Diversidad de genes bacterianos relacionados con promoción del crecimiento vegetal

TRABAJO FIN DE MÁSTER

Enero 2018

Paloma Pizarro Tobías
Profesora Consultora

David Merino Arranz
Carles Ventura Rojo
Profesores responsables de la asignatura

Miguel García Hidalgo
Máster de Bioinformática y Bioestadística
Microbiología, biotecnología y biología molecular

BACTERIAS PROMOTORAS DEL CRECIMIENTO VEGETAL

PLANT GROWTH PROMOTING RHIZOBACTERIA

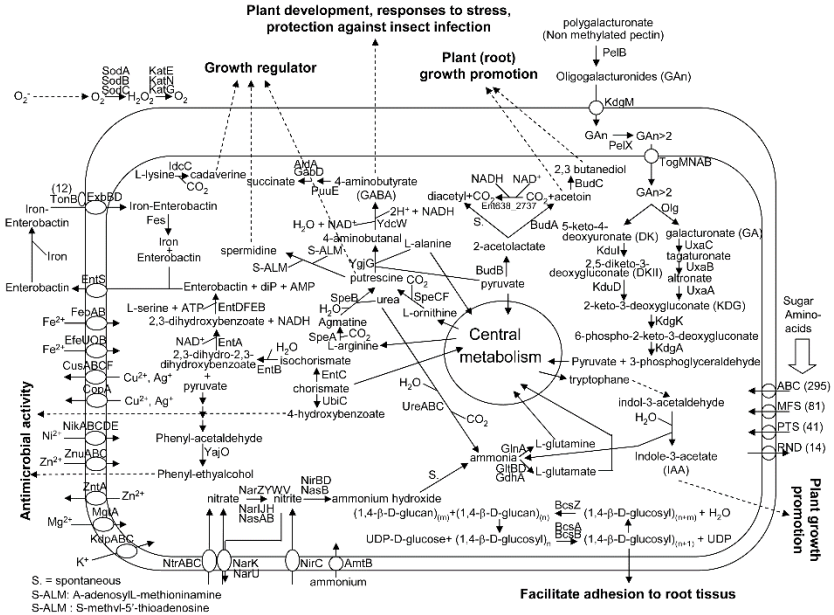
Capítulo 1. Las Bacterias PGPR. Principales mecanismos de Promoción de crecimiento vegetal.

Capítulo 2. Relación filogenética de PGPRs.

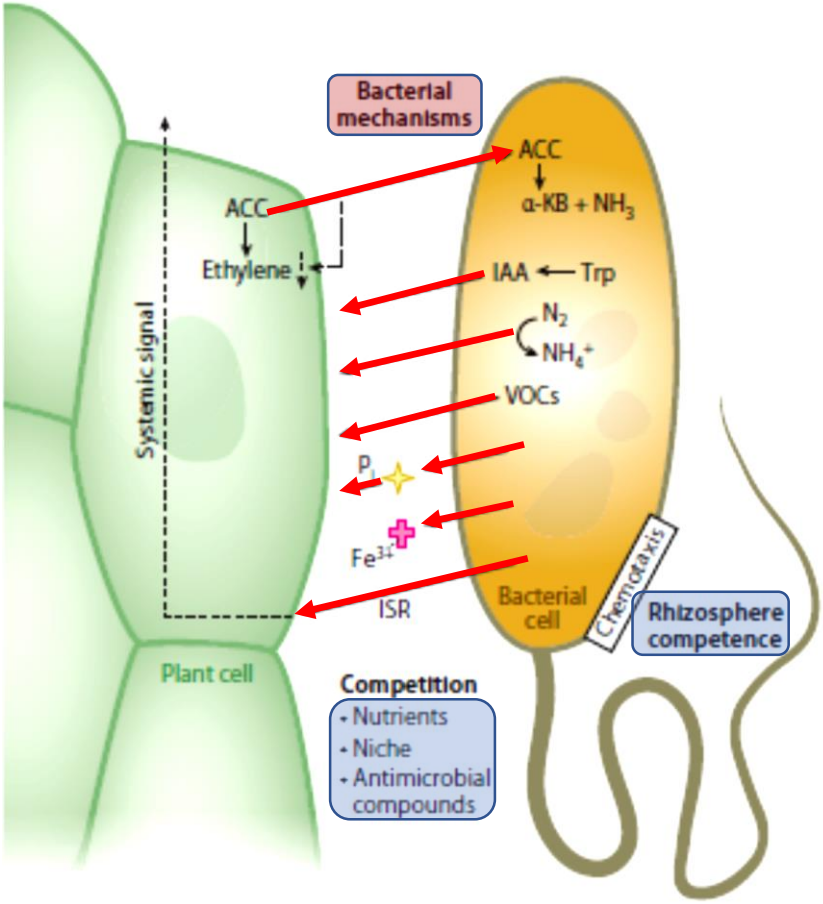
Capítulo 3. Estudio combinado de las relaciones entre mecanismos promotores del crecimiento vegetal, diversidad de PGPRs y cultivos.

Bibliografía.

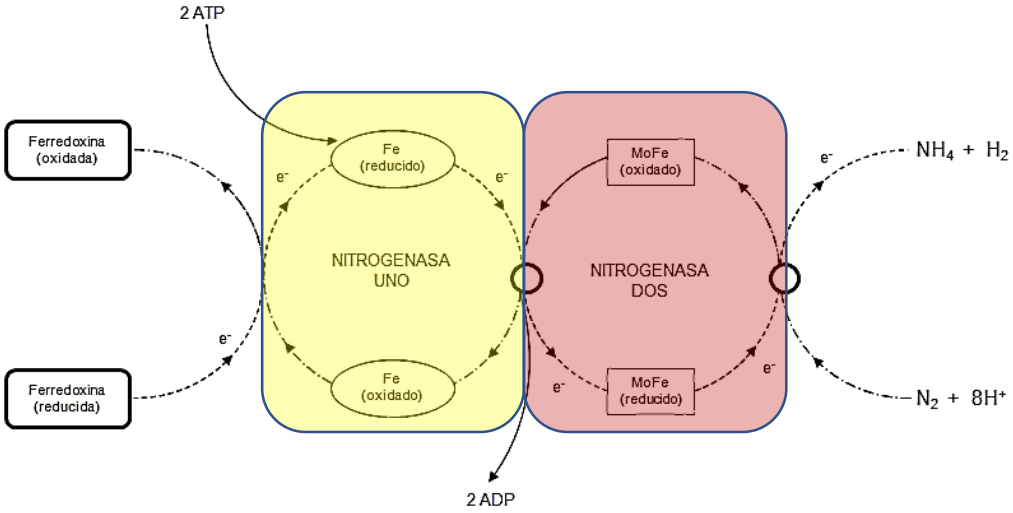
Capítulo 1. Las Bacterias PGPR.
Principales mecanismos de Promoción de crecimiento vegetal

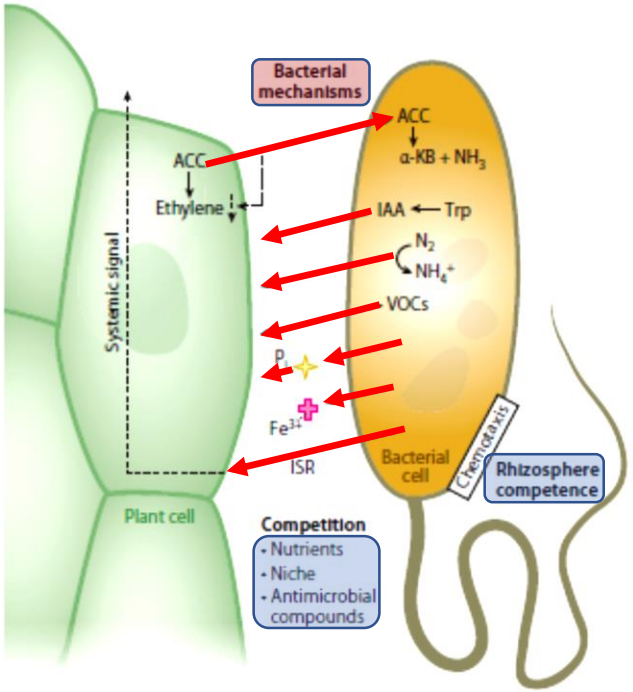


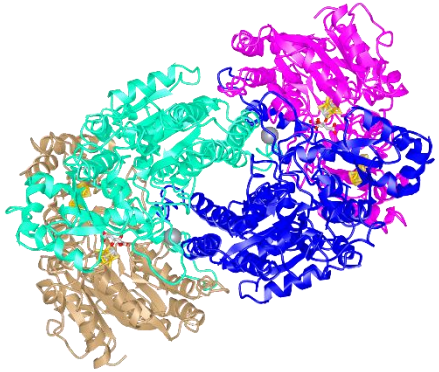
Taghavi S, van der Lelie D, Hoffman A, Zhang Y-B, Walla MD, Vangronsveld J, et al. (2010) Genome Sequence of the Plant Growth Promoting Endophytic Bacterium *Enterobacter* sp. 638. PLoS Genet 6(5): e1000943. https://doi.org/10.1371/journal.pgen.1000943

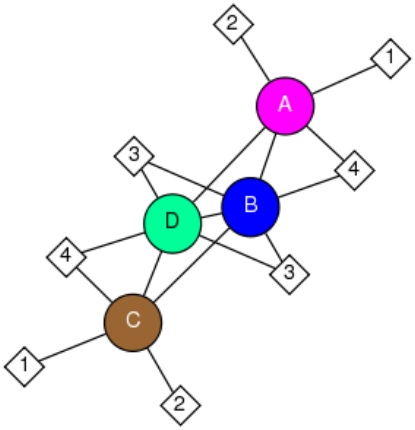


Nitrogenasa - *nifHDK*



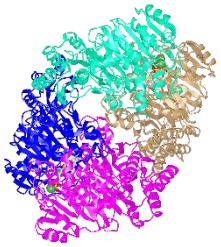


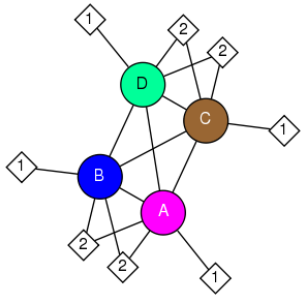


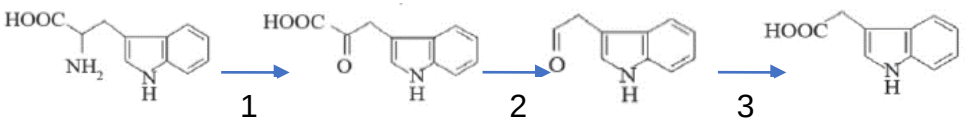


Nitrogenasa Mo-Fe de Azotobacter vinelandii, *estado oxidado.* Compuesta por Fe-prot (A y C) y Fe-Mo prot (B y D). 1: Ácido Homocítrico, 2: Grupo Fe-Mo-S, 3: Ca^{2+} y 4: Grupo Fe-S.

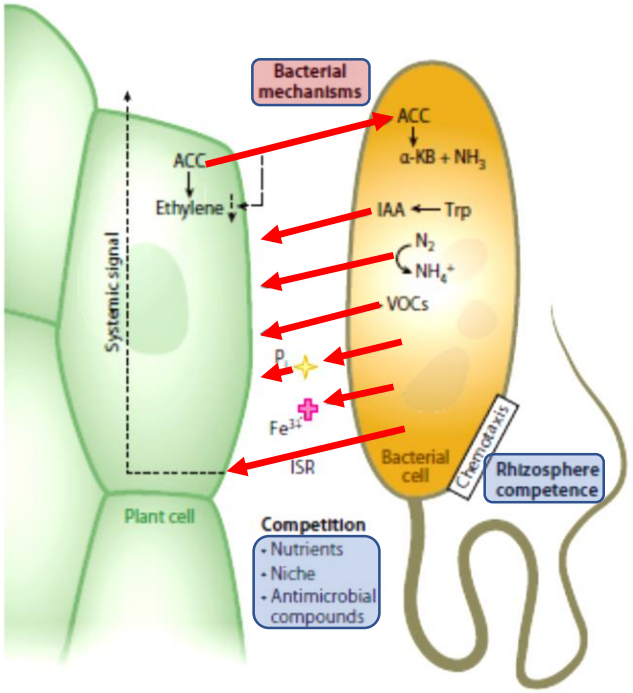
Indolpiruvato Descarboxilasa – *ipdC/ppdC*



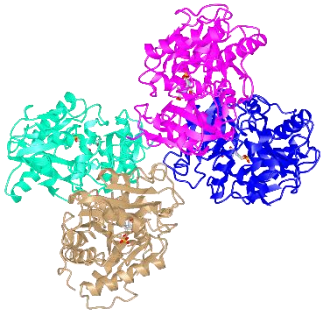


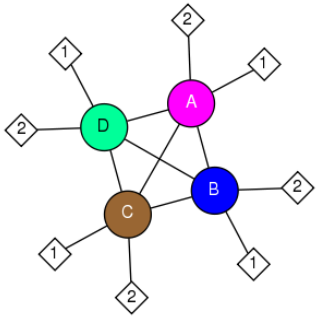


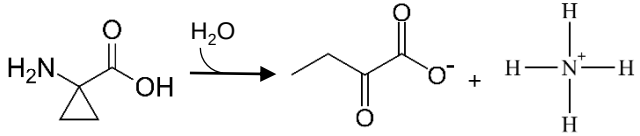
Triptófano — Indol Piruvato — Indol-3-acetaldehído — Ácido Indolacético



ACC Desaminasa - *acdS*

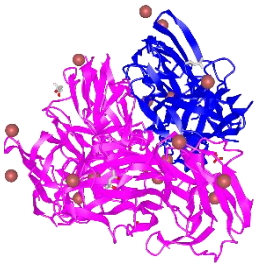


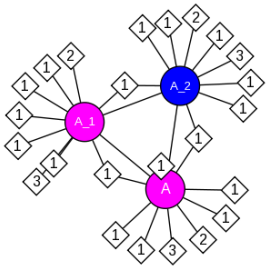




ACC — α-cetobutirato — Amonio

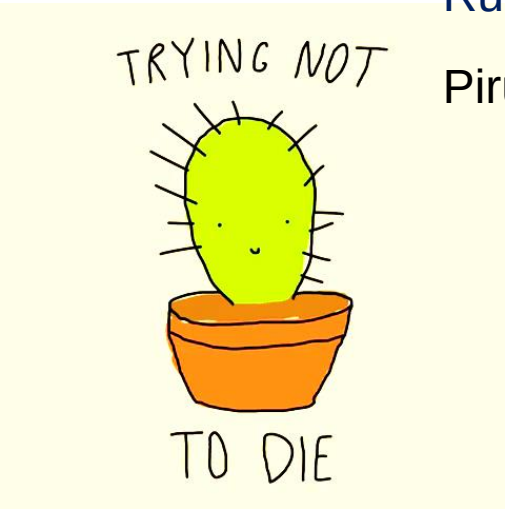
Nitrito Reductasa - *nirK*





NO_2^- ⟶ NO

Respuesta Sistémica Adquirida



Ruta 2-3-butanodiol

Piruvato

→ Acetolactato Sintasa - *budB*

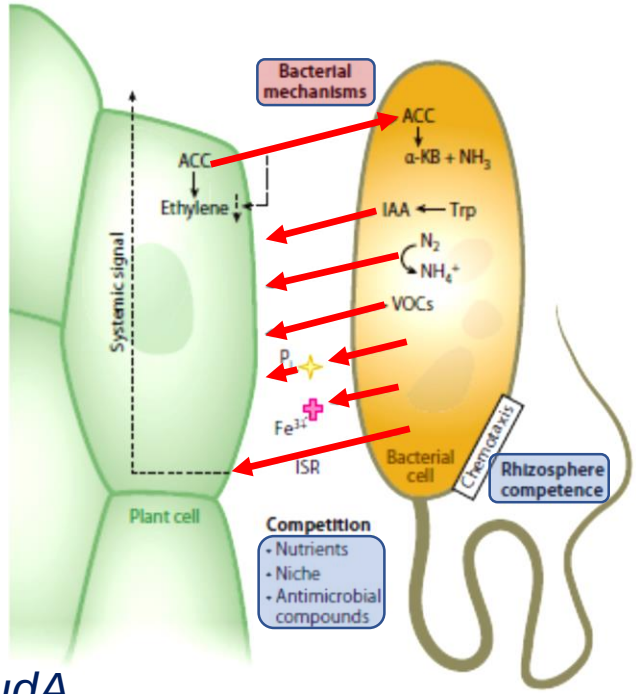
2-acetolactato

→ Acetolactato Descarboxilasa - *budA*

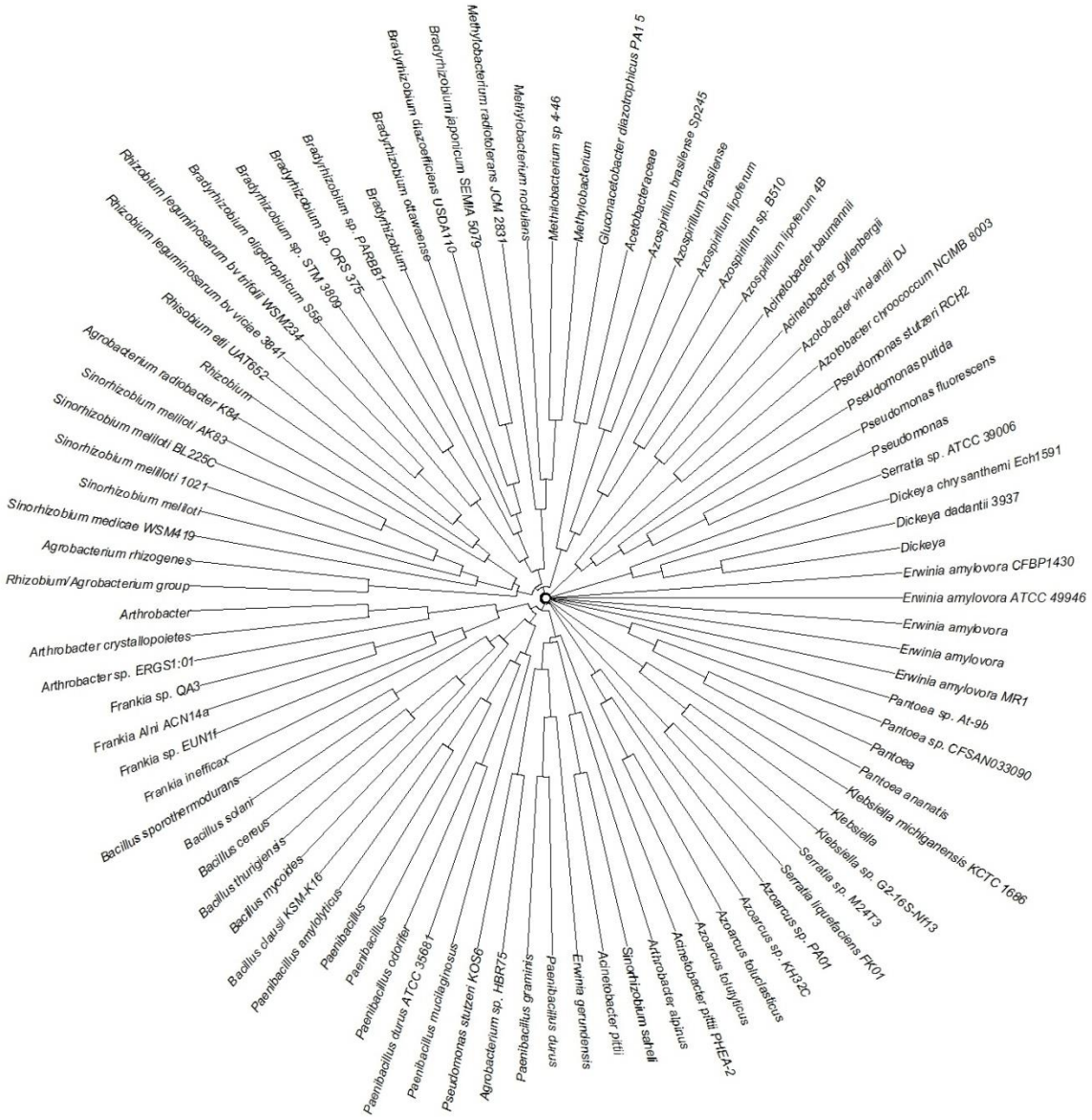
acetoína

→ Diacetil Reductasa - *budC*

2,3-butanodiol



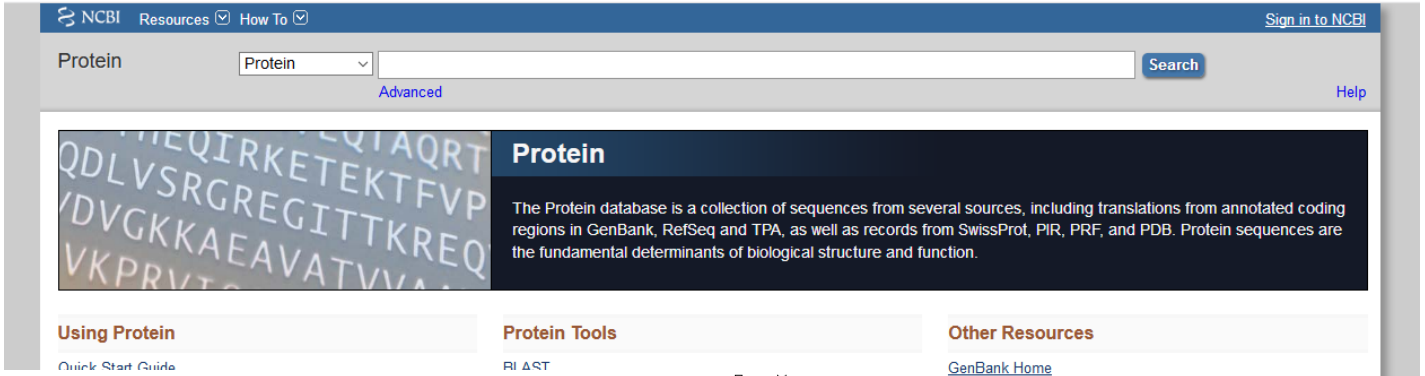
Capítulo 2. Relación filogenética de PGPRs

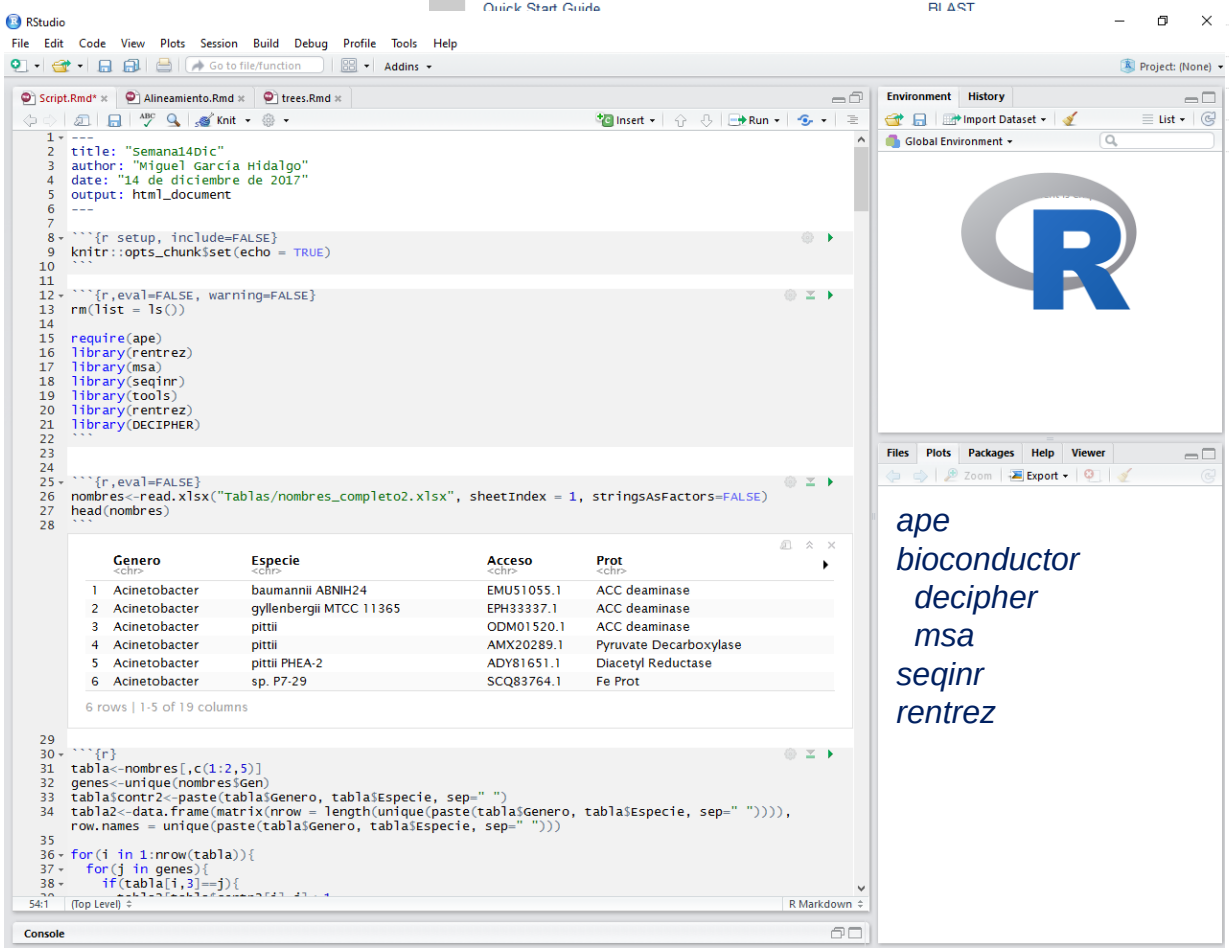


Materiales y métodos

Firmicutes	α Proteobacteria	β Proteobacteria	γ Proteobacteria	Gram + Alto [G+C]
15	34	4	36	11

Cepa	nifH	nifD	nifK	ipdC/ppdC	acdS	nirK	budA	budB	budC	Autor
Acinetobacter baumannii ABNIH24					+					Snitkin,E.S.
Acinetobacter gyllenbergii MTCC 11365					+					Singh,N.K.
Acinetobacter pittii				+	+					Brasiliense,D.M.
Acinetobacter pittii PHEA-2									+	Zhan,Y.
Acinetobacter sp. P7-29	+									Arshad,M.
Acinetobacter sp. P8-1	+									Arshad,M.
Acinetobacter sp. P8-9	+									Arshad,M.
Agrobacterium rhizogenes					+	+				Xiang,T.
Agrobacterium sp.	+									Jha,B.
Agrobacterium sp. P7-7	+									Arshad,M.
Agrobacterium sp. SUL3				+	+	+				Jones,K.J.
Agrobacterium tumefaciens	+				+	+				Poret-Peterson,A.T.
Arthobacter sp. PAMC 25486				+						Jung,J.-H.
Arthrobacter alpinus					+					See-Too,W.S.
Arthrobacter crystallopoietes BAB-32					+					Joshi,M.N.
Arthrobacter sp. CCBAU 10948	+									Luo,W.
Arthrobacter sp. ERGS1:01					+					Kumar,R.
Arthrobacter subterraneus						+				Varghese,N.
Azoarcus sp. KH32C	+	+	+	+		+				Nishizawa,T.
Azoarcus sp. PA01]				+						Junghare,M.
Azoarcus toluclasticus	+			+						NP*
Azoarcus tolulyticus	+	+	+	+						Varghese,N.
Azospirillum brasilense					+	+			+	NP*
Azospirillum brasilense Sp245	+	+	+	+		+				Pothier,J.F.
Azospirillum lipoferum				+	+	+				NP*
Azospirillum lipoferum 4B	+	+	+						+	Wisniewski-Dye,F.
Azospirillum sp. B510	+	+	+		+	+				Kaneko,T.
Azotobacter chroococcum NCIMB 8003	+	+	+		+					Robson,R.L.
Azotobacter vinelandii DJ	+	+	+		+					Setubal,J.C.
Bacillus cereus				+	+		+		+	Hall,Neil.
Bacillus mycoides					+		+			Bleich,R.M.





National Center for Biotechnology Information
NCBI

CLUSTALW

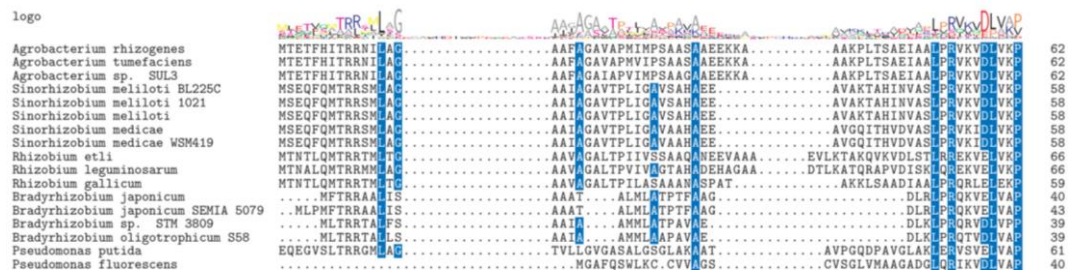
$$V_{n,2} = \frac{n!}{2 * (n-2)!}$$

Feng y Doolitle

$$S_{iden(ij)} = \frac{S_{real(ii)} + S_{real(jj)}}{2}$$

$$S_{eff(ij)} = \frac{S_{real(ij)} + S_{rand(ij)}}{S_{iden(ij)} + S_{rand(ij)}} * 100$$

$$D_{ij} = -lnS_{eff(ij)}$$

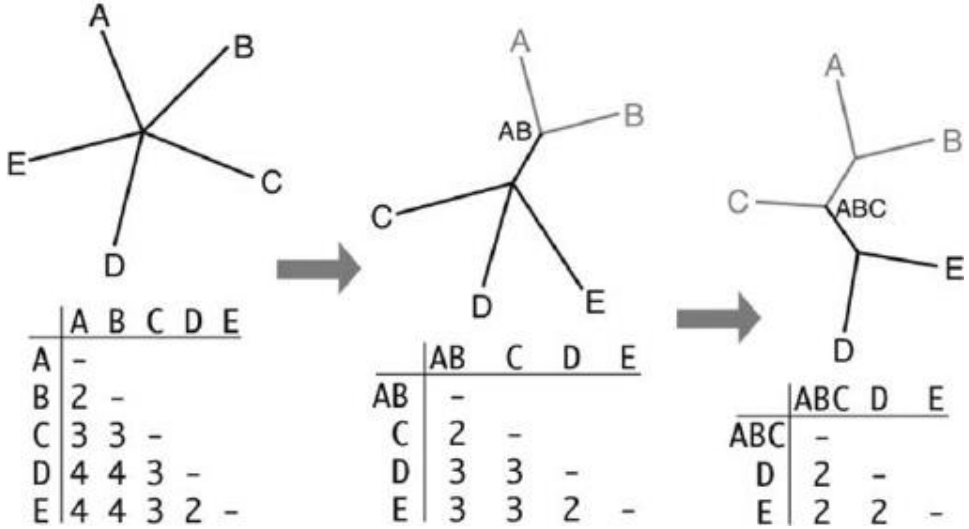


Jukes-Cantor

$$p' = \frac{p}{N}$$

$$d = -\frac{s-1}{s} ln\left(1 - \frac{s}{s-1}p'\right)$$

Neighbour Joining



	A	B	C	D	E
A	-				
B	2	-			
C	3	3	-		
D	4	4	3	-	
E	4	4	3	2	-

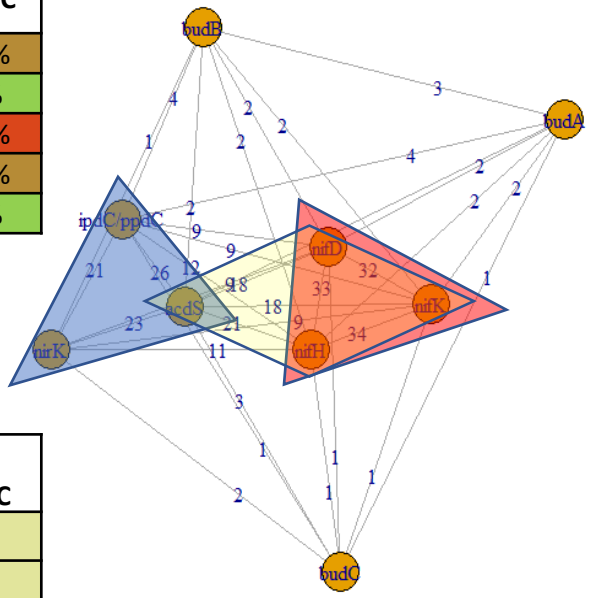
	AB	C	D	E
AB	-			
C	2	-		
D	3	3	-	
E	3	3	2	-

	ABC	D	E
ABC	-		
D	2	-	
E	2	2	-

Resultados

Presencia por gen de estudio

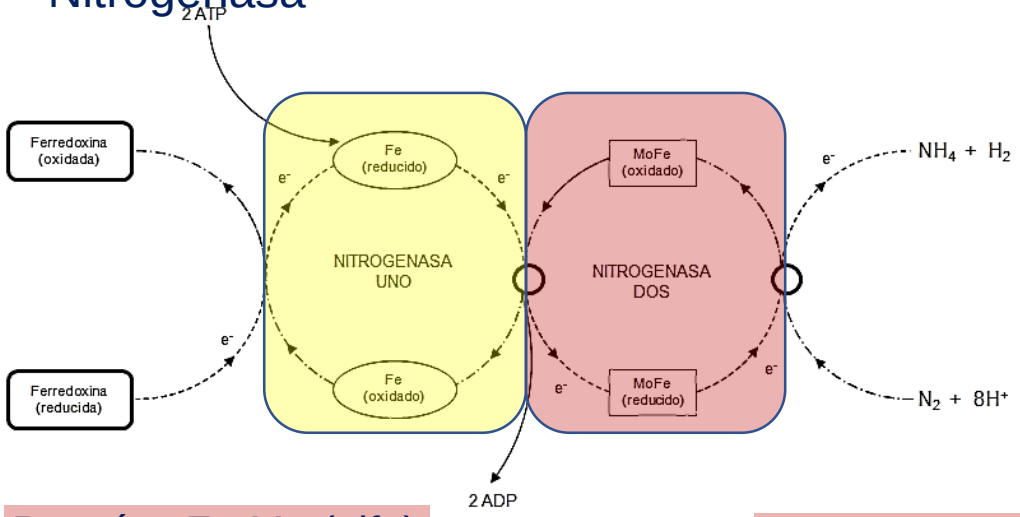
Grupo	nifH	nifD	nifK	ipdC / ppdC	acdS	nirK	budA	budB	budC
Alfaproteobacteria	41%	47%	45%	36%	45%	70%	11%	25%	25%
Betaproteobacteria	7%	6%	6%	10%	0%	4%	0%	0%	0%
Gammaproteobacteria	32%	22%	24%	36%	34%	11%	56%	75%	50%
Firmicutes	11%	16%	15%	13%	6%	11%	33%	0%	25%
Gram+alto [G+C]	9%	9%	9%	5%	15%	4%	0%	0%	0%



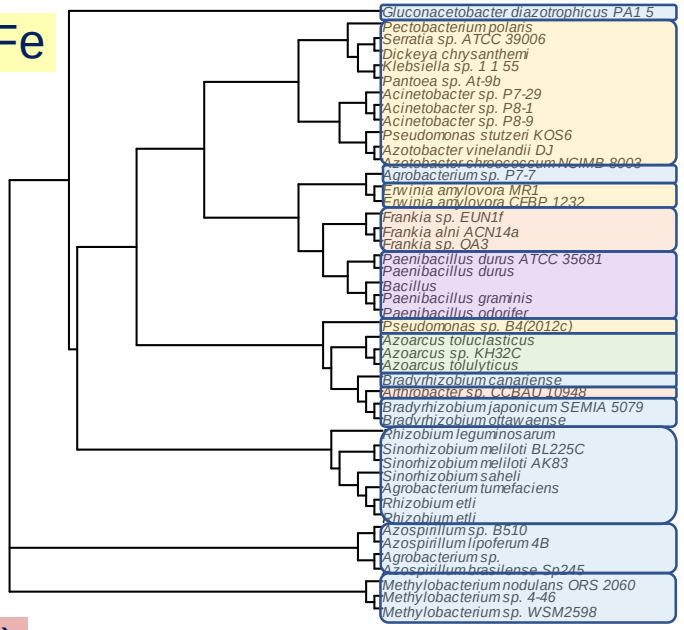
Coocurrencia de genes

Gen	nifH	nifD	nifK	ipdC / ppdC	acdS	nirK	budA	budB	budC
nifH	**44**	33	34	12	21	11	2	2	1
nifD	33	**32**	32	9	18	9	2	2	1
nifK	34	32	**33**	9	18	9	2	2	1
ipdC/ppdC	12	9	9	**39**	26	21	4	4	1
acdS	21	18	18	26	**47**	23	3	2	3
nirK	11	9	9	21	23	**27**	0	1	2
budA	2	2	2	4	3	0	**9**	3	1
budB	2	2	2	4	2	1	3	**8**	0
budC	1	1	1	1	3	2	1	0	**8**

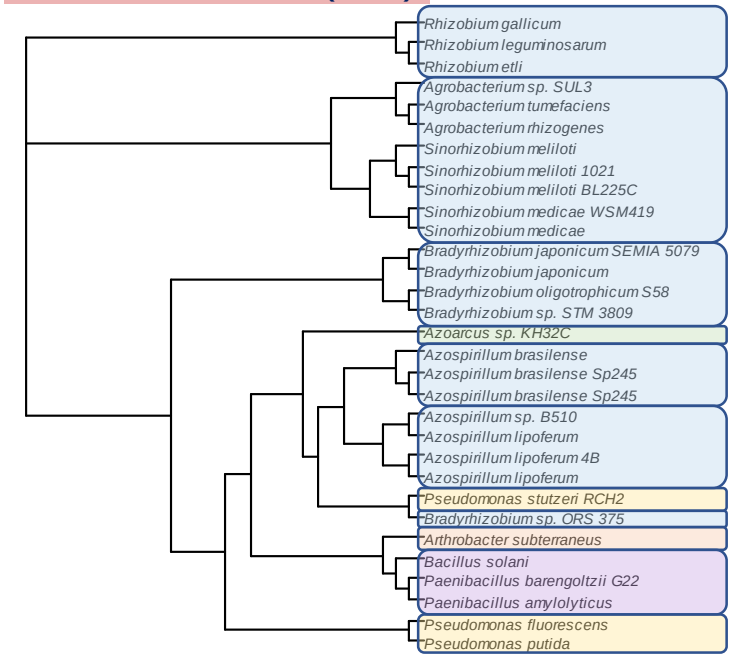
Nitrogenasa



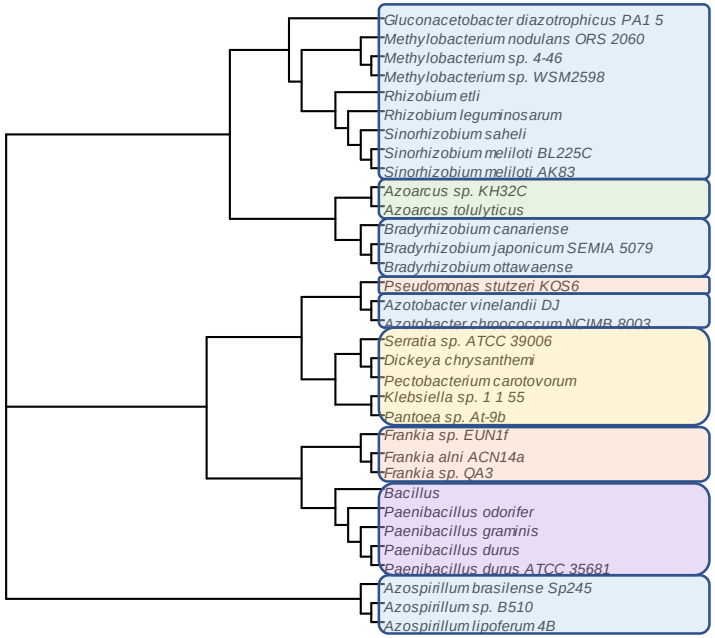
Proteína Fe



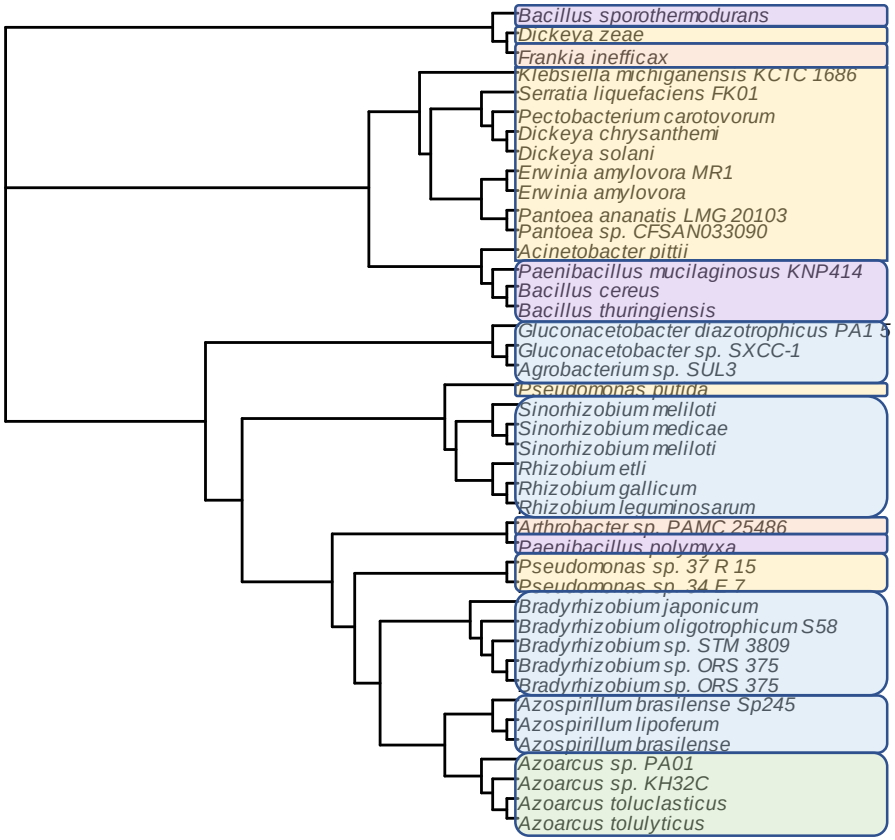
Proteína Fe-Mo (alfa)



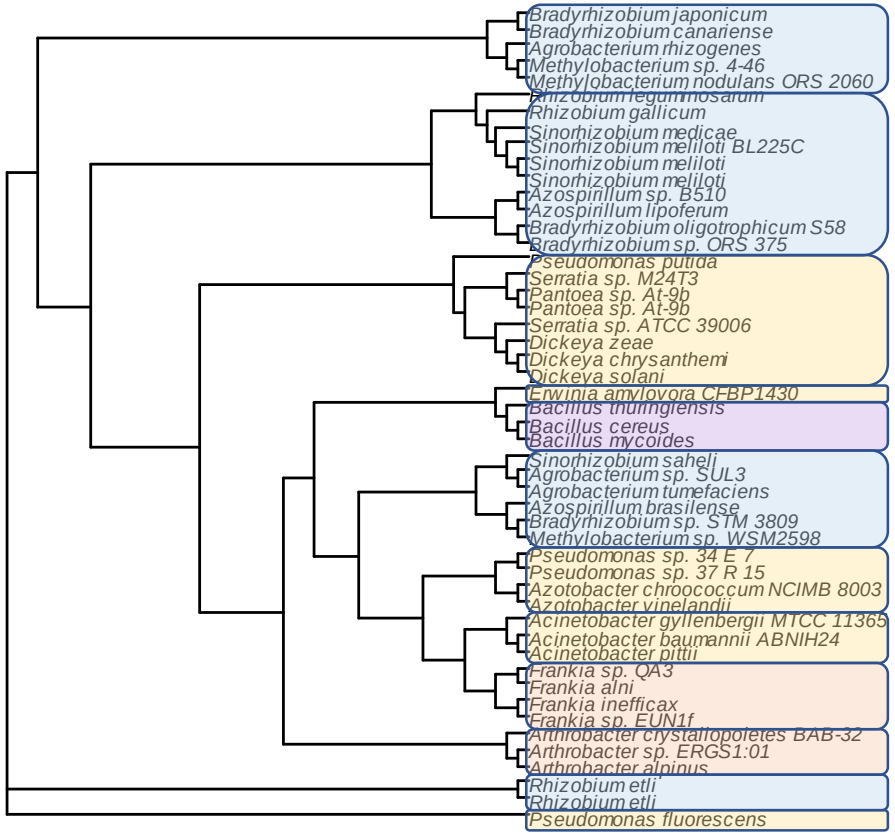
Proteína Fe-Mo (beta)



Indolpiruvato Descarboxilasa

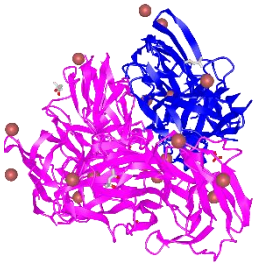


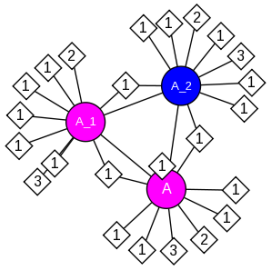
ACC Desaminasa

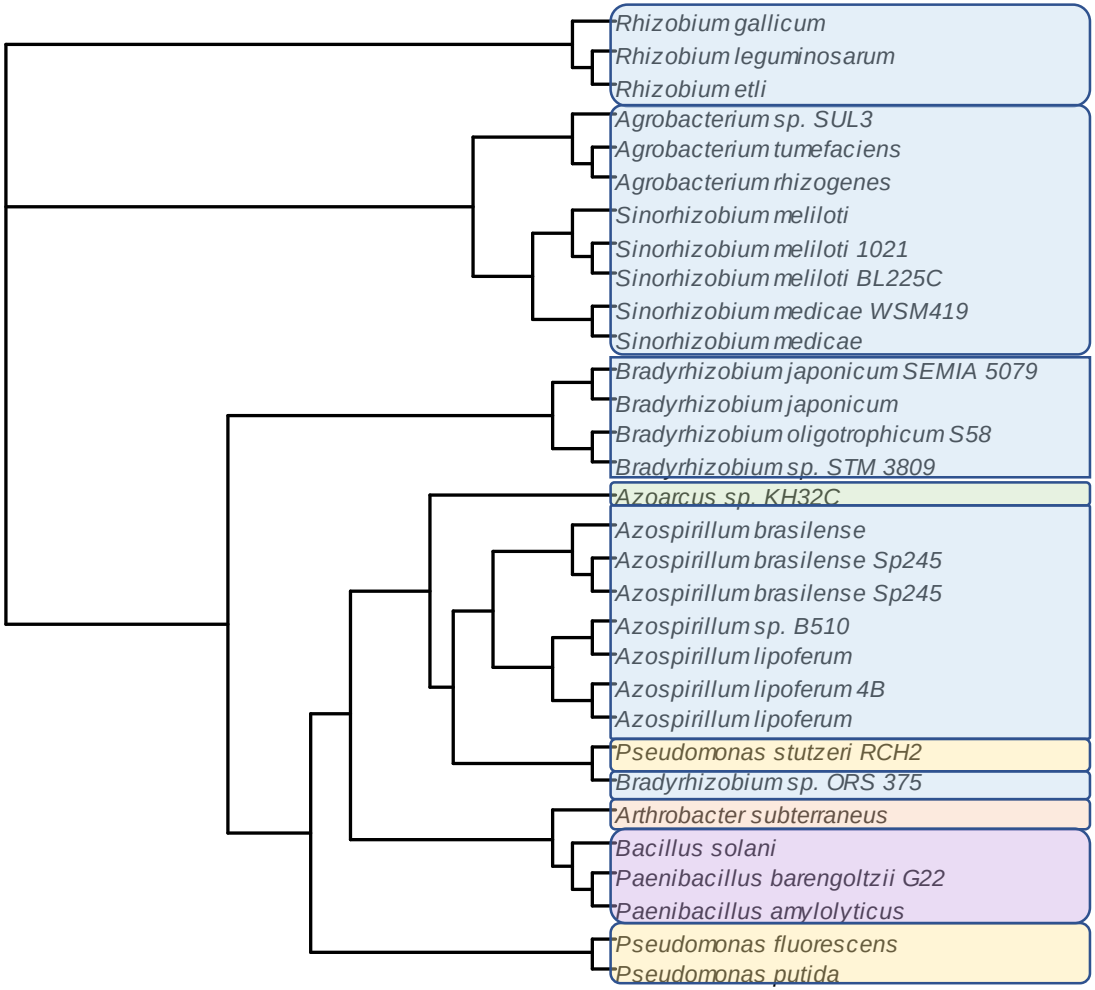


Nitrito Reductasa

NO_2^- ⟶ NO

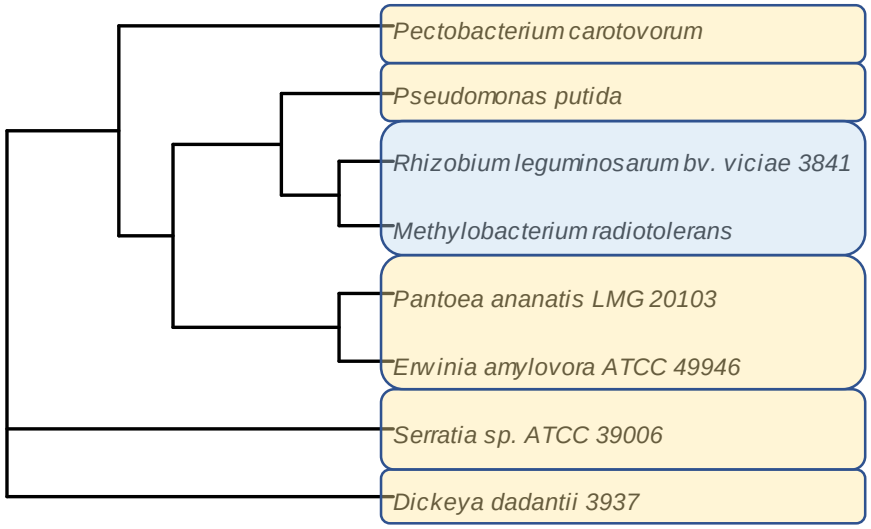




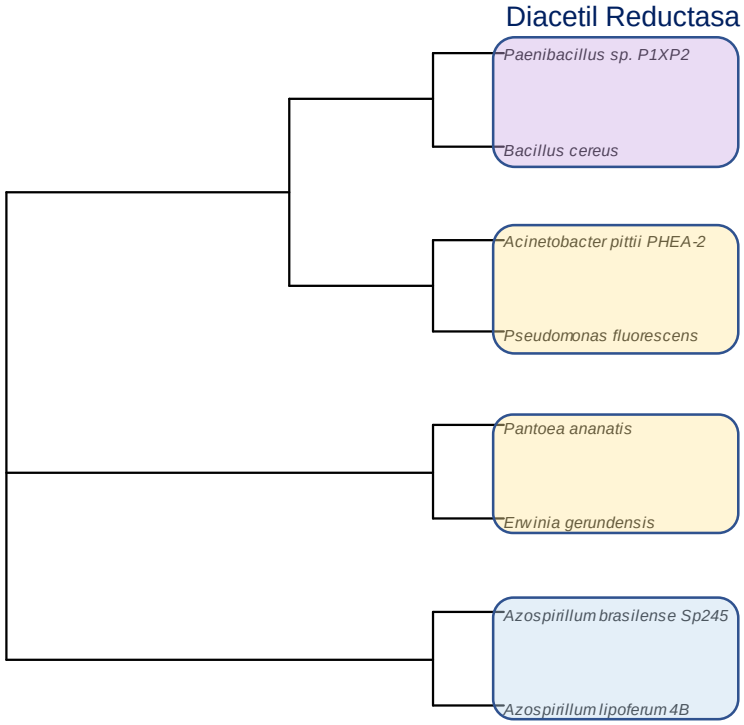


Ruta de 2-3-butanodiol

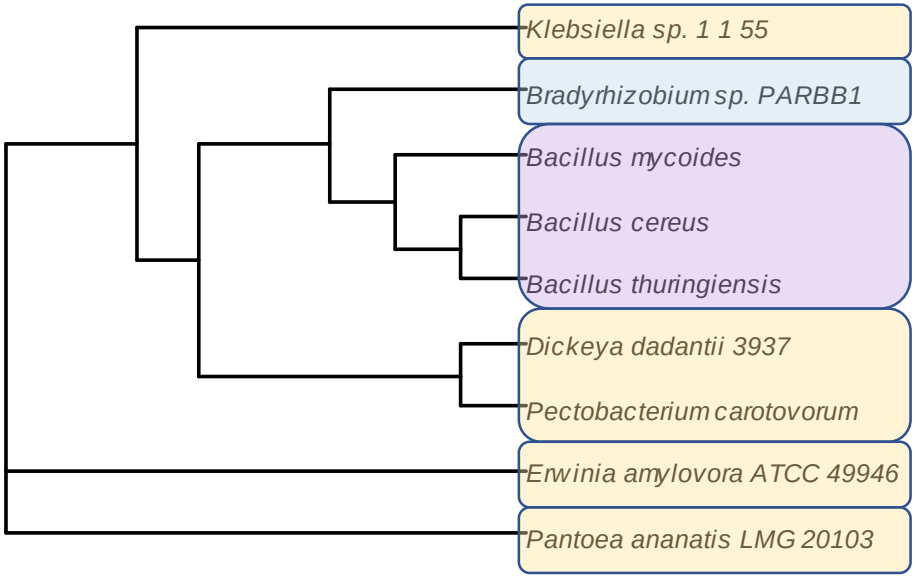
Acetolactato Sintasa



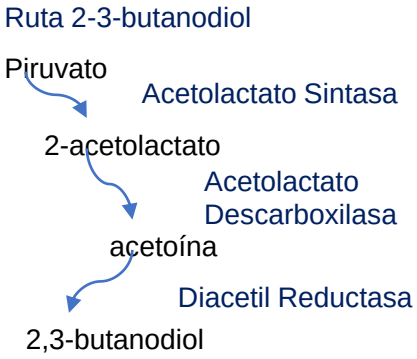
Diacetil Reductasa



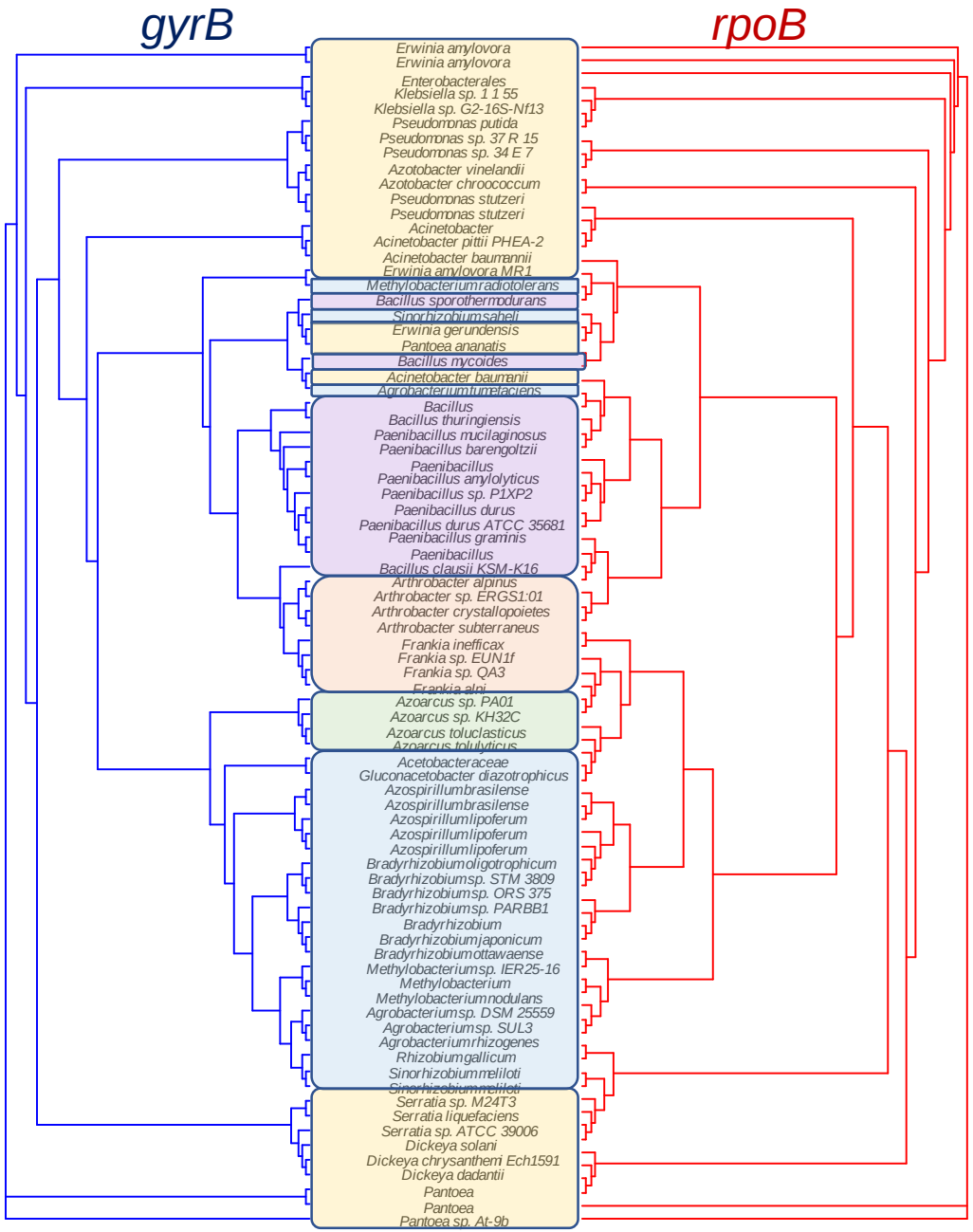
Acetolactato Descarboxilasa



Ruta 2-3-butanodiol



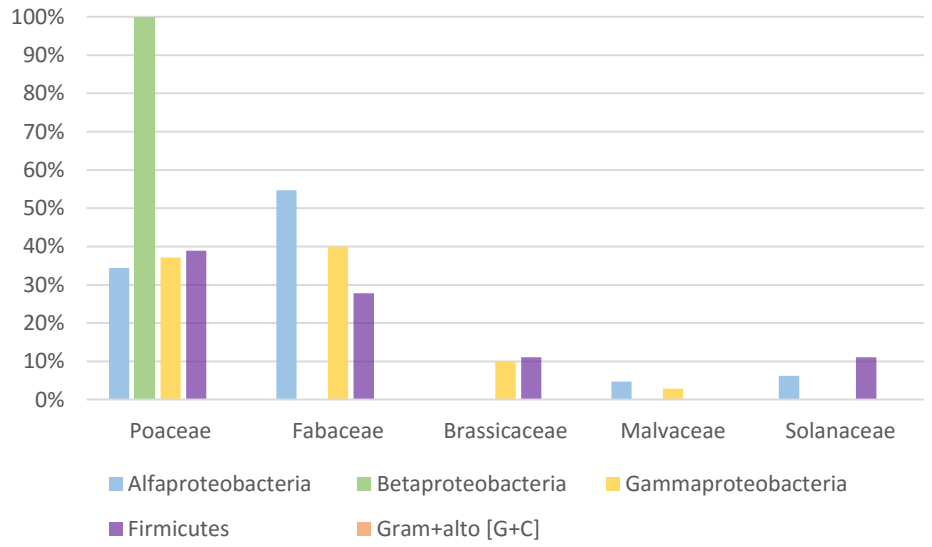
Marcadores filogenéticos



Capítulo 3. Estudio combinado de las relaciones entre mecanismos promotores del crecimiento vegetal, diversidad de PGPRs y cultivos

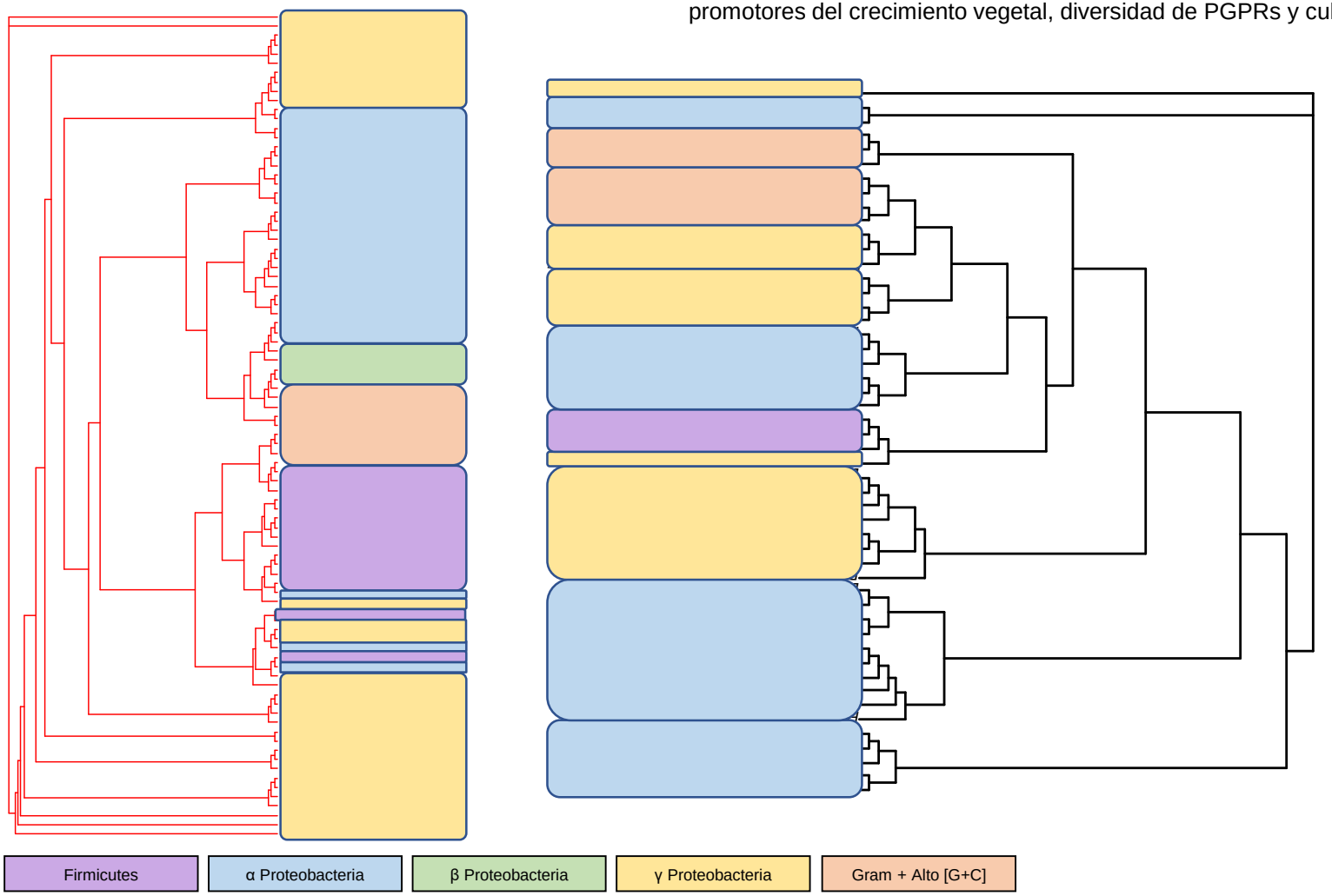
Materiales y métodos

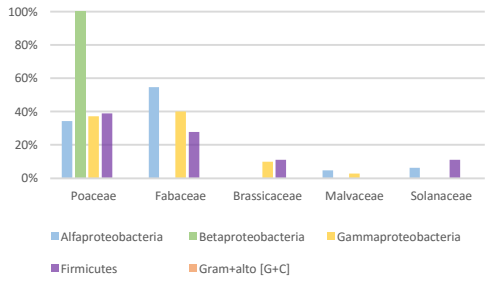
	Arroz	Maíz	Judía Garbanzo	Alcachofa	Brassica	Algodón	Trigo	Cebada	Tomate	Cacahuete	Soja
Alfaproteobacteria	5	13	27	0	0	3	0	4	4	0	8
Betaproteobacteria	4	0	0	0	0	0	0	0	0	0	0
Gammaproteobacteria	7	10	9	0	7	2	9	0	0	7	12
Firmicutes	15	0	0	0	6	0	0	6	6	0	15
Gram+alto [G+C]	0	0	0	0	0	0	0	0	0	0	0



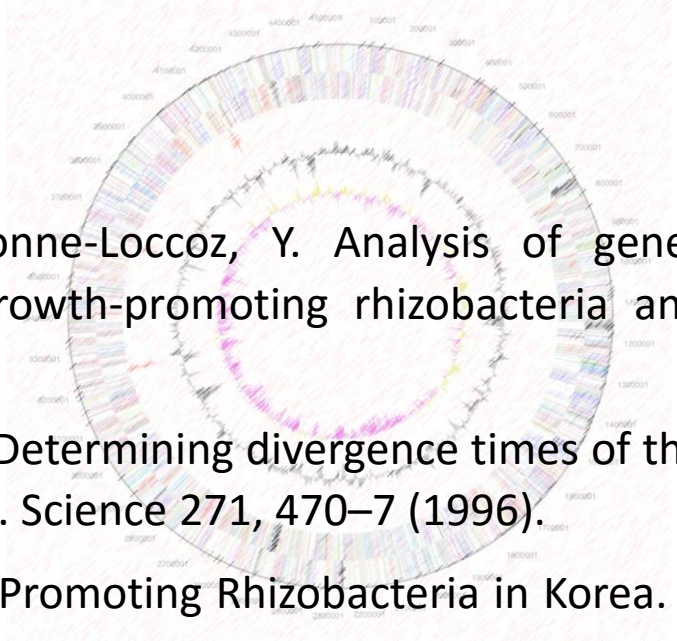
De Souza;Vessey 2003; Ahemad 2014; Pérez-Montaño 2014 y Nadeem 2014

Resultados


Firmicutes
α Proteobacteria
β Proteobacteria
γ Proteobacteria
Gram + Alto [G+C]


100%
80%
60%
40%
20%
0%
Poaceae
Fabaceae
Brassicaceae
Malvaceae
Solanaceae
Alfaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Firmicutes
Gram+alto [G+C]

Bibliografía



- Bruto, M., Prigent-Combaret, C., Muller, D. & Monne-Loccoz, Y. Analysis of genes contributing to plant-beneficial functions in plant growth-promoting rhizobacteria and related Proteobacteria. Sci. Rep. 4, 1–10 (2014).
- Doolittle, R. F., Feng, D. F., Tsang, S., Cho, G. & Little, E. Determining divergence times of the major kingdoms of living organisms with a protein clock. Science 271, 470–7 (1996).
- Kim, W.-I. Genetic Diversity of Cultivable Plant Growth-Promoting Rhizobacteria in Korea. J. Microbiol. Biotechnol. 21, 777–790 (2011).
- Larkin, M. A. et al. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948 (2007).
- NCBI Resource Coordinators. Database Resources of the National Center for Biotechnology Information. Nucleic Acids Res. 45, D12–D17 (2017).
- R Core Team. R: A Language and Environment for Statistical Computing. (2017).
- Shailendra Singh, G. G. Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture. J. Microb. Biochem. Technol. 7, 96–102 (2015).

Diversidad de genes bacterianos relacionados con promoción del crecimiento vegetal

TRABAJO FIN DE MÁSTER

Enero 2018

Miguel García Hidalgo