#### CARRERA DE ESPECIALIZACIÓN EN ESTERILIZACIÓN

#### ASIGNATURA: MICROBIOLOGÍA APLICADA

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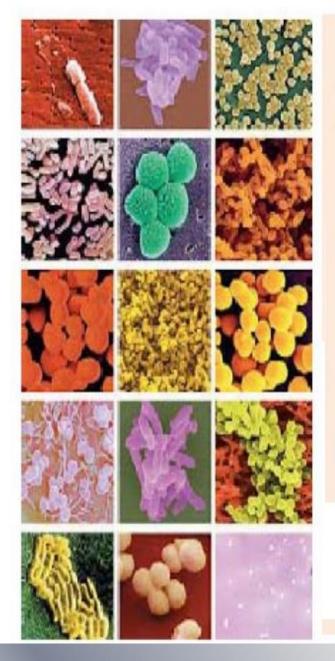


Clase 1 2018 Rosario



# MICROBIOLOGÍA

Deriva de 3 palabras griegas: mikros (pequeño), bios (vida) y logos (ciencia) que conjuntamente significan el estudio de la vida microscópica



"La ciencia encargada del estudio de los microorganismos tan pequeños cuya visualización requiere del microscopio" abarca:

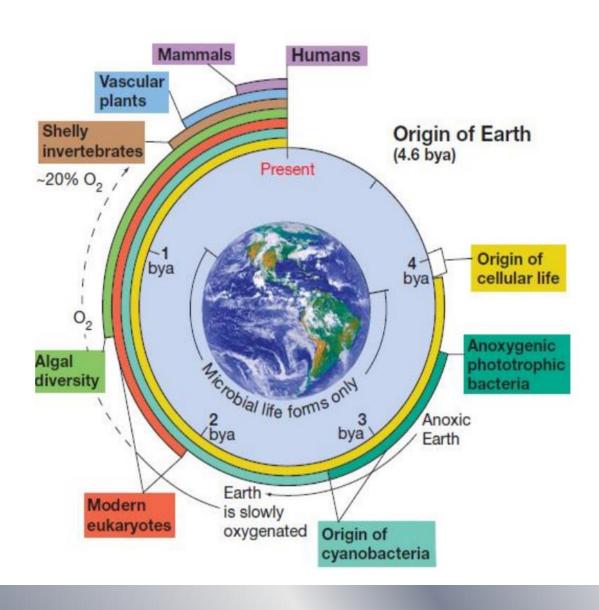
partículas no celulares como los virus, viroides y priones, hasta organismos celulares tan diferentes como las bacterias, los protozoos y parte de las algas y de los hongos.

## ÁMBITO DE LA MICROBIOLOGÍA

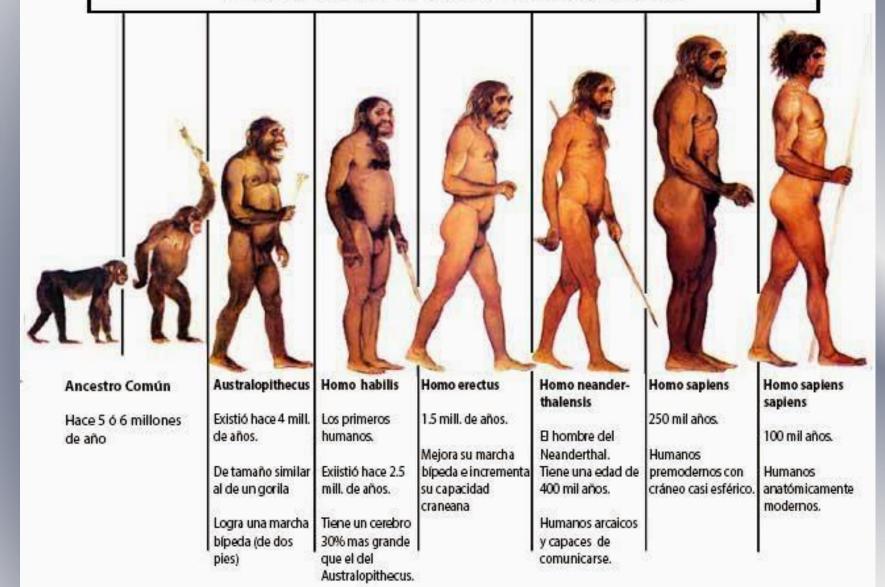
- Todo el ecosistema depende de las bacterias. Éstas influyen en la sociedad humana de muchas formas.
- Repercusión sobre: medicina, agronomía, bromatología, ecología, genética, bioquímica...
- Ocupaciones de un microbiólogo:
- Microbiología médica: identificación del agente causal de una enfermedad infecciosa y planificar medidas para eliminarlo. Descubrir nuevos patógenos, y los mecanismos por los cuales actúan.
- Microbiólogo de Salud Pública: controlar la extensión de enfermedades infecciosas.
- ➤ Inmunólogo: estudiar cómo el sistema inmune actúa frente a agentes patógenos.

- Microbiólogo agrícola: estudia efectos de los microorganismos sobre la agricultura. Trabajan en combatir enfermedades de las plantas que atacan a los cultivos alimentarios. Trabajan en métodos que aumentan fertilidad del suelo. Biopesticidas.
- Ecólogo microbiano: estudian relaciones entre microorganismos y sus hábitats. Tratamiento biológico de basuras.
- Microbiólogo de alimentos: evitar putrefacción de los alimentos y transmisión de enfermedades alimentarias, elaboración de productos por medio de microorganismos (pan), mejorar procesos industriales (conservantes biológicos).
- Microbiólogo industrial: elaboración de antibióticos, vacunas, vitaminas, aminoácidos, liberación minerales valiosos...
- Microbiólogos de investigación básica: fisiología, bioquímica, genética, biología molecular...
- MUCHAS OTRAS INCUMBENCIAS...

# Vida en la Tierra a traves del tiempo



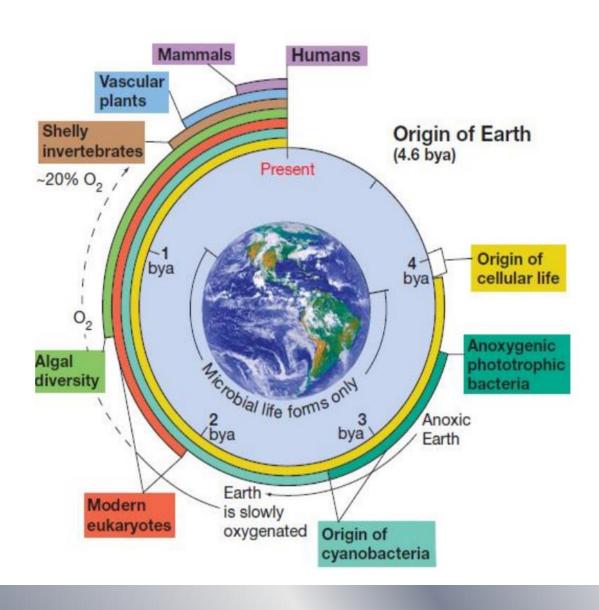
## LA EVOLUCIÓN DEL HOMBRE



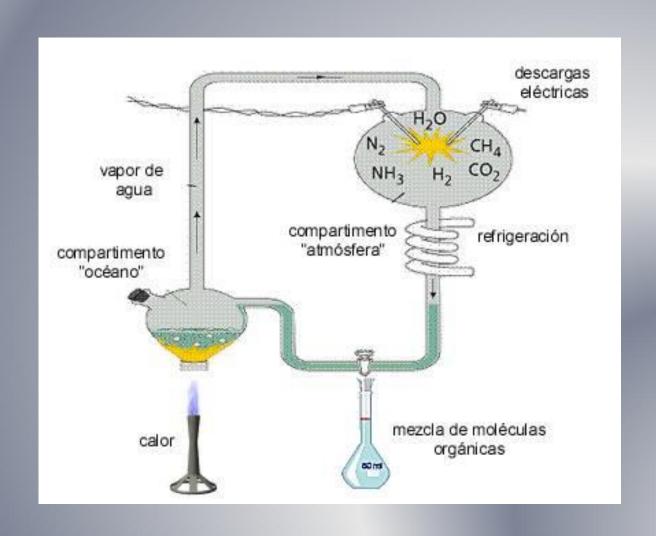
## TEORÍA DE OPARÍN Y HALDANE

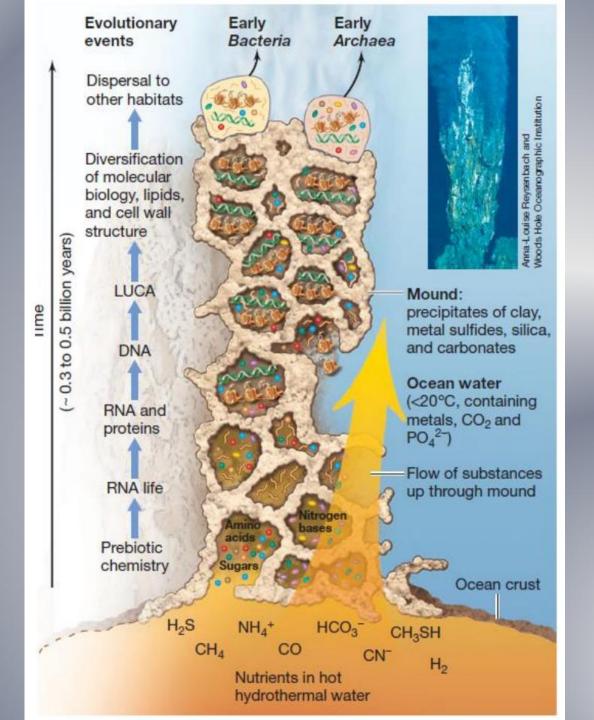


# Vida en la Tierra a traves del tiempo



#### TEORÍA DE OPARÍN Y HALDANE

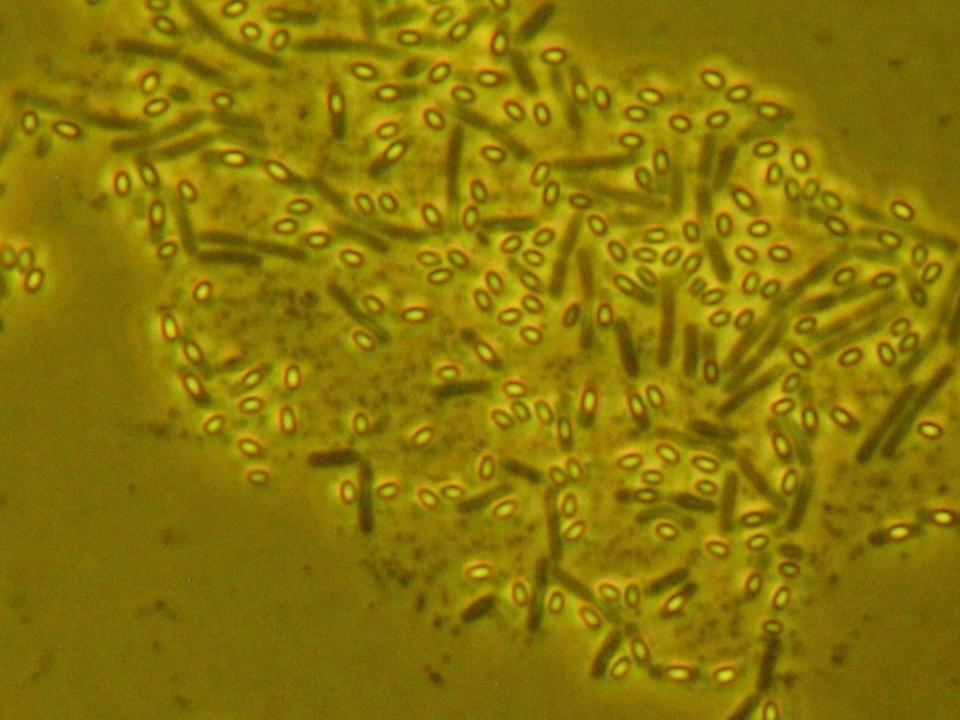


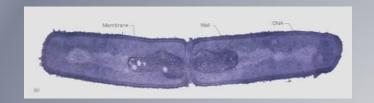


# Estromatolitos en Socompa (Salta)



# OTRA HIPÓTESIS LA LITOPANSPERMIA





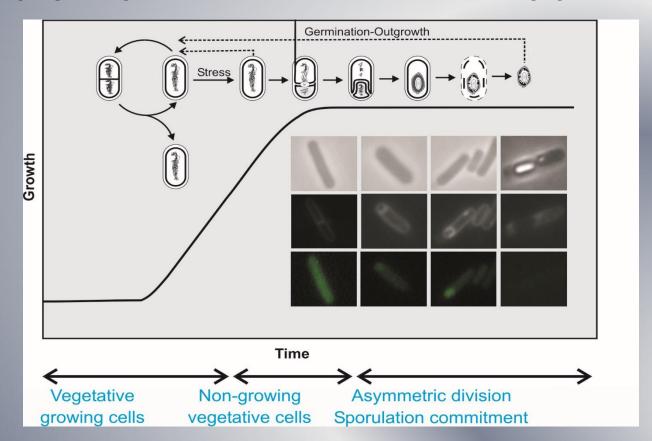


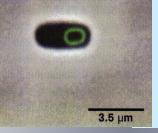


**CÉLULA VEGETATIVA** 

**DESARROLLO** 

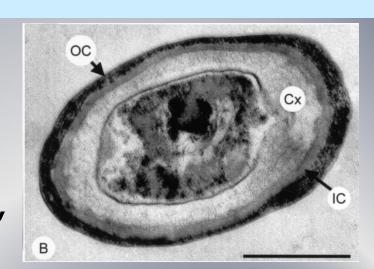
**ESPORA** 



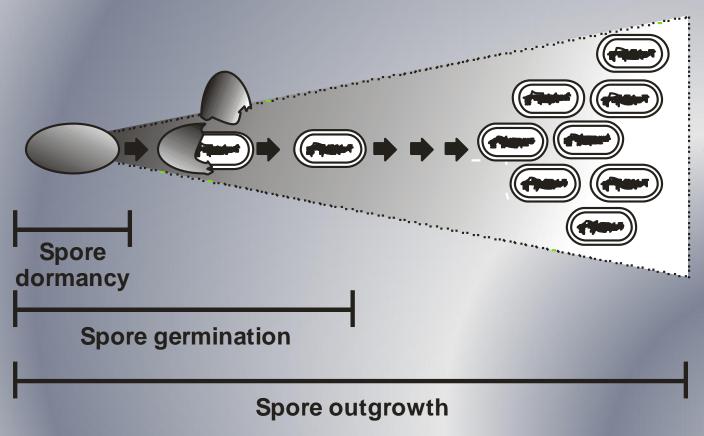


#### **ALGUNAS PROPIEDADES DE LAS ESPORAS BACTERIANAS**

- > Resistencia a las temperaturas extremas,
- > Resistencia a la desecación y la presión,
- > Resistencia a los cambiosa de pH y salinidad,
- Resistencia a la radiación UV y solar,
- > Resistencia a los procesos industriales de fabricación,
- > Resistencia a los ataques por enzimas y detergentes,
- > 100% durmientes, metabolismo cero, agua cero, actividad enzimática cero,
- > Alta longevidad (las esporas bacterianas son prácticamente inmortales e indestructibles)



## GERMINACIÓN, MULTIPLICACIÓN Y DISEMINACIÓN



**SPORE PANSPERMIA** 



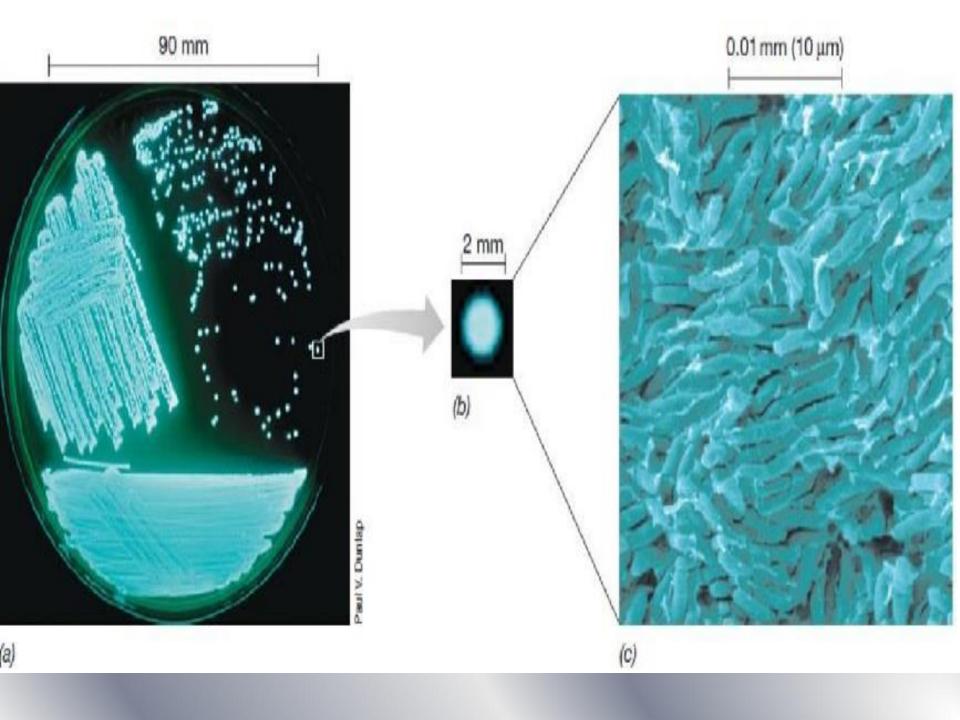
#### Vida en la Tierra a traves del tiempo Mammals Humans Vascular plants Shelly Origin of Earth invertebrates (4.6 bya) Present ~20% O. ARRIBO DE LAS **ESPORAS** bya 02 Anoxygenic Algal phototrophic diversity Tobial life forms only pacteria Anoxic bya bya Earth Earth + Modern is slowly eukaryotes oxygenated Origin of cyanobacteria

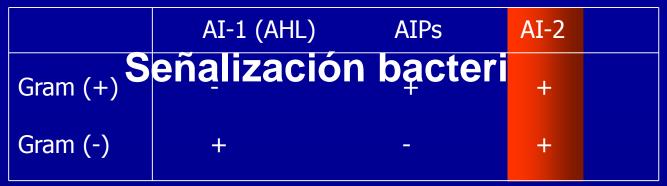
SEA CUAL SEA LA TEORÍA MÁS ACERTADA

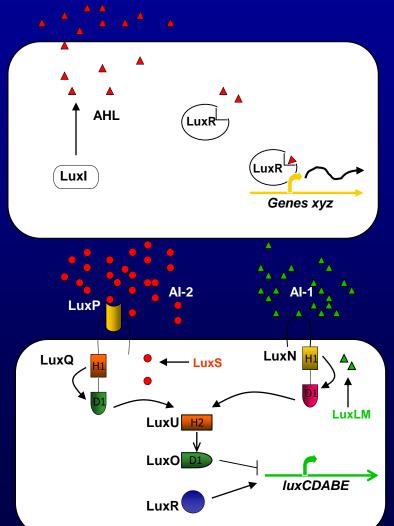
LOS MICROBIOS, PARECIERA SER, ESTUVIERON PRIMERO

Un cultivo puro es aquel en que todos los microorganismos provienen de una sola célula









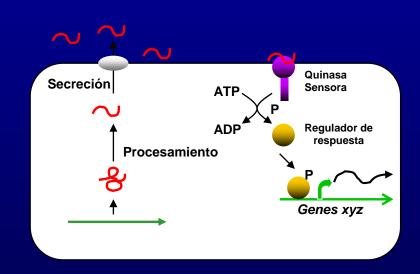


Table 1. Phylogenetic association of known AHL systems

Class	Order	No. families with AHL system/ total families	No. genera with AHL system/ total genera	No. species with AHL system*
Alpha Proteobacteria	Rhodospirillales	0/2	0/24	0
	Rickettsiales	0/3	0/17	0
	Rhodobacterales	1/1	2/23	2
	Sphingomonadales	0/1	0/9	0
	Caulobacterales	0/1	0/4	0
	Rhizobiales	3/10	5/56	8
	All orders (6)	4/18 (22 %)	7/133 (5%)	
Beta Proteobacteria	Burkholderiales	2/5	2/33	3
	Hydrogenophilales	0/1	0/2	0
	Methylophilales	0/1	0/3	0
	Neisseriales	1/1	1/14	1
	Nitrosomonadales	1/3	1/4	1
	Rhodocyclales	0/1	0/8	0
	All orders (6)	4/12 (33 %)	4/56 (7%)	
Gamma Proteobacteria	Chromatiales	0/2	0/30	0
	Acidithiobacillales	0/2	0/2	0
	Zanthomonadales	0/1	0/9	0
	Cardiobacteriales	0/1	0/3	0
	Thiotrichales	0/3	0/15	0
	Legionellales	0/2	0/3	0
	Methylococcales	0/1	0/6	0
	Oceanspirillales	0/2	0/12	0
	Pseudomonadales	1/2	1/28	8
	Altermonadales	0/1	0/12	0
	Vibrionales	1/1	2/6	3
	Aeromonadales	1/2	1/7	2
	Enterobacteriales	1/1	6/41	14
	Pasteurellales	0/1	0/6	0
	All orders (14)	4/22 (18%)	10/180 (5%)	
Delta Proteobacteria	All orders (7)	0/18	0/55	0
Epsilon Proteobacteria	All orders (1)	0/2	0/6	0
Proteobacteria	All orders (34)	12/72 (17%)	21/438 (4%)	42

<sup>\*</sup>Note that lists of bacterial species with AHI. systems are notoriously difficult to keep up-to-date.











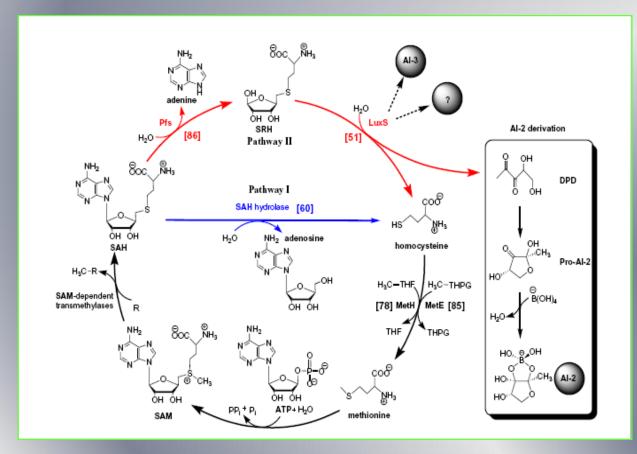


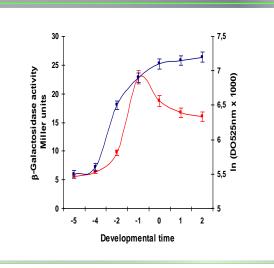


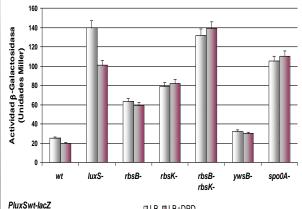


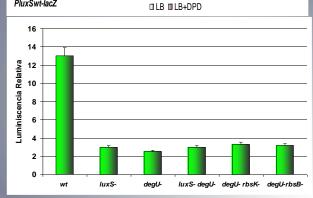
### **Social behavior and cell to cell communication**

	AI-1 (AHL)	AIPs	AI-2	
Gram (+)	-	+	+	
Gram (-)	+	-	+	









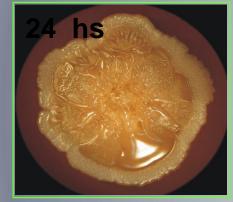
## AI-2, a morphogen like signal for intraspecific behavior





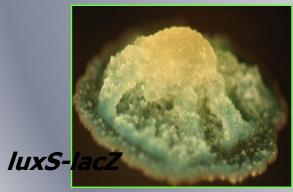


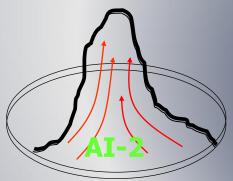


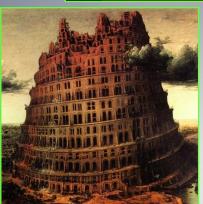




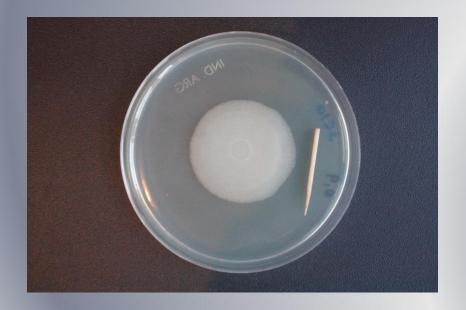






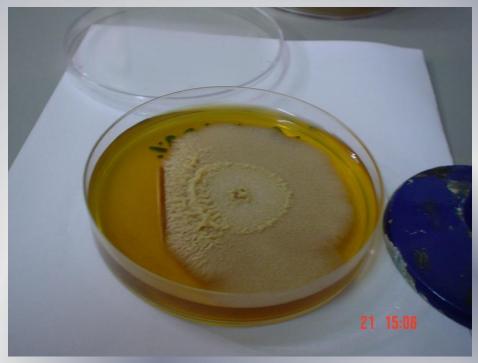










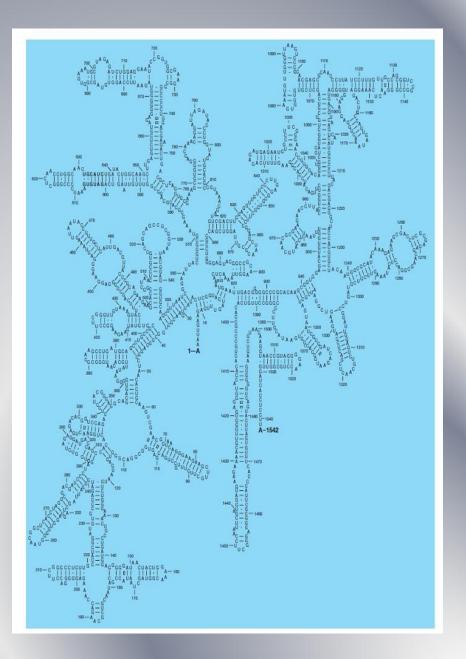


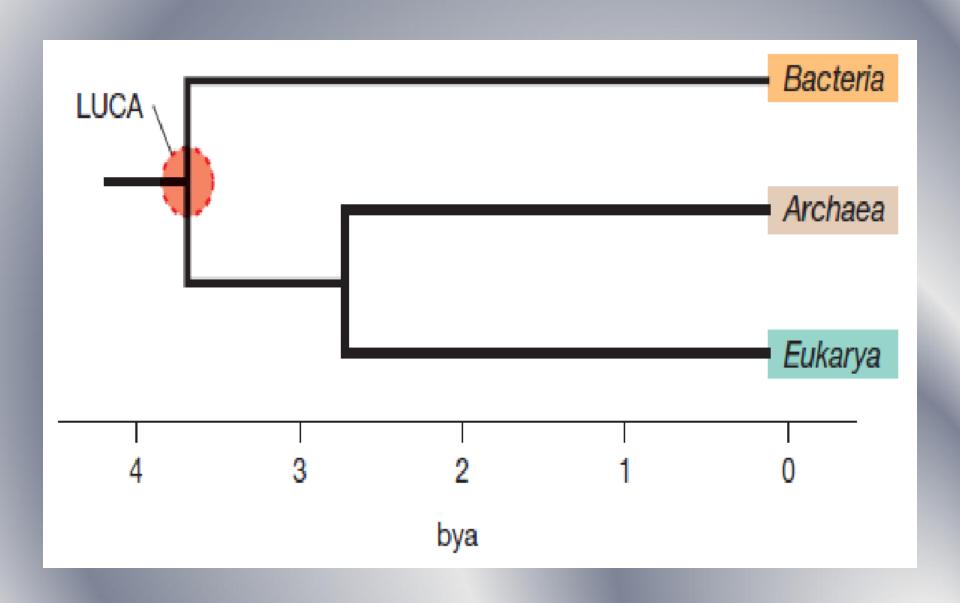
#### **CARL WOESE**

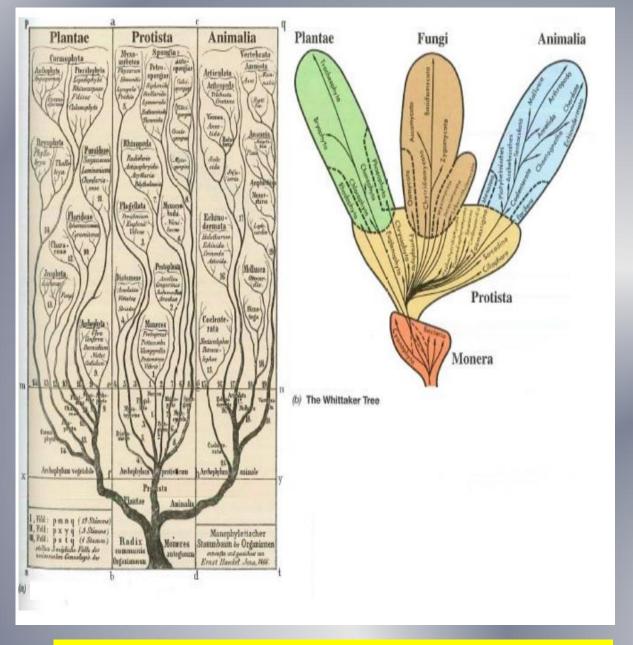


"It's clear to me that if you wiped all multicellular life-forms off the face of the earth, microbial life might shift a tiny bit ... If microbial life were to disappear, that would be it — instant death for the planet."

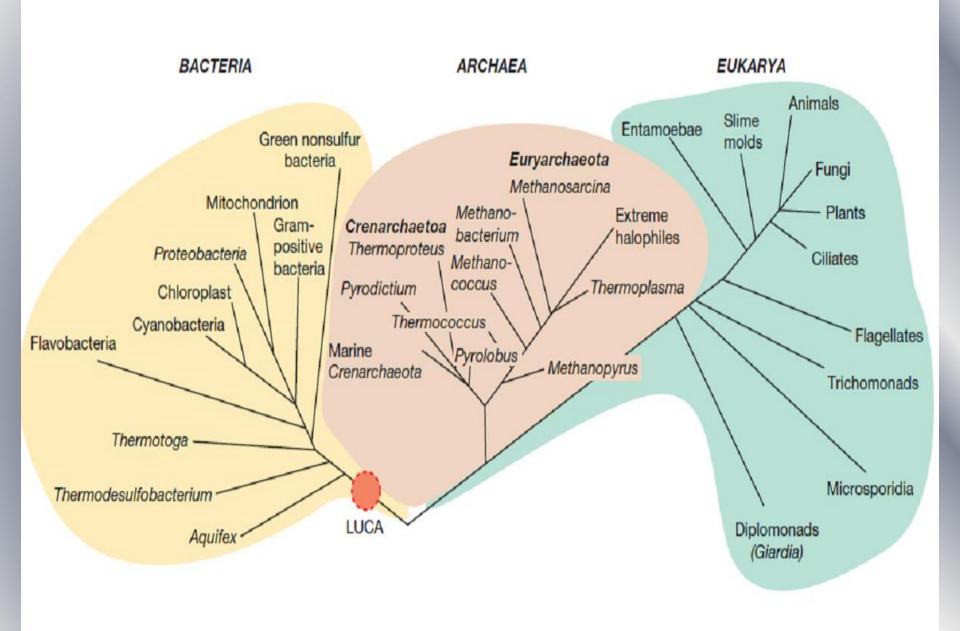
Carl Woese



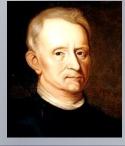




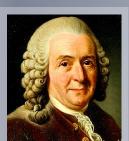
ESTE ÁRBOL ES ABANDONADO POR....



#### HISTORIA DE LA MICROBIOLOGÍA



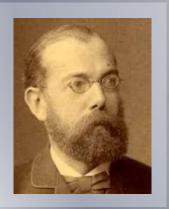
Robert Hooke [1665]
Primera observación de las células.



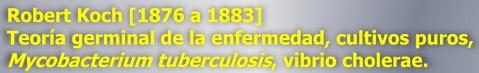
Anton van Leeuwenhoek [1673]
Primera observación de microorganismos vivos.

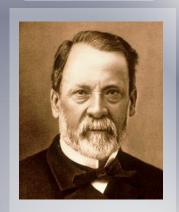


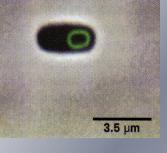
Carl Nilsson Linæus [1735]
Nomenclatura de los microorganismos.



Louis Pasteur [1857 a 1864]
Fermentación, desmoronamiento de la teoría de la generación espontánea, pasteurización.







#### FINES DEL SIGLO XVII DESCUBRIMIENTO DE LAS BACTERIAS

#### PADRE DE LA MICROBIOLOGÍA



Observations, coremunicated to the Publisher by Mr. Antony van Leewenhoeck, in a Dutch Letter of the 5th of Octob. 1676. here English'd: Goncerning little Animals by him observed in Rain-Well-Sea- and Snow water; as also in water wherein Pepper had lain insused.

Which had flood but few days in a new earthen pot, glafed blew within. This invited me to view this water with great attention, especially those little animals appearing to me ten thousand times less than those represented by Mons. Swamerdam, and by him called Water fleas or Water-lice, which may be per-

ceived in the water with the naked eye.

The first fort by me discover'd in the said water, I divers times observed to consist of 5, 6,7, or 8 clear globuls, without being able to differn any film that held them together, or contained them. When these animalcula or living Atoms did move . they put forth two little horns, continually moving themselves: The place between these two horns was flat, though the rest of the body was roundiff, sharpning a little towards the end, where they had a tayl, near four times the length of the whole body of the thickness (b. my Microscope) of a Spidery-web; at the end of which appear da globul, of the bignets of one of those which made up the body; which tay I could not perceive, even in very clear water, to be mov'd by them. These little creatures, if they chanced to light upon the least filament or string, or other fuch particle, of which there are many in water, especially after it hath flood some days, they flook intangled therein, extending their body in a long round, and striving to dis-intangle their tayl; whereby it came to pass, that their whole body lept back towards the globul of the tayl, which then rolled together Serpent-like, and after the manner of Copper- or Iron-wire that having been wound about a flick, and unwound again, retains those windings and turnings. This motion of extension and contraction continued a while; and I have feen feveral hundreds of these poor little creatures, within the space of a grain of gross fand, lye fast cluster'd together in a few filaments.

I also discover'd a second fort, the figure of which was oval; and I imagined their head to stand on the sharp end. These were a little bigger than the former. The inferior part of their body is flat, surnished with divers incredibly thin seet, which moved

SCLA

# AHORA EN EL SIGLO XXI SABEMOS QUE LAS BACTERIAS ESTÁN POR TODAS PARTES MALAS

#### Prokaryotes: The unseen majority

William B. Whitman\*†, David C. Coleman‡, and William J. Wiebe§

Departments of \*Microbiology, ‡Ecology, and §Marine Sciences, University of Georgia, Athens GA 30602

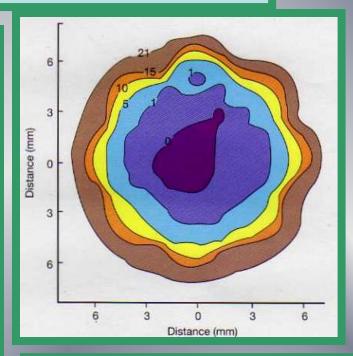
ABSTRACT The number of prokaryotes and the total amount of their cellular carbon on earth are estimated to be  $4-6 \times 10^{30}$  cells and 350-550 Pg of C (1 Pg =  $10^{15}$  g), respectively. Thus, the total amount of prokaryotic carbon is 60-100% of the estimated total carbon in plants, and inclusion of prokaryotic carbon in global models will almost double estimates of the amount of carbon stored in living organisms. In addition, the earth's prokaryotes contain 85-130 Pg of N and 9-14 Pg of P, or about 10-fold more of these nutrients than do plants, and represent the largest pool of these nutrients in living organisms. Most of the earth's prokarvotes occur in the open ocean, in soil, and in oceanic and terrestrial subsurfaces, where the numbers of cells are  $1.2 \times 10^{29}$ ,  $2.6 \times 10^{29}$ ,  $3.5 \times 10^{29}$  $10^{30}$ , and  $0.25-2.5 \times 10^{30}$ , respectively. The numbers of heterotrophic prokaryotes in the upper 200 m of the open ocean, the ocean below 200 m, and soil are consistent with average turnover times of 6-25 days, 0.8 yr, and 2.5 yr, respectively. Although subject to a great deal of uncertainty, the estimate for the average turnover time of prokaryotes in the subsurface is on the order of  $1-2 \times 10^3$  yr. The cellular production rate for all prokaryotes on earth is estimated at  $1.7 \times 10^{30}$  cells/yr and is highest in the open ocean. The large population size and rapid growth of prokaryotes provides an enormous capacity for genetic diversity.

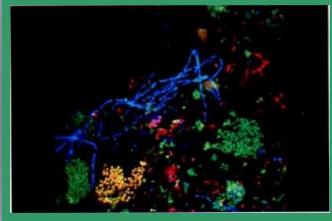
portion of these ce and *Prochlorococ* density of  $4 \times 10$  water contains 5 estimates of volun total of  $3.6 \times 1$  autotrophs, where  $10^{28}$  cells (Table 1

The upper 10 cr in the oceanic hab precipitation, it is water column. Mo continental rise a karyotes were cal cellular densities (ref. 9; Table 1) excluded from this (10).

There are fewer freshwaters and standard freshwaters and standard freshwaters and standard freshwaters are fewer freshwaters and standard freshwaters and standard freshwaters are fewer freshwaters and standard freshwaters and standard freshwaters are fewer freshwaters are fewer freshwaters and standard freshwaters are fewer freshwaters are fewer freshwaters and standard freshwaters are fewer freshwaters are fewer freshwaters and standard freshwaters are fewer freshwaters are fewer freshwaters and standard freshwaters are fewer freshwaters are fewer freshwaters and fewer freshwaters are fewer fresh

In the polar reg and prokaryotes for





#### AHORA QUE LOS DESCUBRIMOS: ¿TODOS LOS MICROBIOS SON MALOS Y RESPONSABLES DE LOS MALES QUE AZOTARON LA HUMANIDAD?

LOS CUATRO
JINETES DEL
APOCALIPSIS:

- 1- LA MUERTE
- 2- LA GUERRA
- 3- EL HAMBRE
- 4- LA PESTE

**Alberto Durero 1498** 



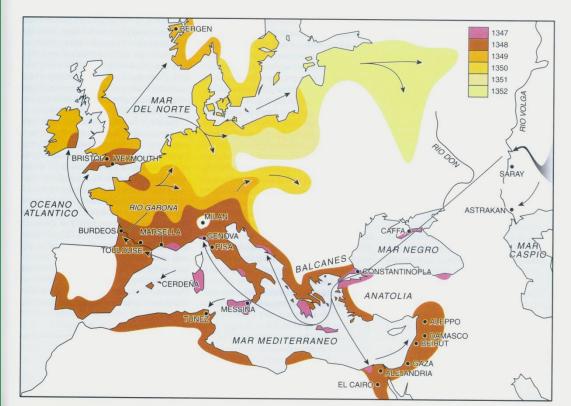
1. LOS CUATRO JINETES DEL APOCALIPSIS: la Peste, la Guerra, el Hambre y la Muerte. Alberto Durero talló en 1498 este grabado en madera, que muestra a la humanidad subyu-

gada por estos azotes implacables. La peste negra que barrió Europa en el siglo XIV quedó en la memoria colectiva como una de las peores mortandades de la época.

#### AHORA QUE LOS DESCUBRIMOS: ¿TODOS LOS MICROBIOS SON MALOS Y RESPONSABLES DE LOS MALES QUE AZOTARON LA HUMANIDAD?



FINES DEL SIGLO XIV, POR LA RUTA DE LA SEDA ENTRADA DE LA PESTE NEGRA



3. LA PESTE NEGRA alcanzó Europa, procedente del Asia Central, a través de la Ruta de la Seda, presentándose en Caffa en torno a 1347. Desde este importante puerto del Mar Negro asaltó las ciudades costeras más importantes de Europa y del nor-

te de Africa. La mayor parte de Europa resultó afectada antes de que la epidemia llegara a su fin, en 1352. Milán fue la más importante de las ciudades que se salvaron, a causa, según se cree, de su lejanía del mar.

# NO, NO TODAS LAS BACTERIAS

**O MICROBIOS** 

SON PERJUDICIALES

# ¿ QUÉ ENTENDEMOS POR FLORA INTESTINAL O COMENSAL?



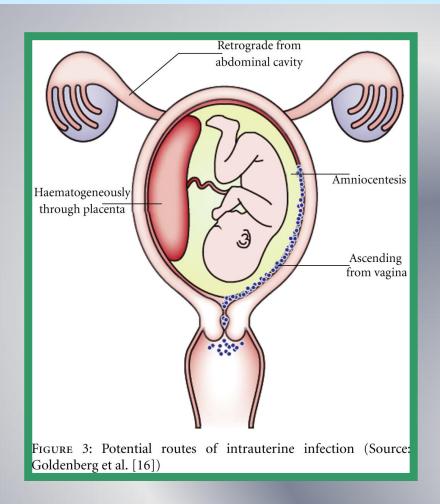
ESTÁ INTEGRADA PRINCIPALMENTE POR BACTERIAS DE LAS "BUENAS"



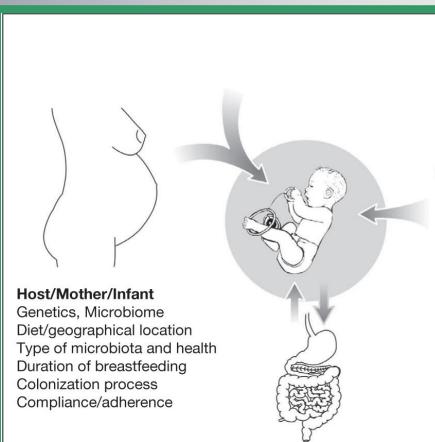
¿ CUÁNDO Y DESDE DÓNDE LA ADQUIRIMOS ?

1 DURANTE EL EMBARAZO "NINGUNA BACTERIA"

EN EL MOMENTO
DEL PARTO POR LA
FLORA VAGINAL DE
LA MADRE



LUEGO DEL
NACIMIENTO
ADQUIRIMOS
PARTE DE LA
FLORA A
TRAVÉS DEL
AMAMANTAMIIENTO



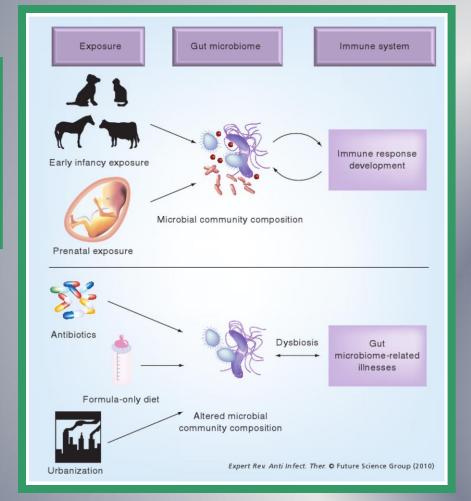
#### Probiotic and its Genome

Strains vary
Dose/administration time
Viability usually poorly assessed
Gene expression
Exposure to food

#### **Environment**

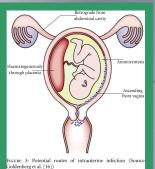
Mode of delivery
Transfer of microbiota
Feeding regimens
Antibiotics
Toxins
Environmental exposure

4
POR ÚLTIMO
DEL MEDIO
AMBIENTE Y
LOS
ALIMENTOS



1 A 4 FACTORES
QUE AFECTAN
LA FLORA
INTESTINAL DEL
INFANTE Y EL
DESARROLLO
TEMPRANO DEL
SISTEMA
INMUNOLÓGICO

# "5" Desde la placenta???







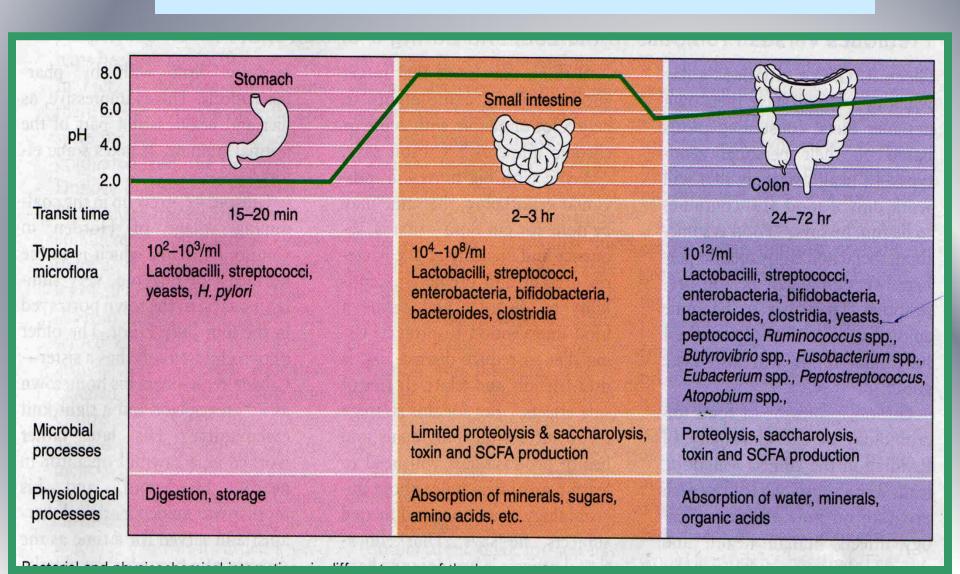
The placenta harbors a unique microbiome (Mayo de 2014)

Humans and their microbiomes have coevolved as a physiologic community composed of distinct body site niches with metabolic and antigenic diversity. The placental microbiome has not been robustly interrogated, despite recent demonstrations of intracellular bacteria with diverse metabolic and immune regulatory functions. A population-based cohort of placental specimens collected under sterile conditions from 320 subjects with extensive clinical data was established for comparative 16S ribosomal DNA-

subjects with extensive clinical data was established for comparative 16S ribosomal DNA-based and whole-genome shotgun (WGS) metagenomic studies. Identified taxa and their gene carriage patterns were compared to other human body site niches, including the oral, skin, airway (nasal), vaginal, and gut microbiomes from nonpregnant controls. We characterized a unique placental microbiome niche, composed of nonpathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla. In aggregate, the placental microbiome profiles were most akin (Bray-Curtis dissimilarity <0.3) to the human oral microbiome. 16S-based operational taxonomic unit analyses revealed associations of the placental microbiome with a remote history of antenatal infection (permutational multivariate analysis of variance, P = 0.006), such as urinary tract infection in the first trimester, as well as with preterm birth <37 weeks (P = 0.001).

Comment on "the placenta harbors a unique microbiome". Y CONTINUA......

#### INTERACCIÓN ENTRE LA FLORA INTESTINAL Y EL SISTEMA INMUNOLÓGICO



#### INTERACCIÓN ENTRE LA FLORA INTESTINAL Y EL SISTEMA INMUNOLÓGICO

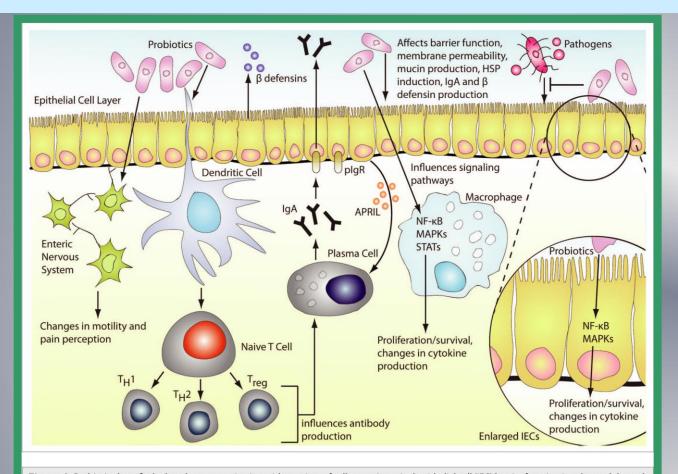


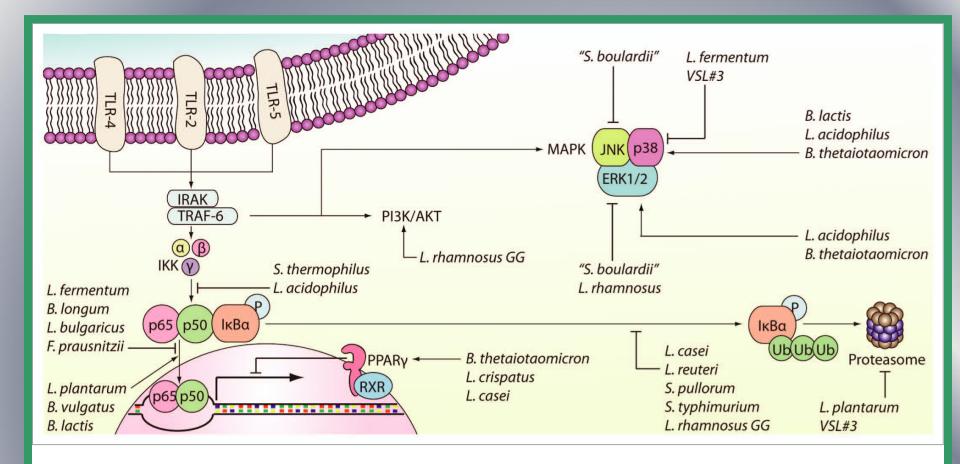
Figure 1. Probiotics benefit the host by communicating with a variety of cell types. Intestinal epithelial cell (IEC) barrier function is enhanced through probiotic modulation of tight junctions as well as enhanced mucin production. Probiotics interfere with pathogens by increasing β defensin secretion from IECs and IgA from plasma cells and by directly blocking the signaling pathways hijacked by pathogens. Cytokine secretion by IECs, macrophages and dendritic cells is regulated by probiotics through modulation of key signaling pathways such as NFκB and MAPKs. Changes in these pathways can also affect proliferation and survival of target cells. Through interactions with dendritic cells, probiotics can influence T cell subpopulations and skew them towards a Th1, Th2 or Treg response. Probiotics can also cause changes in gut motility and pain perception by modulating pain receptor expression and secreting potential neurotransmitter molecules. APRIL, a proliferation-inducing ligand; hsp, heat shock protein; IEC, intestinal epithelial cell; Ig, immunoglobulin; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor-kappaB; plgR, polymeric immunoglobulin receptor; STAT, signal transducers and activator of transcription; Tred, T regulatory cell.

#### **Probiotics-host communication**

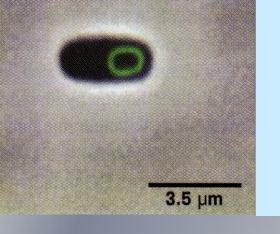
Modulation of signaling pathways in the intestine

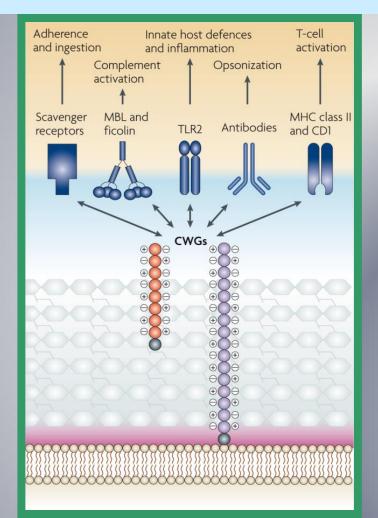
Carissa M. Thomas<sup>1,2</sup> and James Versalovic<sup>1-3,\*</sup>

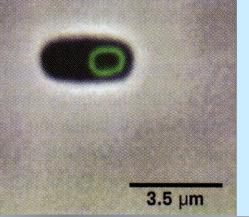
<sup>1</sup>Interdepartmental Program of Cell and Molecular Biology; and <sup>2</sup>Department of Pathology and Immunology; Baylor College of Medicine; Houston, TX USA; <sup>3</sup>Department of Pathology; Texas Children's Hospital; Houston, TX USA

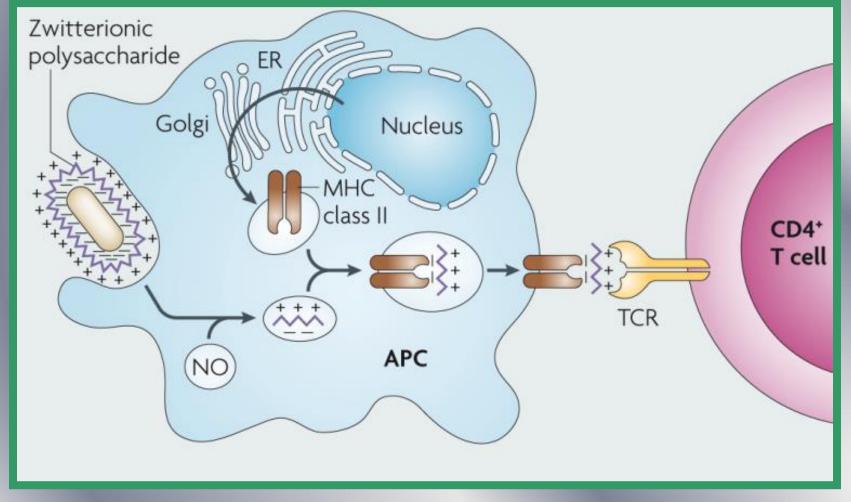


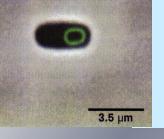
**Figure 2.** Probiotics modulate key signaling pathways in intestinal epithelial cells. Various probiotics prevent NFκB activation by inhibiting IκBα phosphorylation, ubiquitination, proteasomal degradation, or translocation of NFκB into the nucleus (suppression is indicated by a block sign "+"). Probiotics can also enhance RelA export from the nucleus via PPARγ. Other probiotics increase NFκB activation through enhanced translocation into the nucleus (activation is indicated by an arrow sign "+"). Apoptosis of intestinal epithelial cells can be prevented by probiotic modulation of the PI3K/Akt pathway. Probiotic-induced changes in phosphorylation levels of p38, JNK, and ERK1/2 MAPKs can affect cytokine secretion and apoptosis. ERK, extracellular signal-regulated kinases; IκBα, inhibitor of NFκB α; IKK, IκB kinase; IRAK, interleukin-1 receptor-associated kinase; JNK, c-Jun N-terminal kinase; P, phosphorylation; PPARγ, peroxisome proliferator activated receptor-γ; RXR, retinoid X receptor; TLR, Toll-like receptor; Ub, ubiquitin.









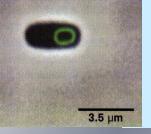


## ALGUNOS MICROBIOS SON MUY BUENOS COMPAÑEROS

# The gut flora as a forgotten organ

Ann M. O'Hara¹ & Fergus Shanahan¹,2+

Alimentary Pharmabiotic Centre, University College Cork, National University of Ireland, Cork, Ireland



## ALGUNOS MICROBIOS SON MUY BUENOS COMPAÑEROS

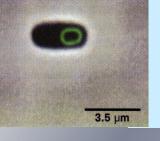
EXISTE UNA RELACIÓN ENTRE LA FLORA NORMAL Y EL SISTEMA INMUNOLÓGICO



EXISTE UNA RELACIÓN ENTRE EL SISTEMA INMUNOLÓGICO Y EL SISTEMA NERVIOSO

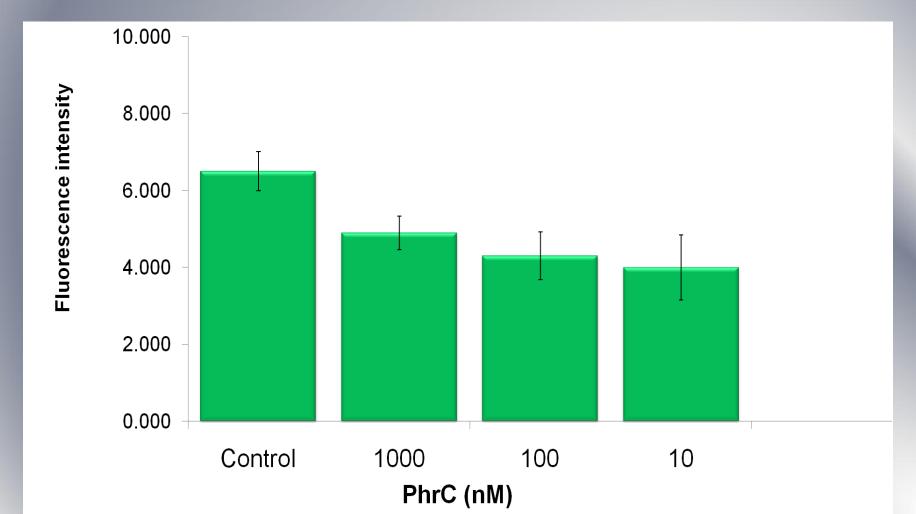


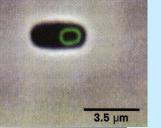
ENTONCES DEBE EXISTIR UNA RELACIÓN (DIRECTA O INDIRECTA) ENTRE LA FLORA NORMAL Y EL SISTEMA NERVIOSO



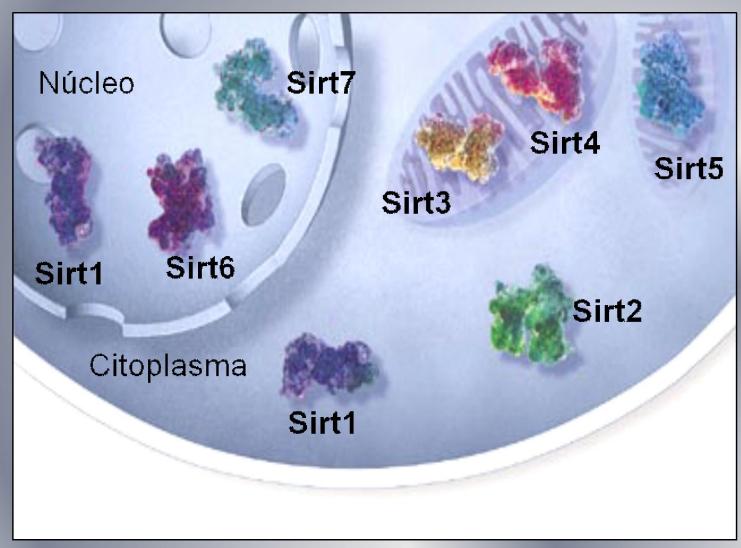
# ¿CUÁLES SON LOS EFECTOS BENEFICIOSOS DE LOS MICROORGANISMOS PROBIÓTICOS?

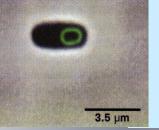
# B. SUBTILIS DISMINUYE LA GENERACIÓN DE ROS EN NEURONAS DE CEREBELO ¿AUMENTO DEL LIFESPAN?





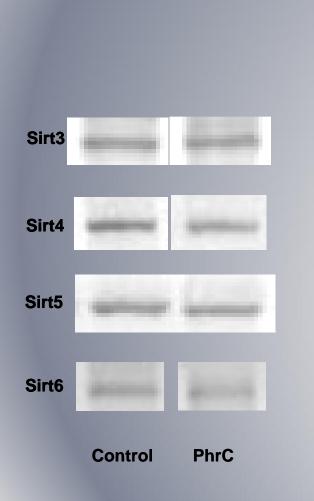
# ¿CUÁLES SON LOS EFECTOS BENEFICIOSOS DE LOS MICROORGANISMOS PROBIÓTICOS?

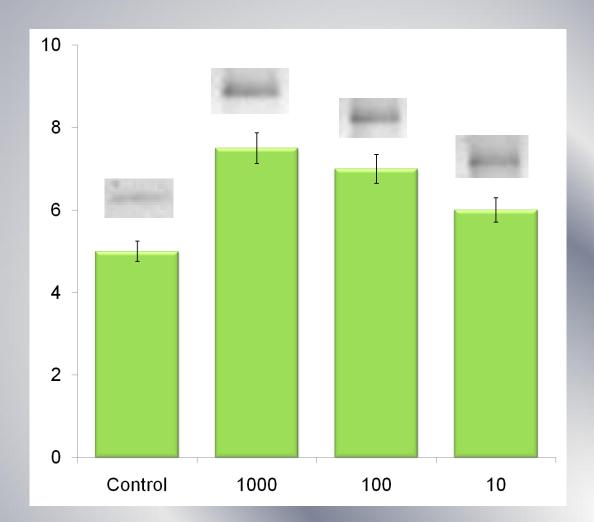


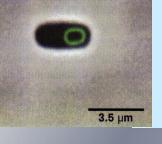


# ¿CUÁLES SON LOS EFECTOS BENEFICIOSOS DE LOS MICROORGANISMOS PROBIÓTICOS?

B. Subtilis estimula la producción de Sirt7 en cultivo de neuronas



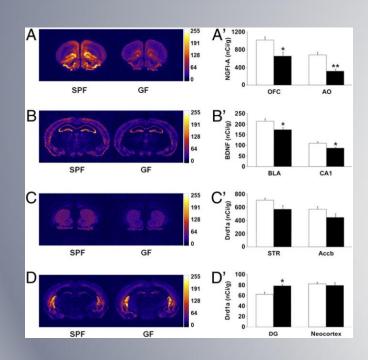




# ALGUNOS MICROBIOS SON MUY BUENOS COMPAÑEROS

### Normal gut microbiota modulates brain development and behavior

Rochellys Diaz Heijtz<sup>a,b,1</sup>, Shugui Wang<sup>c</sup>, Farhana Anuar<sup>d</sup>, Yu Qian<sup>a,b</sup>, Britta Björkholm<sup>d</sup>, Annika Samuelsson<sup>d</sup>, Martin L. Hibberd<sup>c</sup>, Hans Forssberg<sup>b,e</sup>, and Sven Pettersson<sup>c,d,1</sup>



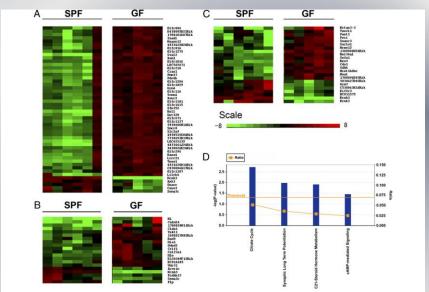
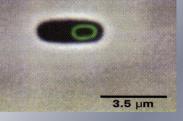
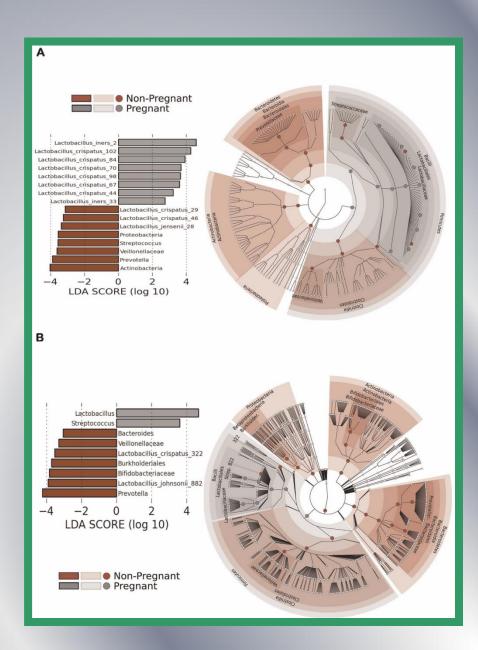


Fig. 5. Expression profiling of GF mice and SPF mice brains. A heatmap of genes showing statistically significant (q < 5%) and fold change (>2) differences, between SPF (n = 0) and GF (n = 5) mice in the hippocampus (A), frontal cortex (β), and striatum (C). Each row represents the relative levels of expression of a single gene across all mice; each column represents the levels of expression for a single mouse. The colors red and green denote high and low expression, respectively. Differentially expressed genes were investigated for functional clustering by using Ingenuity Pathway Analysis software for canonical pathways (O), as described in Experimental Procedures.

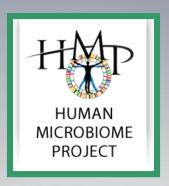


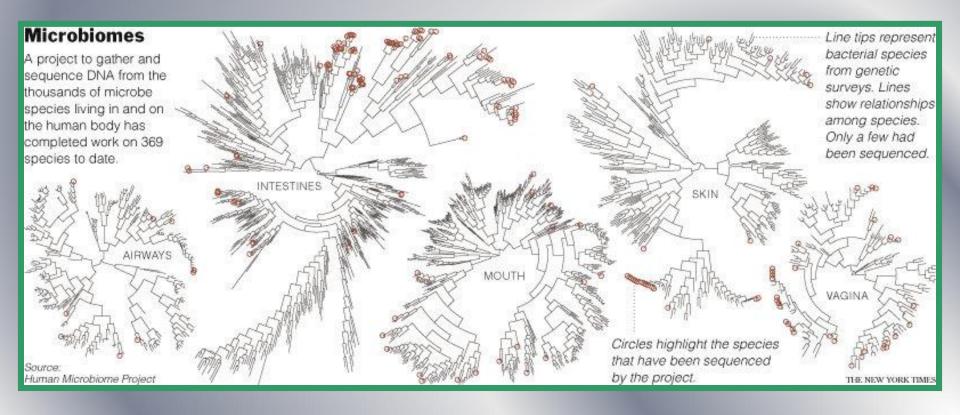
POR CADA CÉLULA HUMANA DE NUESTRO CUERPO PORTAMOS 10 BACTERIAS

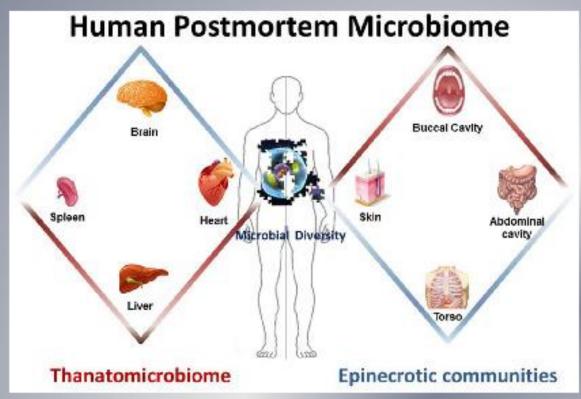
SOMOS NUESTRAS CÉLULAS (HUMANAS) MÁS NUESTRAS BACTERIAS COMENSALES QUE INTERACCIONAN ENTRE SÍ Y CON NUESTRAS CÉLULAS Y QUE NOS CONFORMARÍAN COMO SUJETO



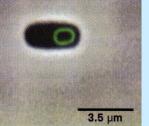
#### PROYECTO MICROBIOMA HUMANO SECUENCIACIÓN DE LOS GENES TOTALES DE NUESTRA FLORA COMENSAL







The human postmortem microbiome. The components of the human postmortem microbiome include the thanatomicrobiome (the microbiome of internal organs of cadavers) and epinecrotic microbial communities (the microbiome on surfaces of decaying remains).



# ¿ QUÉ BUSCAMOS?



1 3 9 16 30 54 69 93 130

# AÑOS DE VIDA

#### Muchas gracias!

#### Roberto Ricardo Grau

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