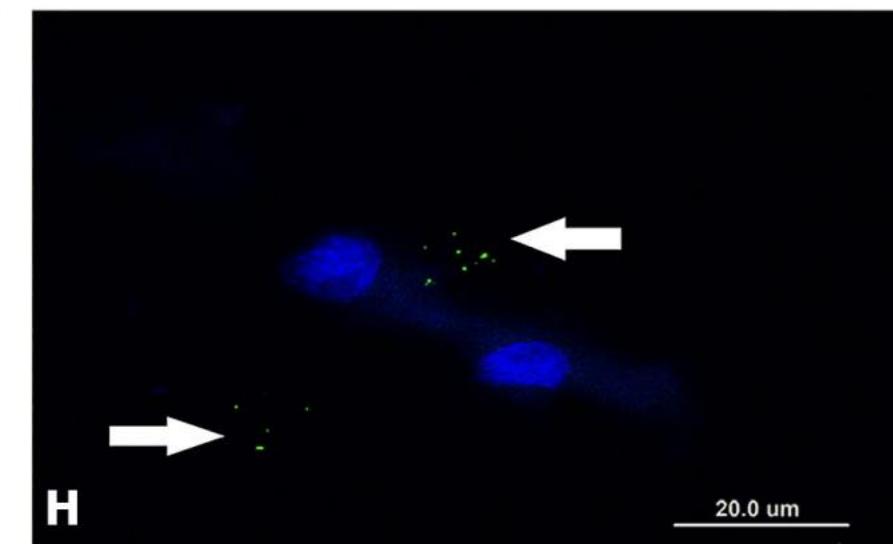
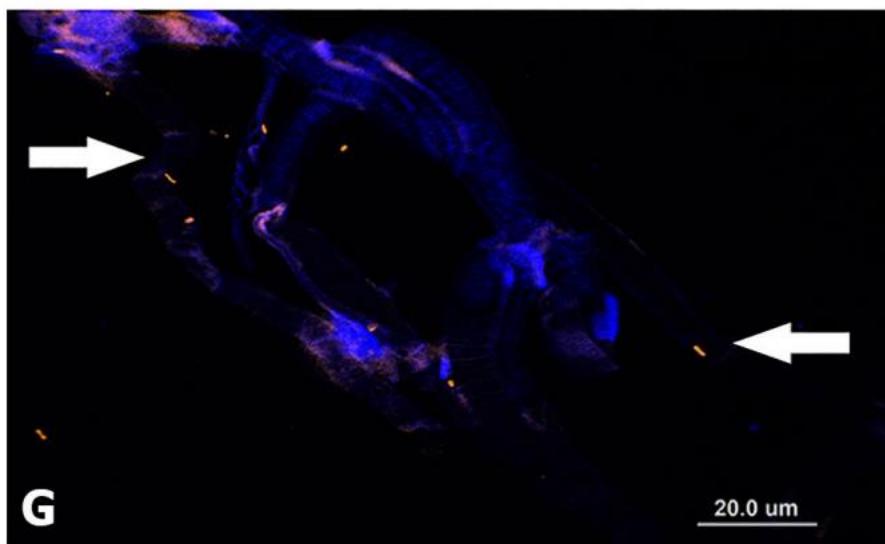
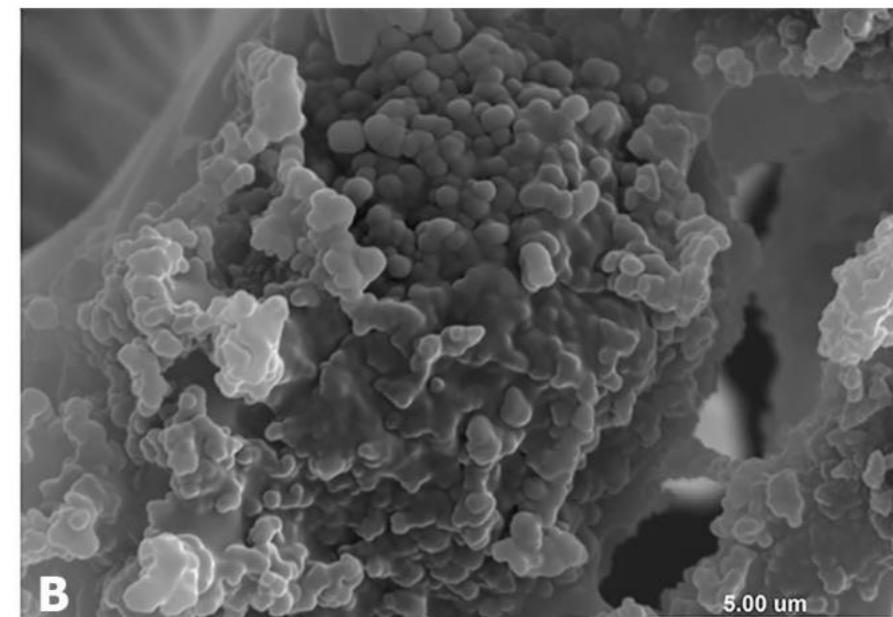
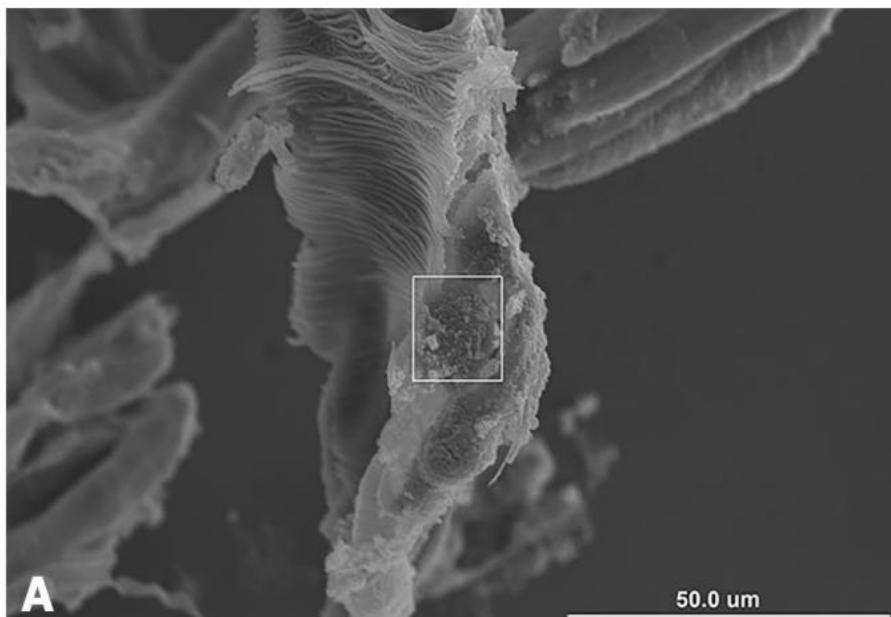






**A****B**





Análisis de genomas de bacterias en cultivo

- Objetivo: Describir la diversidad de genes de resistencia a los antibióticos y los elementos genéticos móviles presentes en el genoma de una colección de bacterias de la familia Enterobacteriaceae aisladas de puntos con diferente influencia urbana en el Río Virilla.



PRODUCCIÓN DE ESPECIES
REACTIVAS DE OXÍGENO
(ROS)



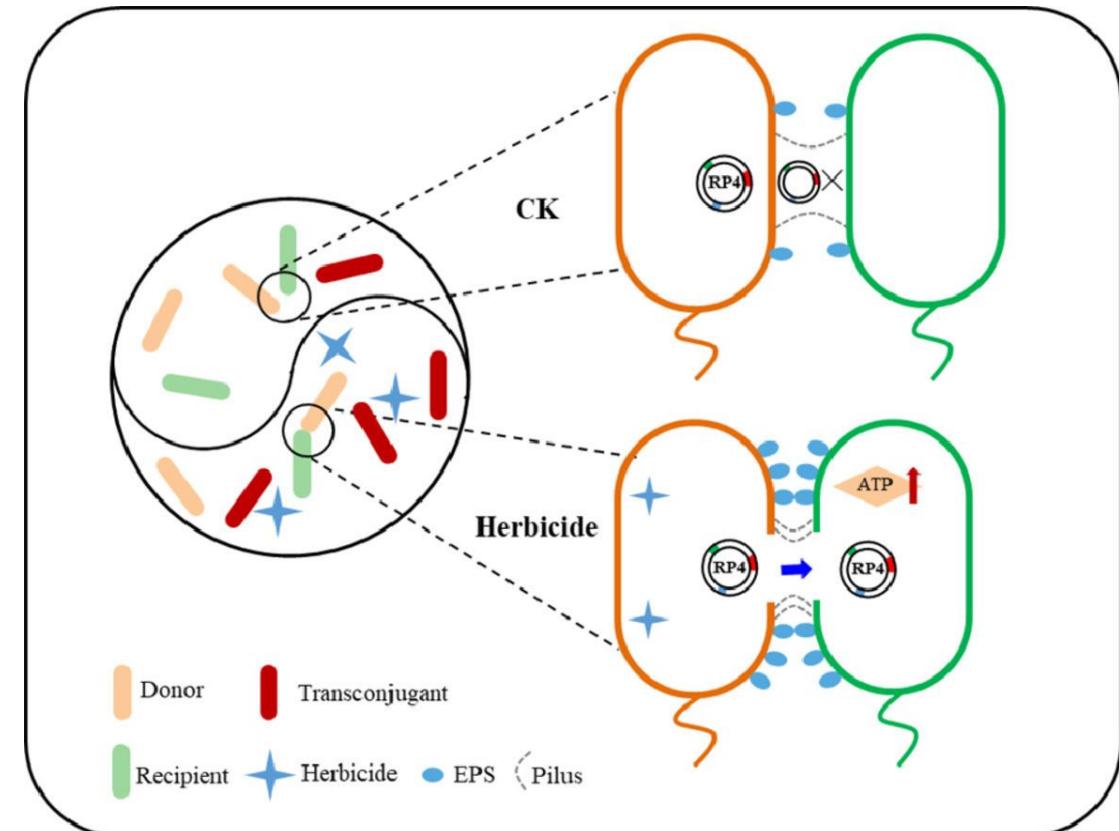
SOBREEXPRESIÓN DE
GENES



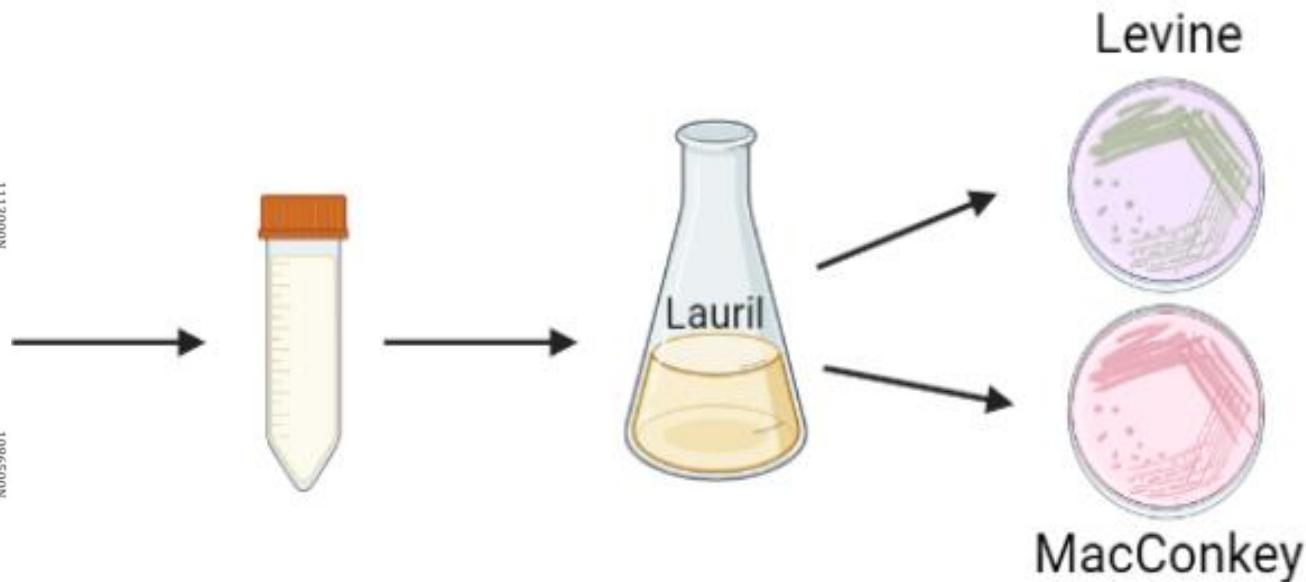
SISTEMA SOS

Enterobacteriaceae

- Preocupante resistencia a los antibióticos betalactámicos (Carbapenémicos)
- Acetaminofén promueve la transferencia de plásmidos en *E. coli* (Jia Y et al., 2021)



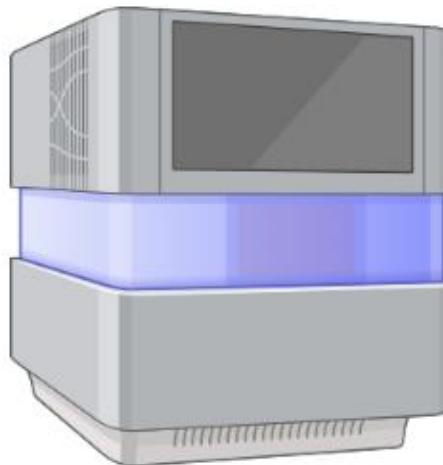
Li et al. 2022



- Accesibilidad, altura, índice BMWP-CR e índice Holandés (SINIGIRH)
- 1, 8 y 15 de noviembre del 2021



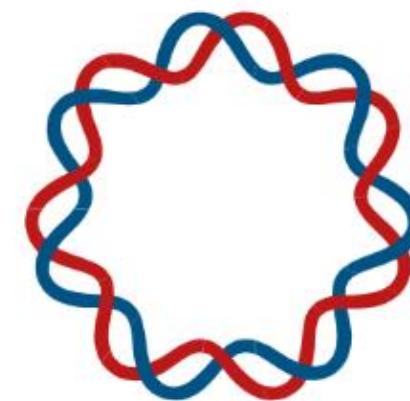
Secuenciación, ensamblaje y anotación



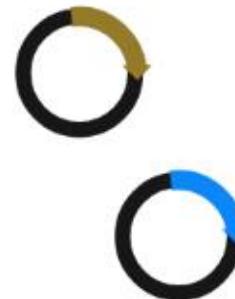
Secuenciación Illumina
y PacBio*



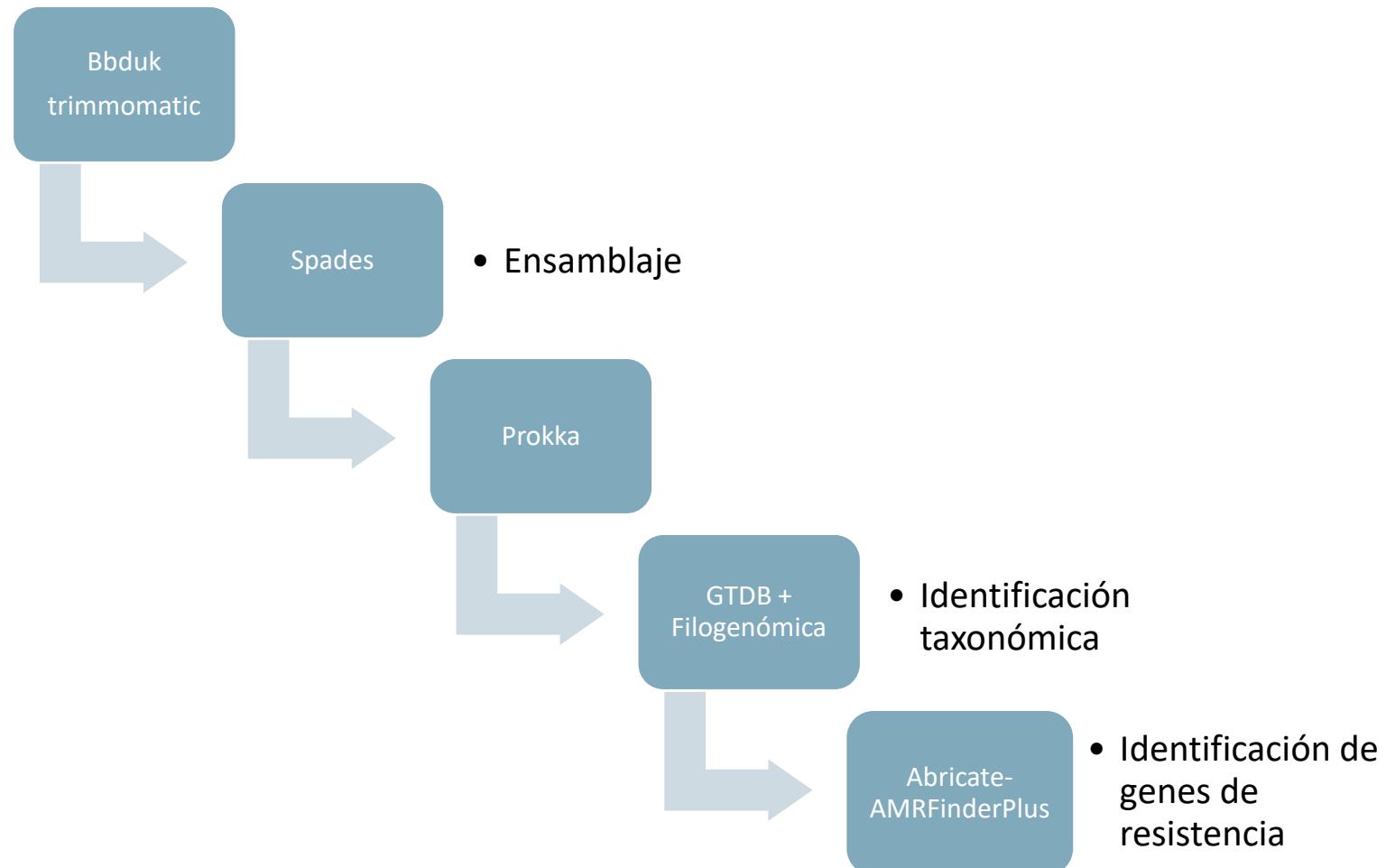
Análisis Bioinformáticos



Ensamblaje de genoma y
reconstrucción de EGM

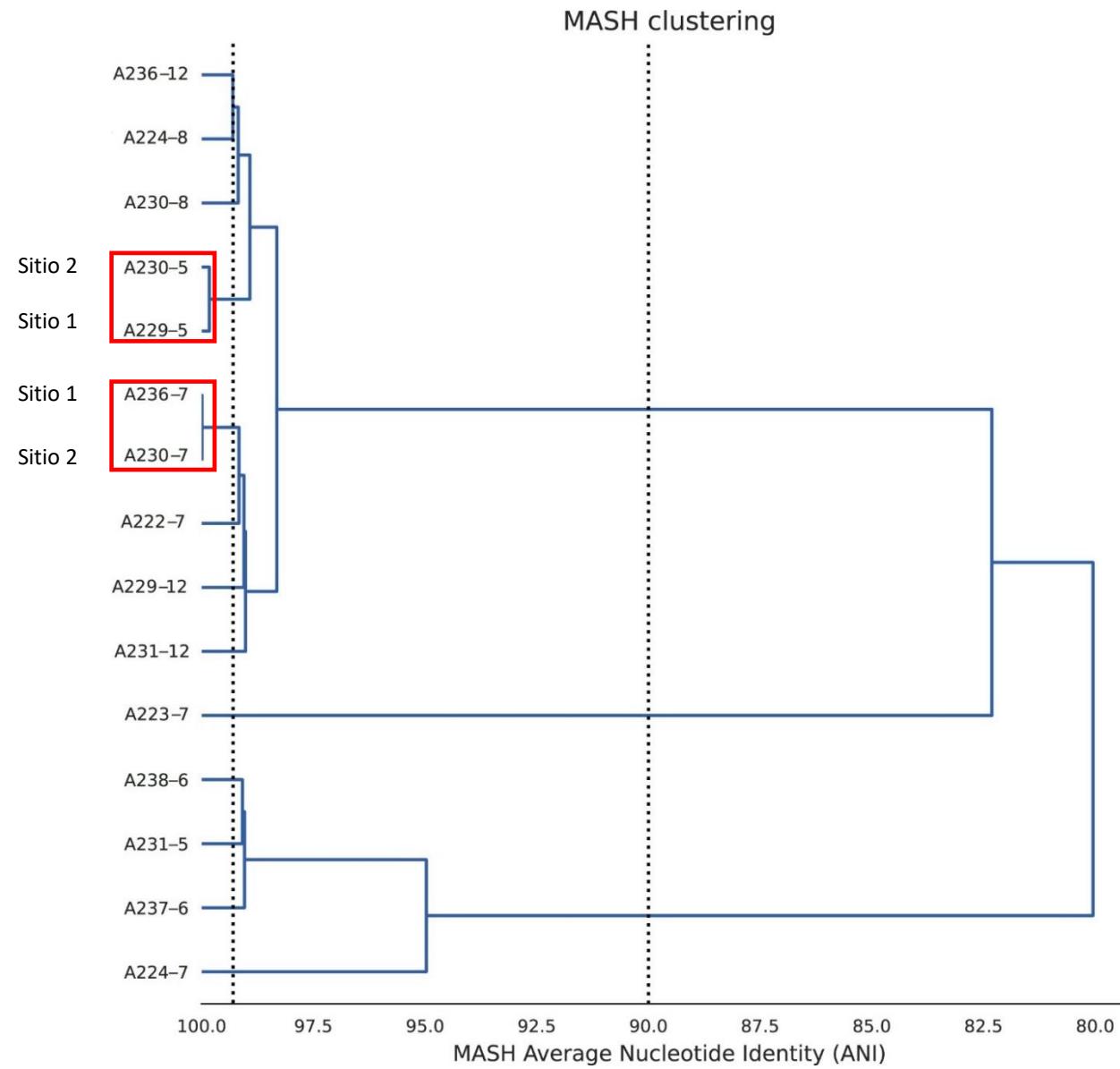


Workflow bioinformático

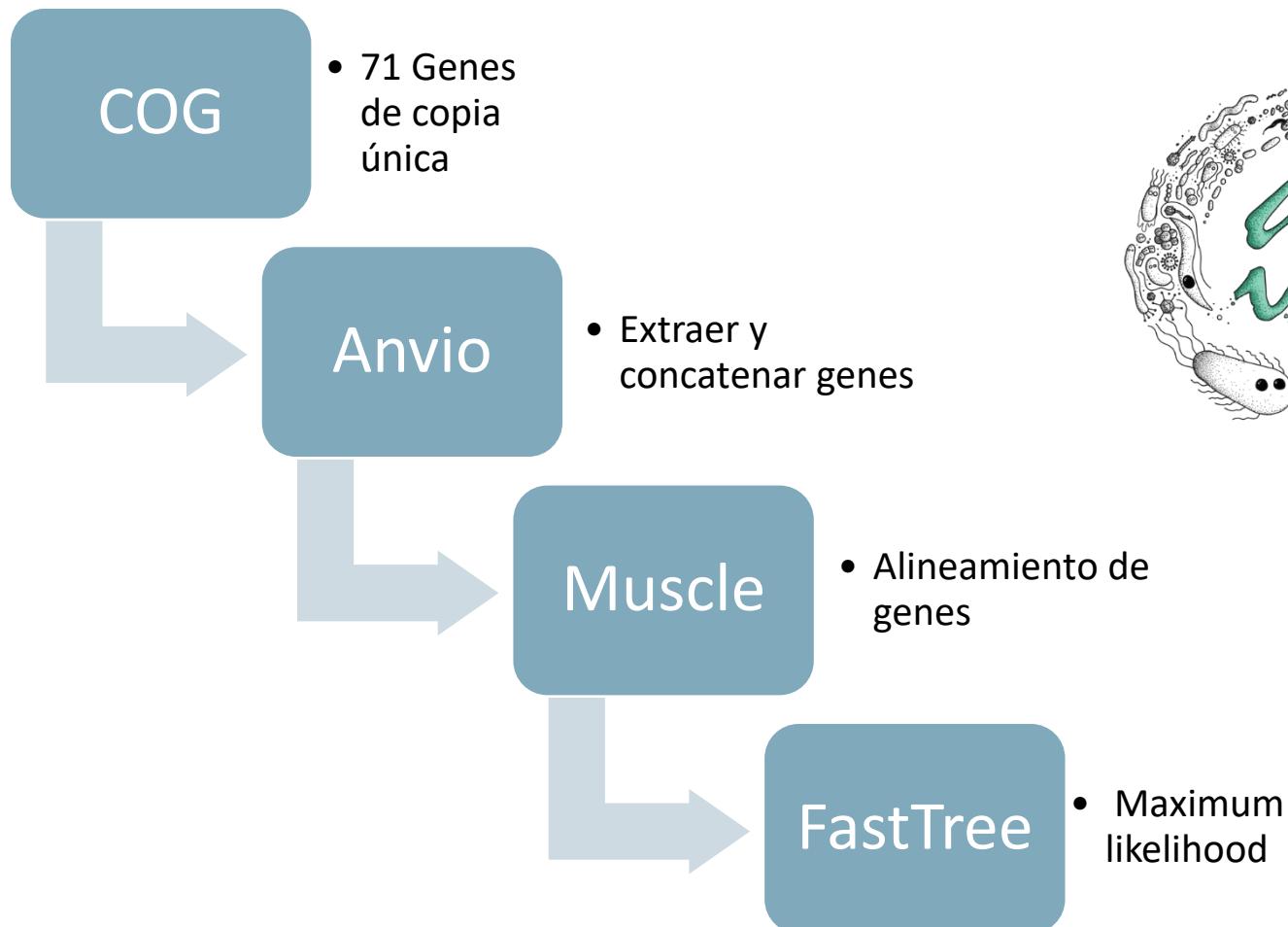


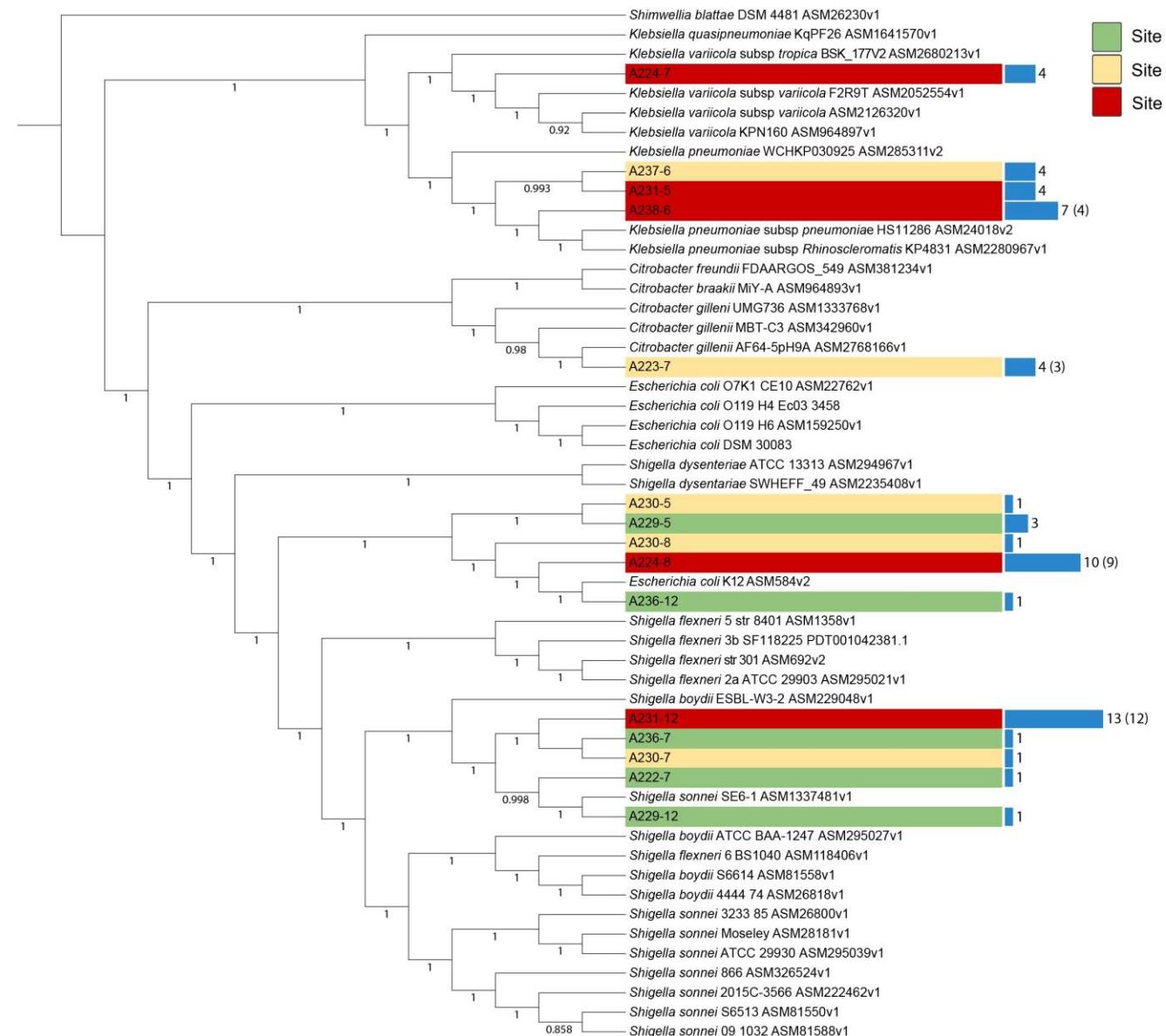
Calidad de los genomas

Aislamiento (No. Sitio de aislamiento)	Ensamblaje	Contigs	Size (Mpb)	N50	L50	%GC	Compleitud	Redundancia	Taxonomía según GTDB (ANI)
A222-7 (1)	Illumina	73	4,9	130684	11	50,61	100	0	<i>Shigella flexneri</i> (97,81)
A229-5 (1)	Illumina	127	5,05	78655	22	50,89	87,32	2,8	<i>Shigella flexneri</i> (97,49)
A229-12 (1)	Illumina	99	4,75	103479	15	50,78	100	1,4	<i>Shigella flexneri</i> (97,90)
A236-7 (1)	Illumina	64	4,8	167951	10	50,7	100	0	<i>Shigella flexneri</i> (97,75).
A236-12 (1)	Illumina	73	4,7	147946	8	50,59	100	0	<i>Shigella flexneri</i> (97,76)
A223-7 (2)	Illumina	45	4,97	413561	6	52,48	100	1,4	<i>Citrobacter gillenii</i> (98,96)
A230-5 (2)	Illumina	143	4,96	59516	25	50,56	78,87	1,4	<i>Shigella flexneri</i> (97,80)
A230-7 (2)	Illumina	77	4,8	158798	11	50,7	100	1,4	<i>Shigella flexneri</i> (97,63)
A230-8 (2)	Illumina	159	4,96	77565	23	50,62	100	0	<i>Shigella flexneri</i> (97,66)
A237-6 (2)	Illumina	67	5,22	188161	10	57,56	100	0	<i>Klebsiella pneumoniae</i> (99,06)
A224-7 (3)	Illumina	84	5,6	141929	14	57,21	100	0	<i>Klebsiella variicola</i> (98,94)
A224-8 (3)	Illumina	101	4,7	109905	16	50,65	100	0	<i>Shigella flexneri</i> (97,74)
A231-5 (3)	Illumina	51	5,47	215170	9	57,27	100	0	<i>Klebsiella pneumoniae</i> (99,05)
A231-12 (3)	Illumina	60	5	226574	8	50,55	100	0	<i>Shigella flexneri</i> (97,96)
A238-6 (3)	Illumina	75	5,54	191124	10	57,19	100	0	<i>Klebsiella pneumoniae</i> (98,96)
A224-8 (3)	Híbrido	5	4,7	4712205	1	50,73	100	0	<i>Shigella flexneri</i> (97,74)
A231-12 (3)	Híbrido	4	5,08	4757699	1	50,61	100	0	<i>Shigella flexneri</i> (97,96)

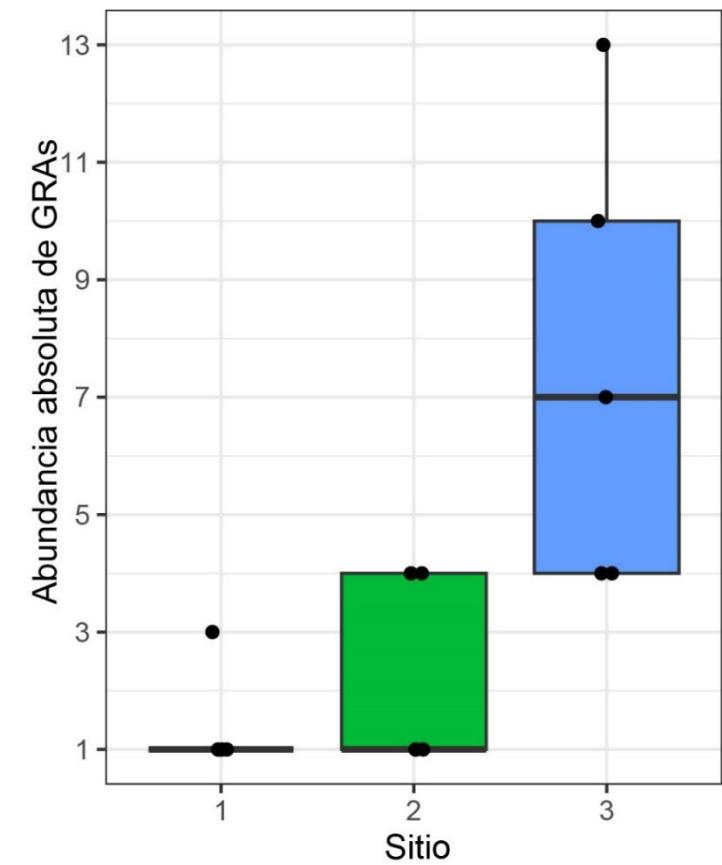
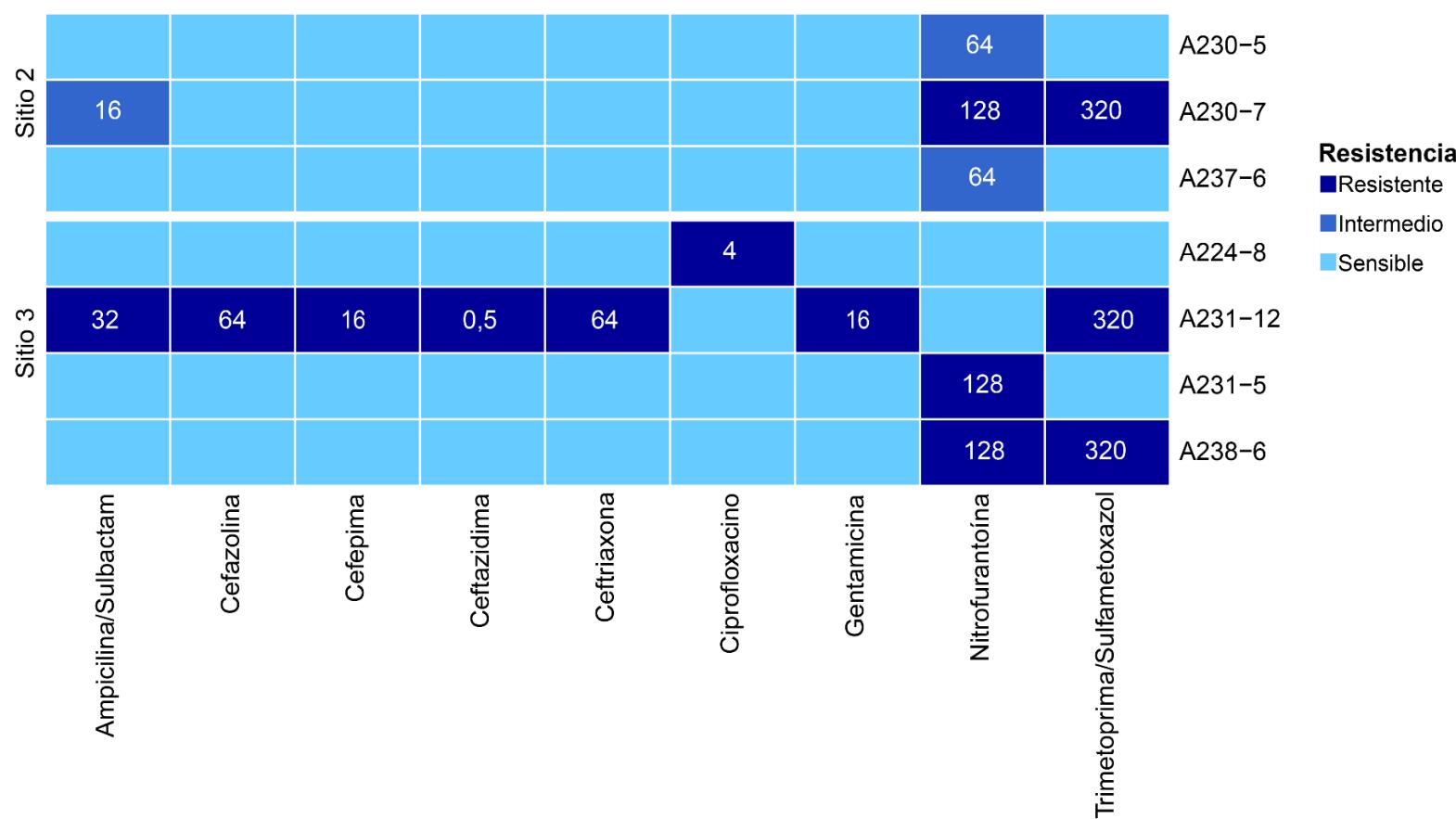


Establecer las relaciones filogenómicas de los aislamientos obtenidos, con diferentes cepas del mismo género y familia distribuidas a nivel global.





Perfiles de resistencia a antibióticos



Kruskall Wallis (Chi=9,45, p=0,009, Fig. 4)

Búsqueda de mutaciones en genes asociados a resistencia

- Comparación de aislamiento A224-8 contra *Escherichia coli* str. K-12 susceptible
- gyrA: Ser-83→Leu

Query	61	KAYKKSARVVGDVIGKYHPHGDLAVYNTIVRMAQPFSLRYMLVDGQGNFGSIDGDSAAAM	120
		KAYKKSARVVGDVIGKYHPHGD	AVY+TIVRMAQPFSLRYMLVDGQGNFGSIDGDSAAAM
Sbjct	61	KAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDSAAAM	120

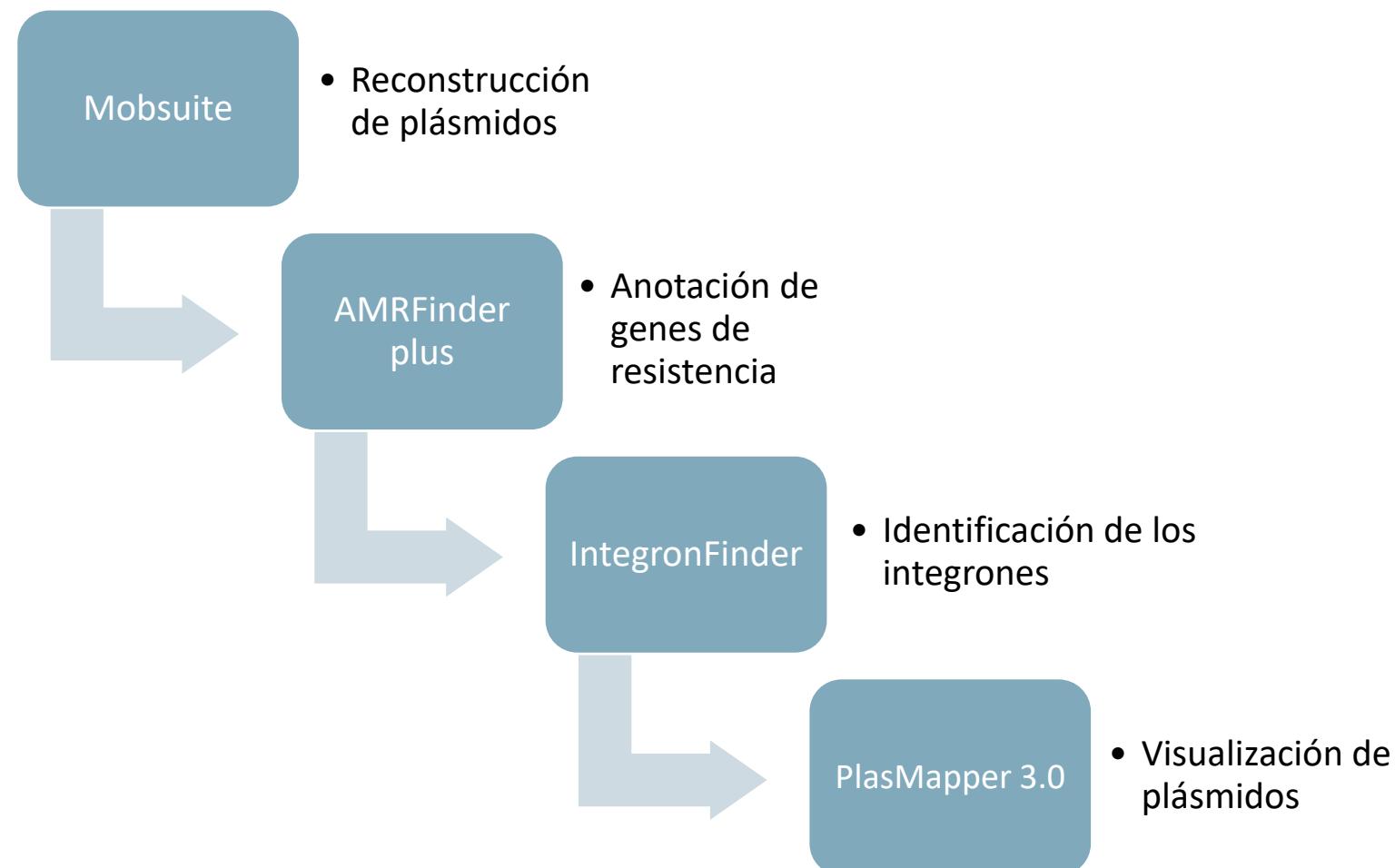
Búsqueda de mutaciones en genes asociados a resistencia

Aislamiento (No. de aislamiento)	Fenotipo	Especie	Mutaciones en <i>nfsA</i>	Mutaciones en <i>nfsB</i>	Genes asociados al fenotipo de resistencia a nitrofurantoina
A238-6 (3)	Resistente	<i>K. pneumoniae</i>	E29A, G125W, G204S		<i>oqxB, oqxA</i>
A231-5 (3)	Resistente	<i>K. pneumoniae</i>	Q195L		<i>oqxB11, oqxA5</i>
A237-6 (2)	Resistente	<i>K. pneumoniae</i>	K222R		<i>oqxB25, oqxA6</i>
A224-7 (3)	Sensible	<i>K. variicola</i>	R59Q, T117I, E144A, G25S, V167I R180H, E191D		<i>oqxB15, oqxA6</i>
A230-7 (2)	Resistente	<i>Shigella</i> sp.		M75I, V93A, H107R	
A230-5 (2)	Resistente	<i>Shigella</i> sp.		V93A, H107R	
A231-12 (3)	Sensible	<i>Shigella</i> sp.		M75I, V93A, H107R	
A230-8 (2)	Sensible	<i>Shigella</i> sp.		H107R	
A236-12 (2)	Sensible	<i>Shigella</i> sp.		H107R	
A236-7 (2)	Sensible	<i>Shigella</i> sp.	L157F	M75I, V93A, H107R	
A229-12 (1)	Sensible	<i>Shigella</i> sp.	E28G	M75I, V93A, H107R	
A224-8 (3)	Sensible	<i>Shigella</i> sp.		H107R	
A222-7 (1)	Sensible	<i>Shigella</i> sp.		M75I, V93A, H107R	
A229-5 (1)	Sensible	<i>Shigella</i> sp.		V93A, H107R	<i>oqxB11 oqxA10</i>

Relación fenotipo-genotipo

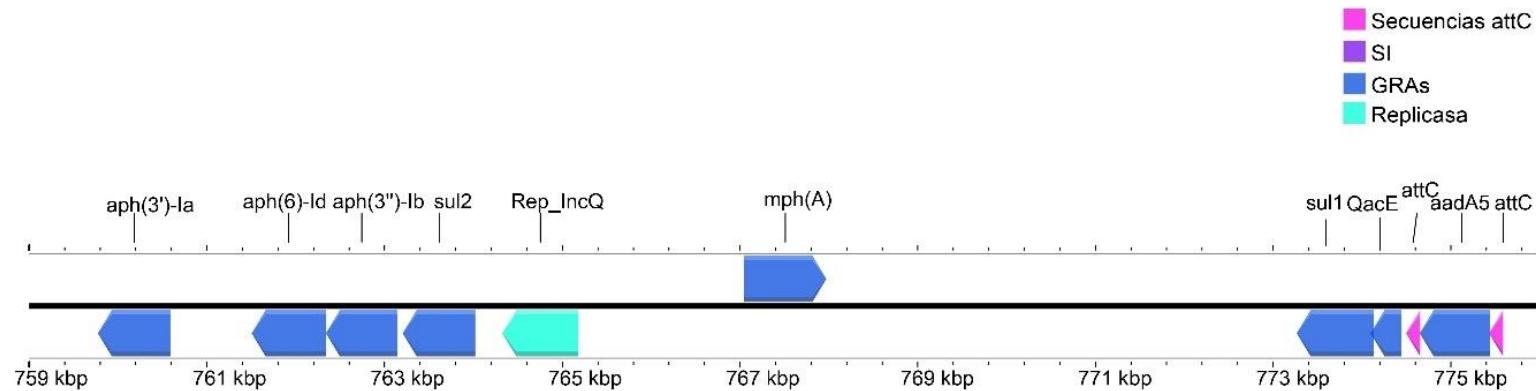
Aislamiento	Fenotipo de resistencia	Genotipo responsable
A230-5	Nitrofurantoína	No encontrado*
A230-7	Ampicilina/Sulbactam, Trimetoprima/sulfametoxazol y Nitrofurantoína	No encontrado*
A237-6	Nitrofurantoína	<i>oqxB25, oqxA6, nfsB</i> : K222R
A224-8	Ciprofloxacina	<i>gyrA</i> : S83L
A231-5	Nitrofurantoína	<i>oqxB11, oqxA5, nfsA</i> : Q195L
A238-6	Trimetoprima/sulfametoxazol Nitrofurantoína	<i>sul2, dfrA26</i> <i>oqxB, oqxA, nfsA</i> : E29A, G125W, G204S
A231-12	Betalactámicos	<i>blaEC-18, blaCTX-M-115, blaTEM-1</i>
	Gentamicina	<i>aac(3)-IId</i>
	Trimetoprima/sulfametoxazol	<i>sul1, sul2, dfrA12</i>

2) Describir la estructura de los elementos genéticos móviles presentes en las Enterobacterias aisladas y su relación con los genes de resistencia a antibióticos



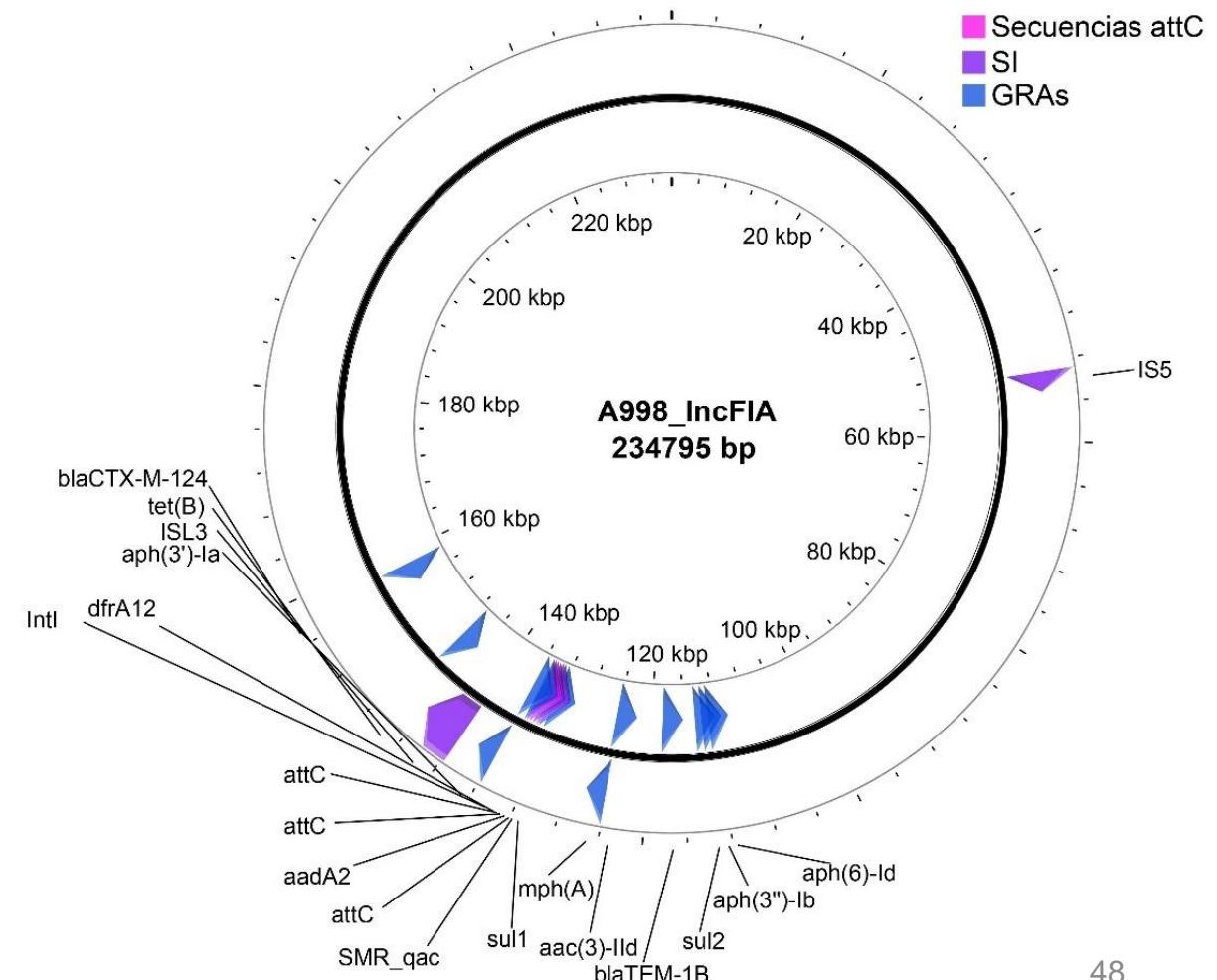
Shigella sp. A224-8 sitio 3 plásmido AC802

- Incorporado en el genoma
- Replicasa asociada a familia IncQ1
- 7 ARGs
- Integron clase 1 CALIN (Sin integrasa) con dos ARGs
- Plásmido *E. coli* EAHEC pASL01a 27kb (cobertura: 57%; identidad: 99,88%)

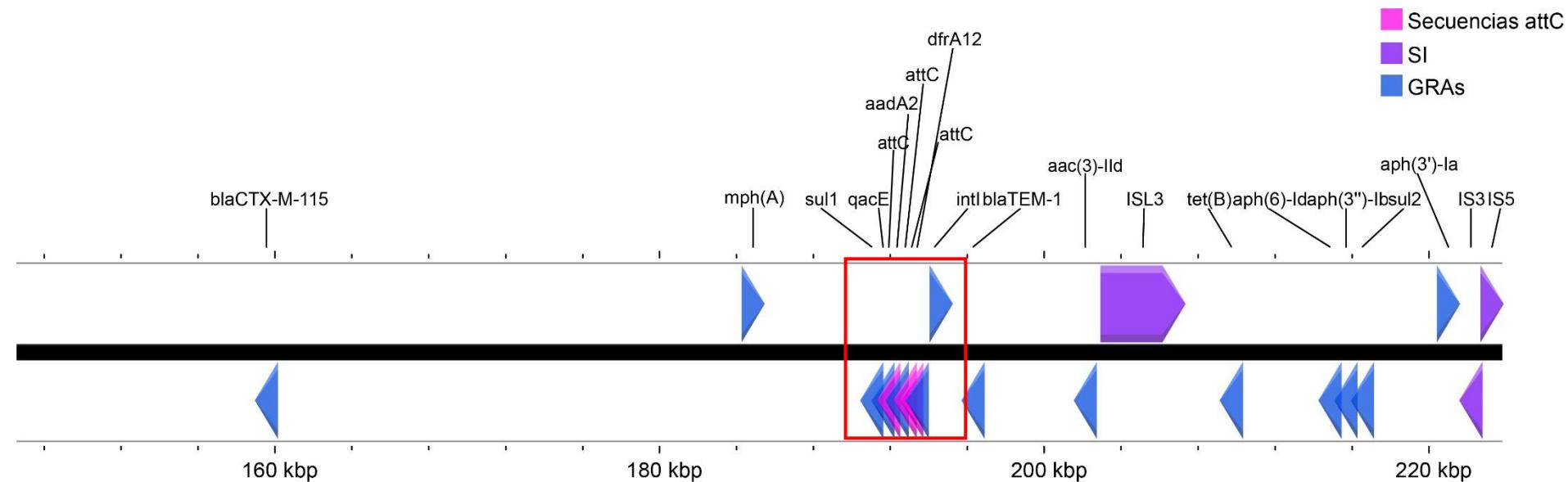


Shigella sp. A231-12 sitio 3 plásmido A998

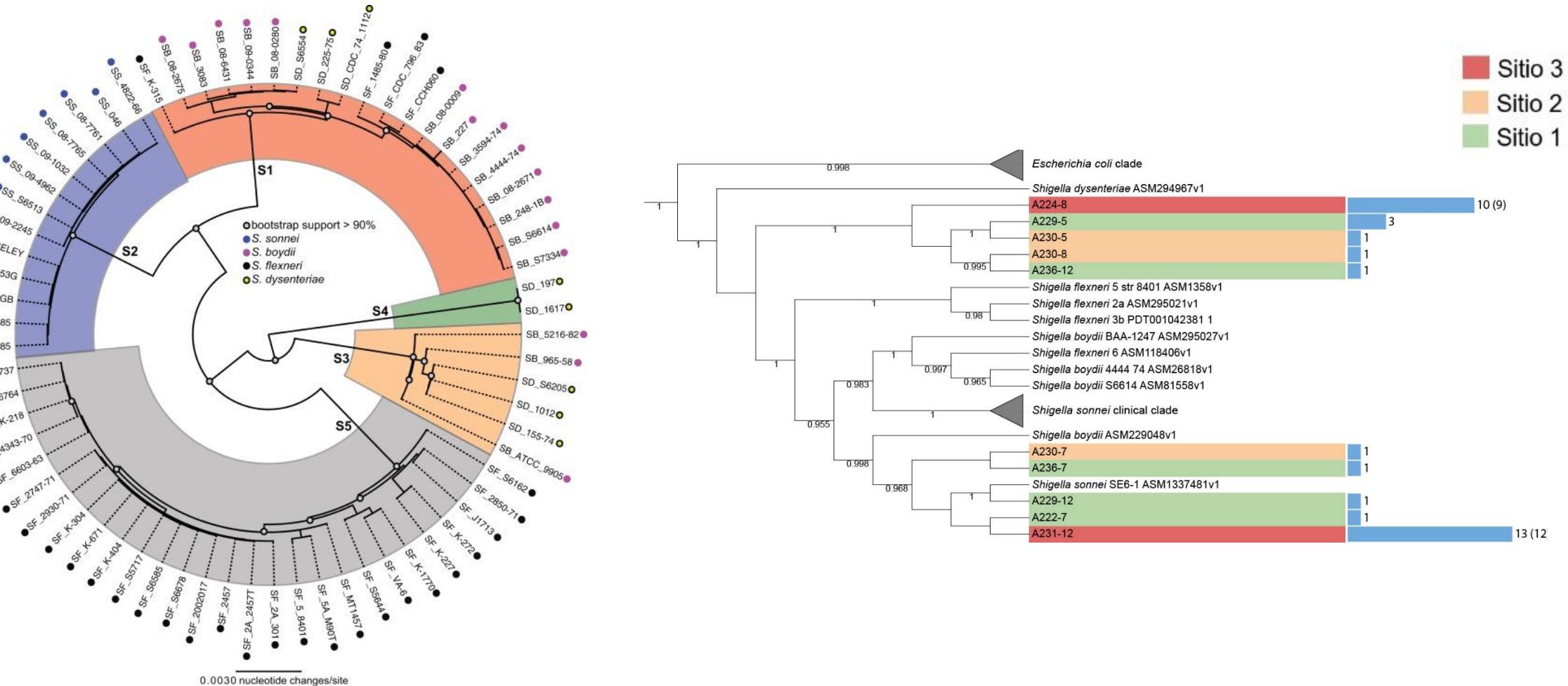
- Plásmido conjugativo
- Familia de replicasas IncFIA e IncQ1
- 12 ARGs
- 1 integron completo
- Plásmido pSRC27-H *Salmonella entérica*
205kb (cobertura: 89%; identidad: 99,98%)



Shigella sp. A231-12 sitio 3, plásmido A998



Aislamientos asignados al género *Shigella*

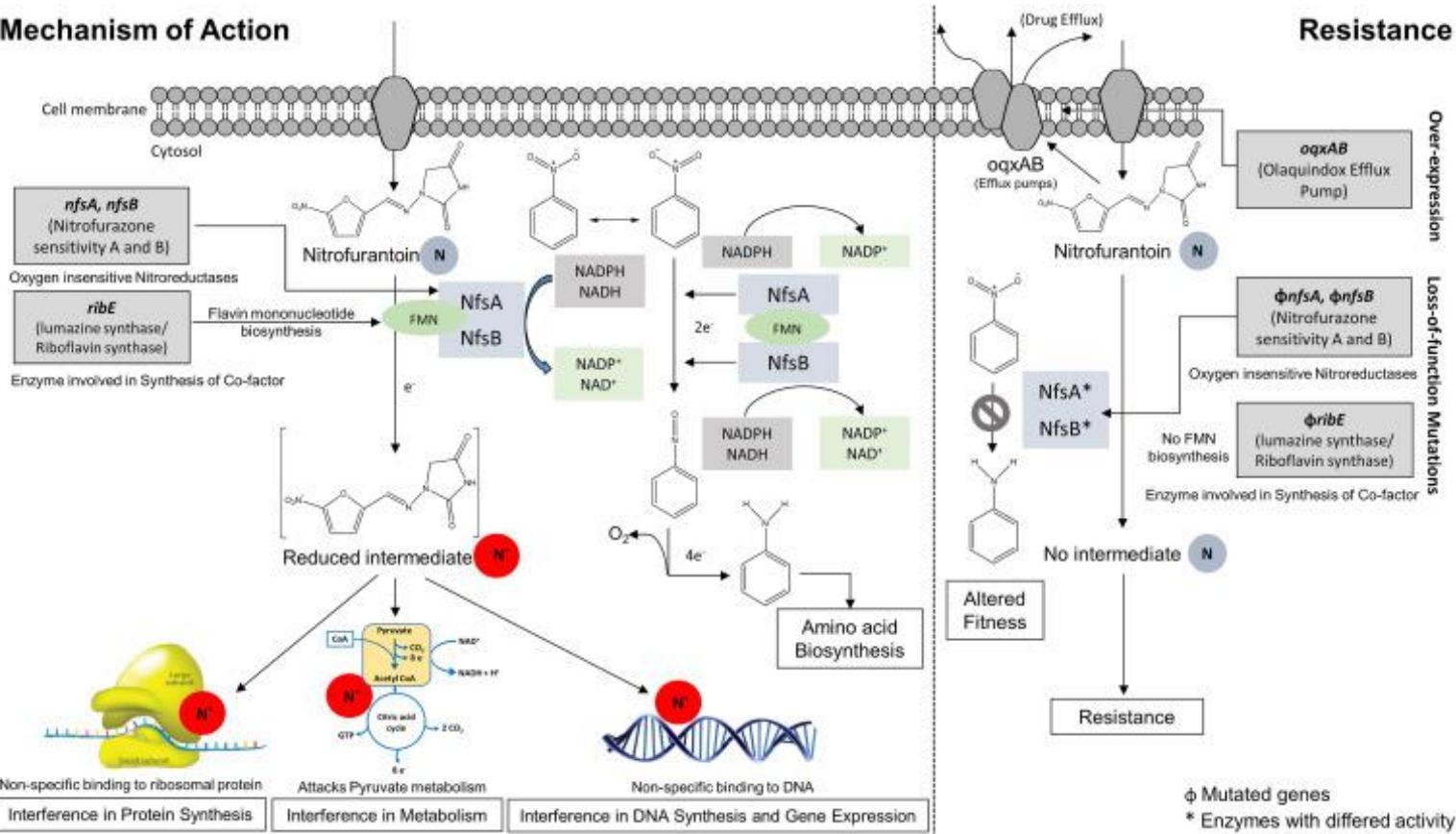


ShigaPass

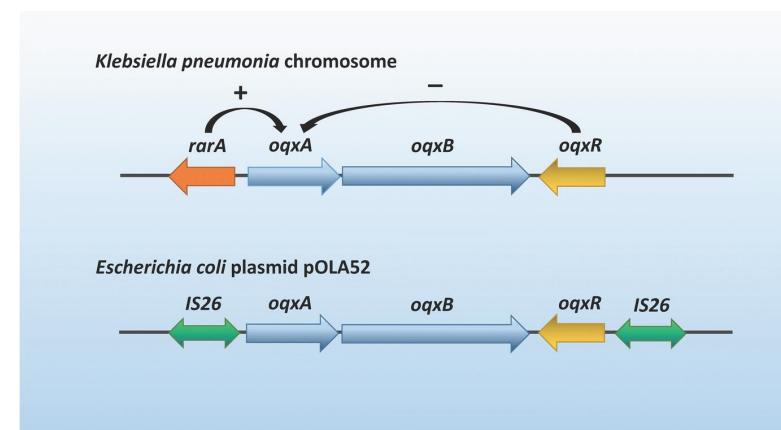
Name	rfb	rfb_hits,(%)	MLST	fliC	CRISPR	ipaH	Predicted_Sero type	Predicted_Flex Serotype
A224-8	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A231-12	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A230-7	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A230-8	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A229-12	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A231-5	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A231-12	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A236-7	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A236-12	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A237-6	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A238-6	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A222-7	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A223-7	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A224-7	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A224-8	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A229-5	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	
A230-5	ND	ND,(0.0%)	ND	ND	ND	ipaH-	Not Shigella/EIEC	

Resistencia a nitrofurantoína

Mechanism of Action



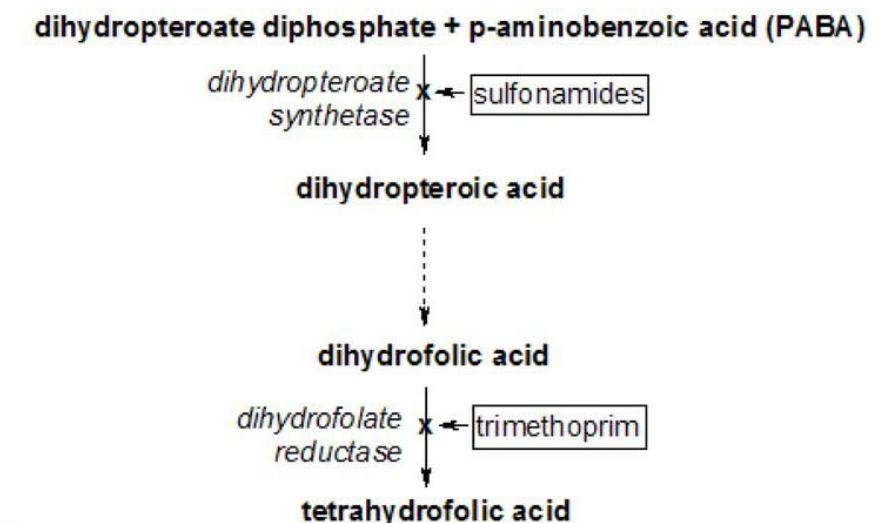
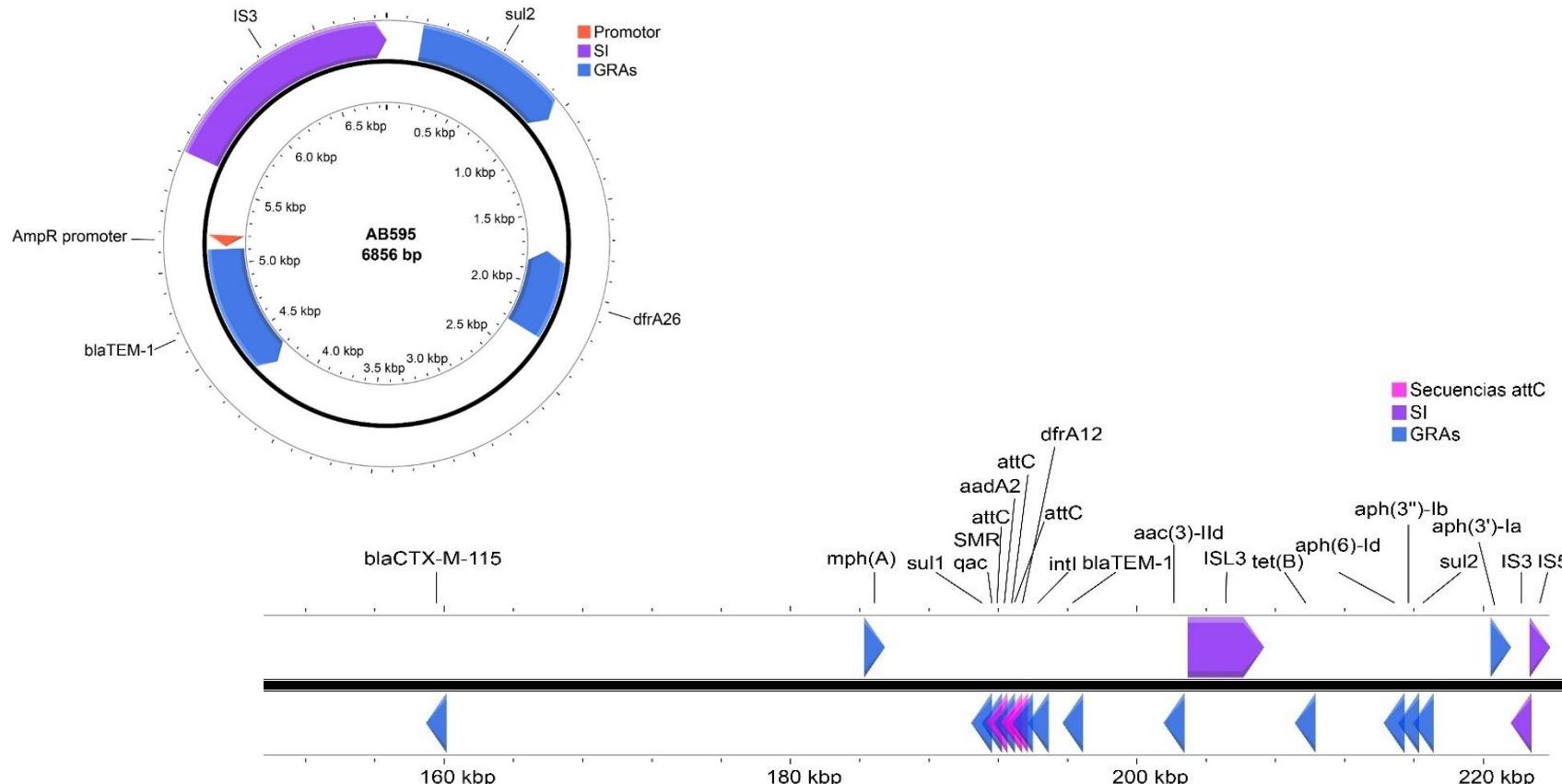
A238-6 (3)	<i>K. pneumoniae</i>	E29A, G125W, G204S	<i>oqxB, oqxA</i>
A231-5 (3)	<i>K. pneumoniae</i>	Q195L	<i>oqxB11, oqxA5</i>
A237-6 (2)	<i>K. pneumoniae</i>	K222R	<i>oqxB25, oqxA6</i>



Li et al. 2019.

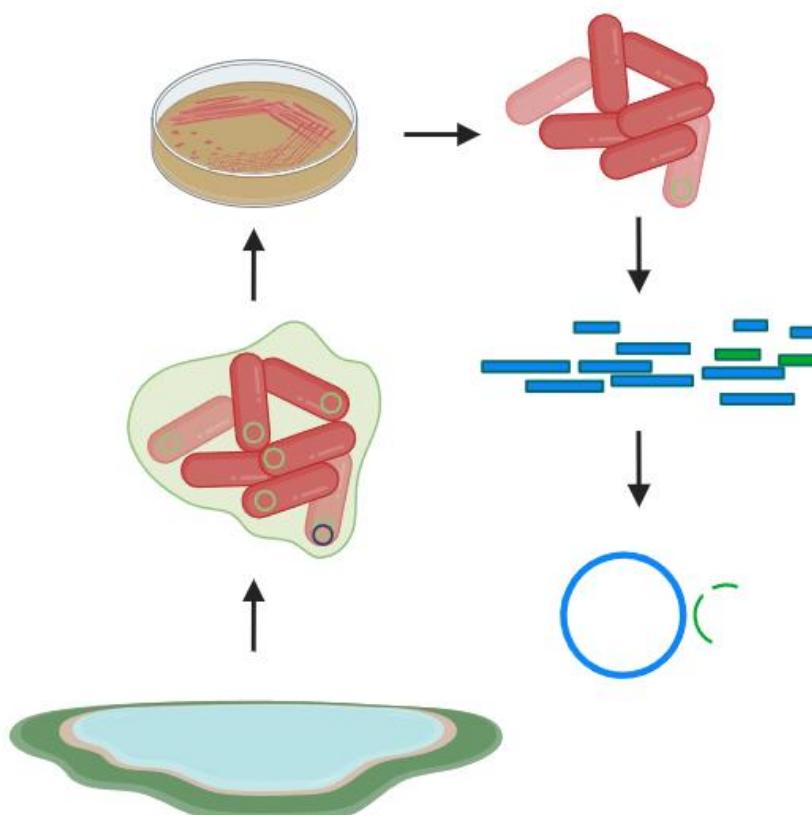
Resistencia a Trimetoprima/Sulfamexotazol

- Presencia de genes *sul* y *dfrA* en plásmidos de los aislamientos A231-12 y A238-6



Falta de genotipo asociado a resistencia

- Baja cantidad de células con plásmidos
- Plásmidos con bajo número de copias



Aislamiento	Fenotipo de resistencia	Genotipo responsable
A230-5	Nitrofurantoína	No encontrado*
A230-7	Ampicilina/Sulbactam, Trimetoprima/sulfametoxazol y Nitrofurantoína	No encontrado*

Whole genome sequencing (WGS) fails to detect antimicrobial resistance (AMR) from heteroresistant subpopulation of *Salmonella enterica*

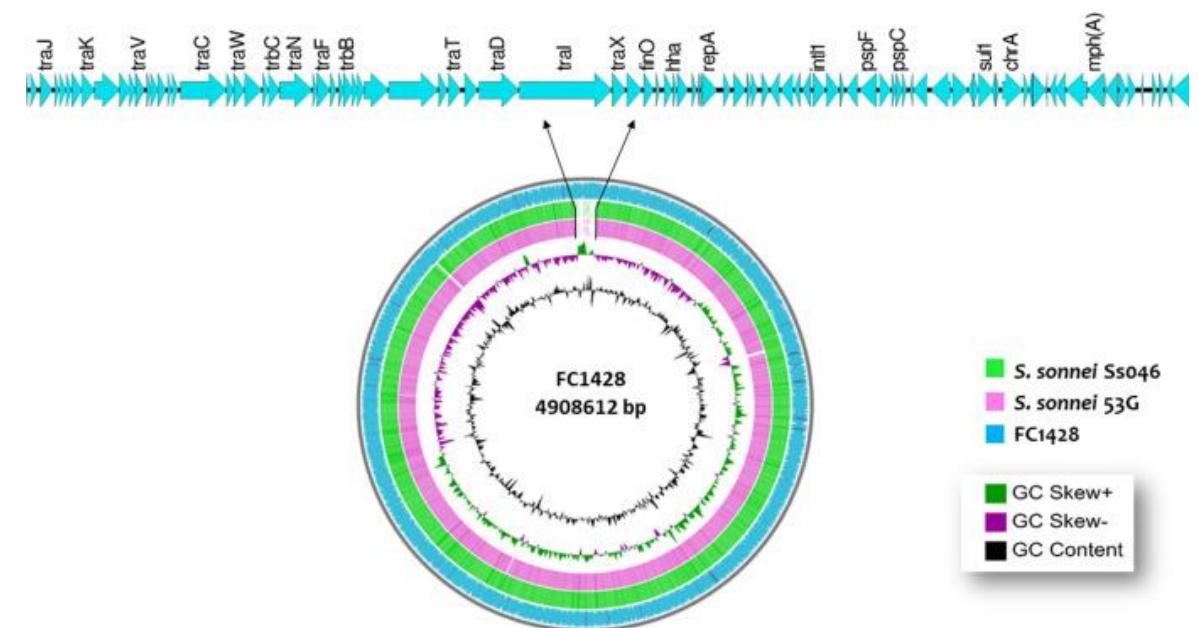
Ye Htut Zwe ¹, Seow Fong Chin ², Gurjeet Singh Kohli ³, Kyaw Thu Aung ⁴, Liang Yang ⁵, Hyun-Gyun Yuk ⁶

Integrative Analysis of Whole Genome Sequencing and Phenotypic Resistance Toward Prediction of Trimethoprim-Sulfamethoxazole Resistance in *Staphylococcus aureus*

Aislamiento A224-8

- Resistencia a Ciprofloxacina, S83L: Mutación relacionada con grado de contaminación y mayores niveles de MIC (Johning et al. 2015).
- Incorporación de plásmido en el cromosoma

Hybrid genome assembly of *Shigella sonnei* reveals the novel finding of chromosomal integration of an IncFII plasmid carrying a *mphA* gene

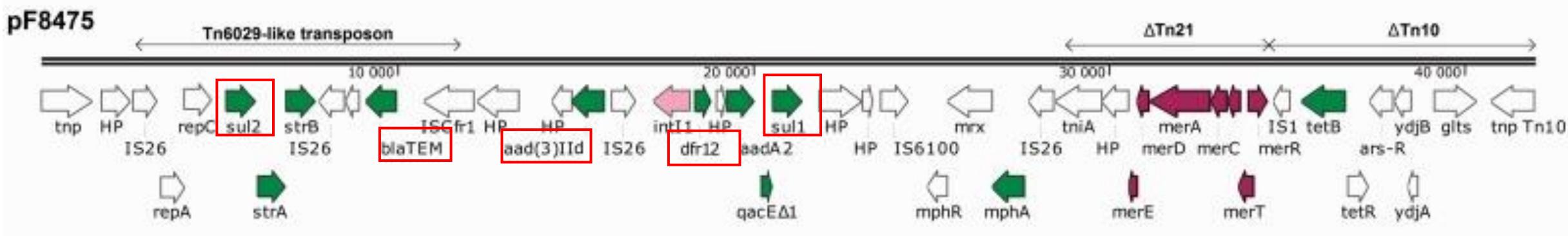


Muthuirulandi Sethuve et al. 2021

Aislamiento A231-12

- Resistencia a 7 antibióticos (5 betalactámicos)
- Betalactamasas TEM-1 y CTX

A231-12	Betalactámicos	<i>blaEC-18*</i> , <i>blaCTX-M-115</i> , <i>blaTEM-1</i>
	Gentamicina	<i>aac(3)-IId</i>
	Trimetoprima/sulfam etoxazol	<i>sul1</i> , <i>sul2</i> , <i>dfrA12</i>



Kubasova et al. 2016

Arreglo genético con múltiples GRAs

- Arreglo *dfrA12-aadA2-qac-sul1*

Table 2. Gene cassette arrays in class 1 integrons from trimethoprim-sulfamethoxazole resistant isolates from urine specimens.

Isolates	Gene cassette arrays (No. of isolates)	<i>sul1</i> only	<i>sul2</i> only	<i>sul1 + sul2</i>	<i>sul3</i>
<i>E. coli</i> (34)	<i>dfrA17-aadA5</i> (27)	5		22	
	<i>dfrA12-aadA2</i> (3)	3			
	<i>dfrA1-aadA1</i> (2)	2			
	<i>aadA2</i> (1)				1
	<i>aacA4-arr3-dfrA27</i> (1)	1			
<i>K. pneumoniae</i> (10)	<i>dfrA1-ofrC</i> (6)	1		5	
	<i>aadA2</i> (2)		1	1	
	<i>aac(6')-lb-oxa-1-aadA2</i> (1)				1
	<i>dfrA1-aadA1</i> (1)	1			
<i>Acinetobacter</i> spp. (10)	<i>aacA4-catB8-aadA1</i> (7)	6		1	
	<i>aacA44-IMP1-oxa-2</i> (3)	3			
<i>P. aeruginosa</i> (8)	<i>aadB-cmlA-oxa-10-aadA1</i> (7)	7			
	<i>aadA2</i> (1)	1			

Shin et al. 2015

Relación entre contaminación y GRAs

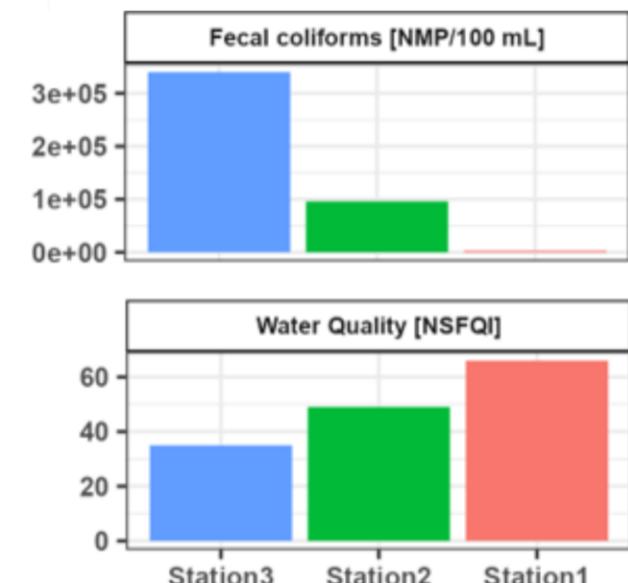
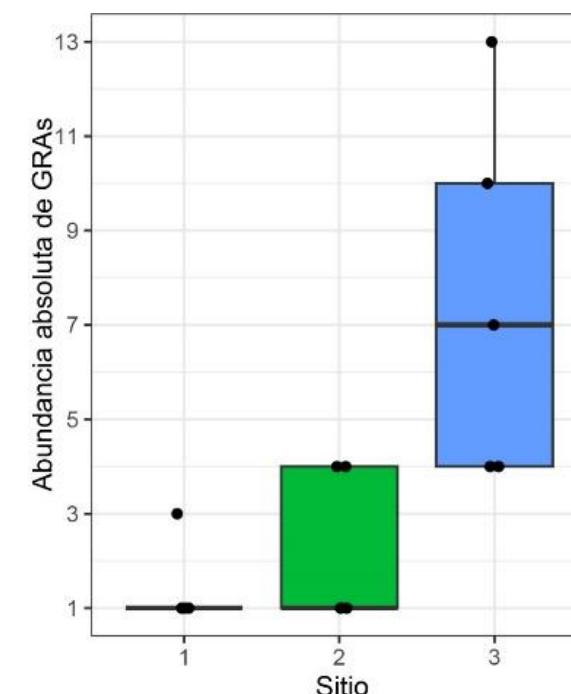
- Mayores niveles de contaminación parecen relacionarse con incremento en EGMs y GRAs:

Fecal Pollution Drives Antibiotic Resistance and Class 1 Integron Abundance in Aquatic Environments of the Bolivian Andes Impacted by Mining and Wastewater

by Jorge Agramont 1,* , Sergio Gutiérrez-Cortez 1,* , Enrique Joffré 2,3 ,
Asa Sjöling 2,3  and Carla Calderon Toledo 1 

Environmental antimicrobial resistance is associated with faecal pollution in Central Thailand's coastal aquaculture region

Thunchanok Thongsamer^c, Rattikan Neamchan^c, Adrian Blackburn^a, Kishor Acharya^a,
Sawannee Sutheeworapong^b, Bundit Tirachulee^b, Pavinee Pattanachan^b,
Soydha Vinitnontharat^c, Xin-Yuan Zhou^d, Jian-Qiang Su^d, Yong-Guan Zhu^d, David Graham^a,
David Werner^a 



Take-Home Message

- Necesidad de estudiar las dinámicas resistencia en cepas ambientales a nivel local
- Datos ambientales como insumo para instituciones que realizan la vigilancia epidemiológica a nivel nacional





Preguntas? ☺