Nuevos Avances en Bio-estimulantes (Bioinoculantes) de Uso Foliar para la Producción Agrícola.

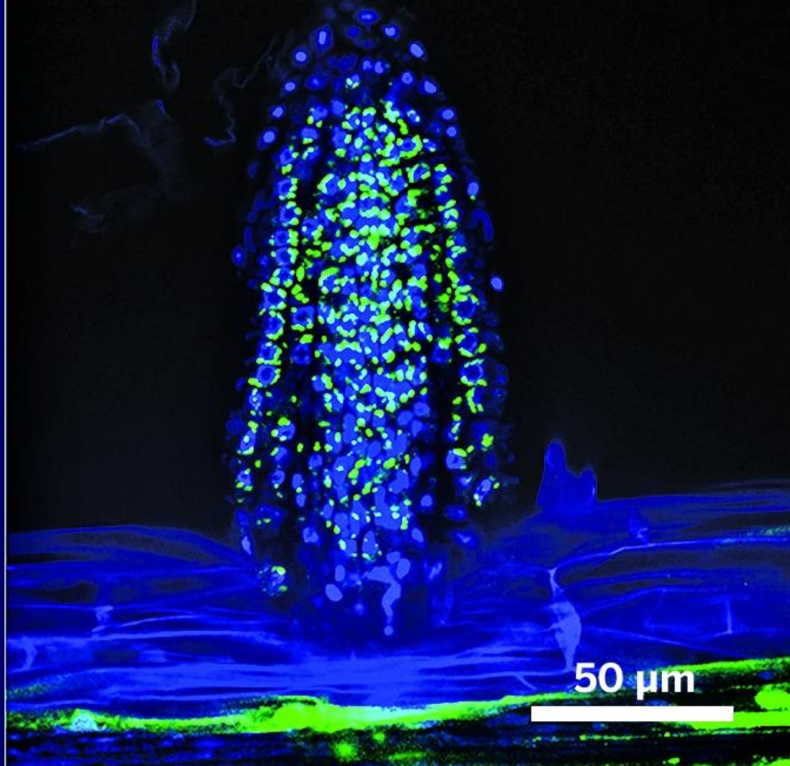
Ing. Agr. Alejandro Perticari
INTA – IMYZA CICVyA

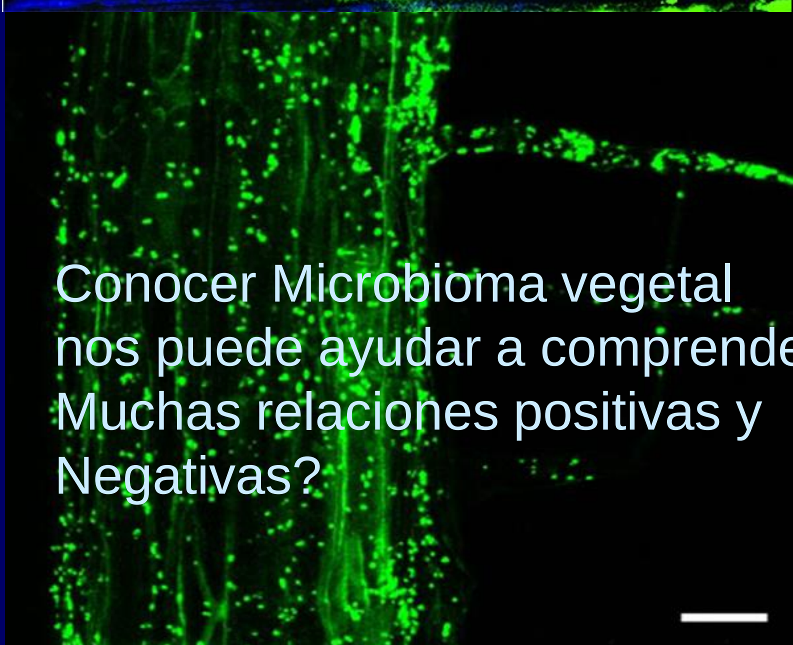
Abundancia de organismos del suelo

Organismo	Número por gramo suelo acre)	Biomasa (kg/ha)
Lombrices	>5	110 – 1,700
Artrópodos	1-10	6 – 170
Nematodes	10 – 100	11 – 170
Protozoarios	>10000	22 – 220
Algas	>1000	11 – 550
Hongos	>200000	1,100 – 17,000
Actinomycetes	> 100 millones	440 – 5,500
Bacteria	100 millones a 1 billon	440 – 5,500

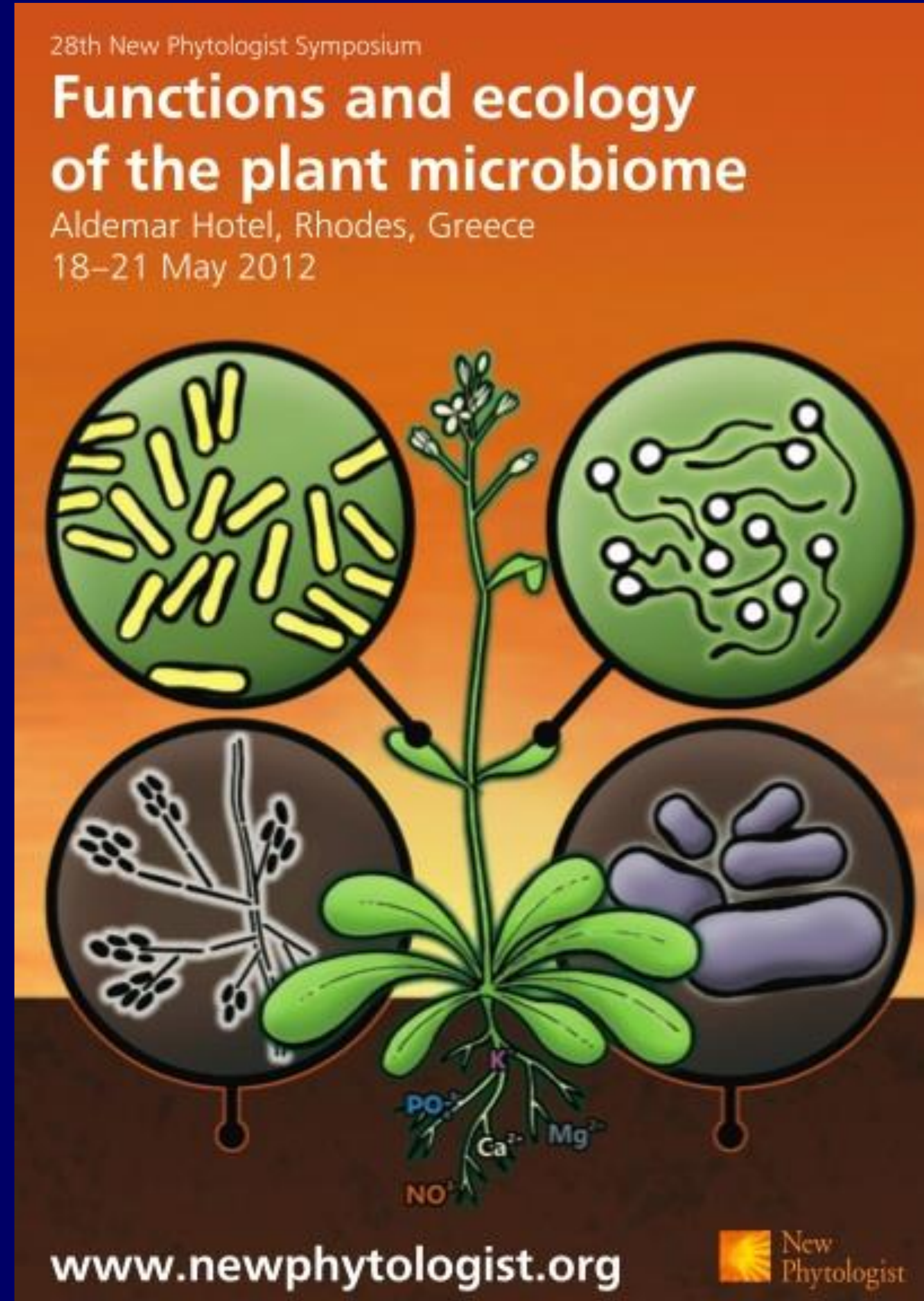
Richard Stehouwer, 2010. Unv. Pensylvania. USA

Microbioma vegetal.

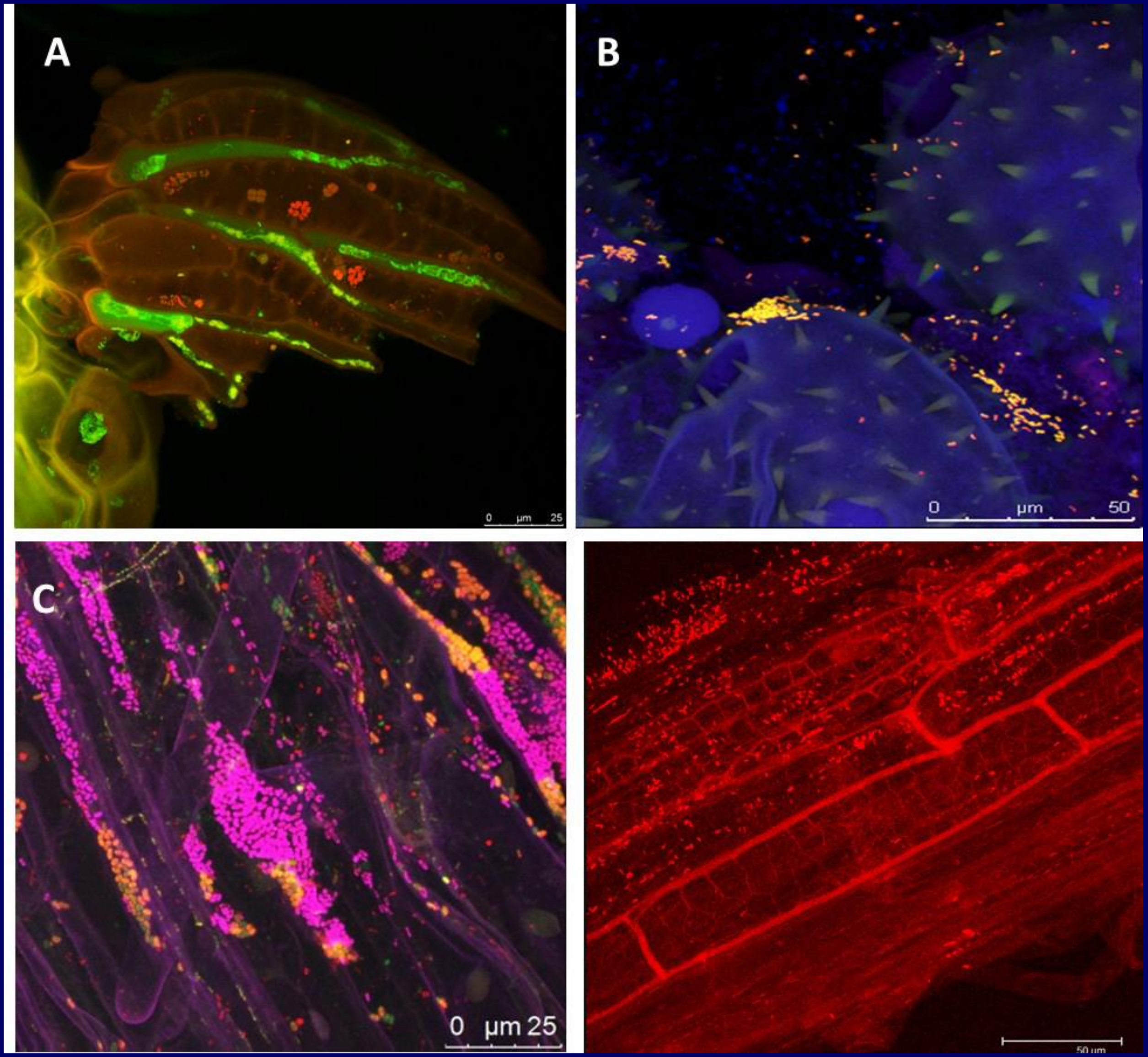




Conocer Microbioma vegetal nos puede ayudar a comprender Muchas relaciones positivas y Negativas?



Collectively known as the plant microbiome, plant-associated microbes can help plants defend off disease, stimulate growth, occupy space that would otherwise be taken up by pathogens, promote stress resistance, and influence crop yield and quality. Therefore, the plant microbiome is a key determinant of plant health and productivity.



Next-Generation Bio-Products Sowing the Seeds of Success for Sustainable Agriculture. Gabriele Berg et al 2013

American Journal of Botany

American Journal of Botany 100(9): 1726–1737. 2013.

SPECIAL INVITED PAPER—RHIZOSPHERE INTERACTIONS

Bacterial endophytes enhance competition by invasive plants[1]

Marnie E. Rout[2,3,6], Thomas H. Chrzanowski[4], Tara K. Westlie[2], Thomas H. DeLuca[5], Ragan M. Callaway[2], and William E. Holben[2,3]

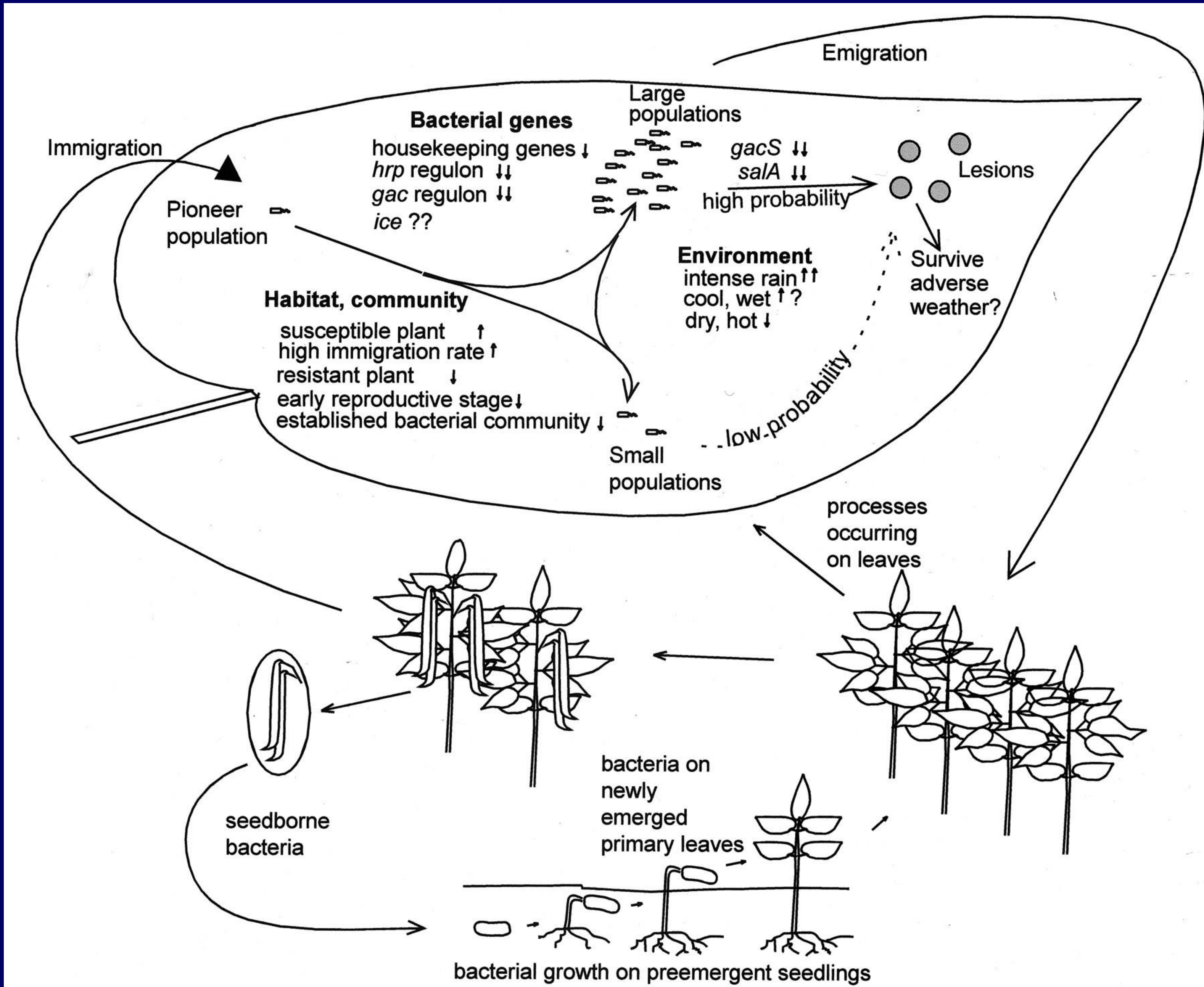
Diferentes partes de la planta? Diferentes microbiomas

Filosfera

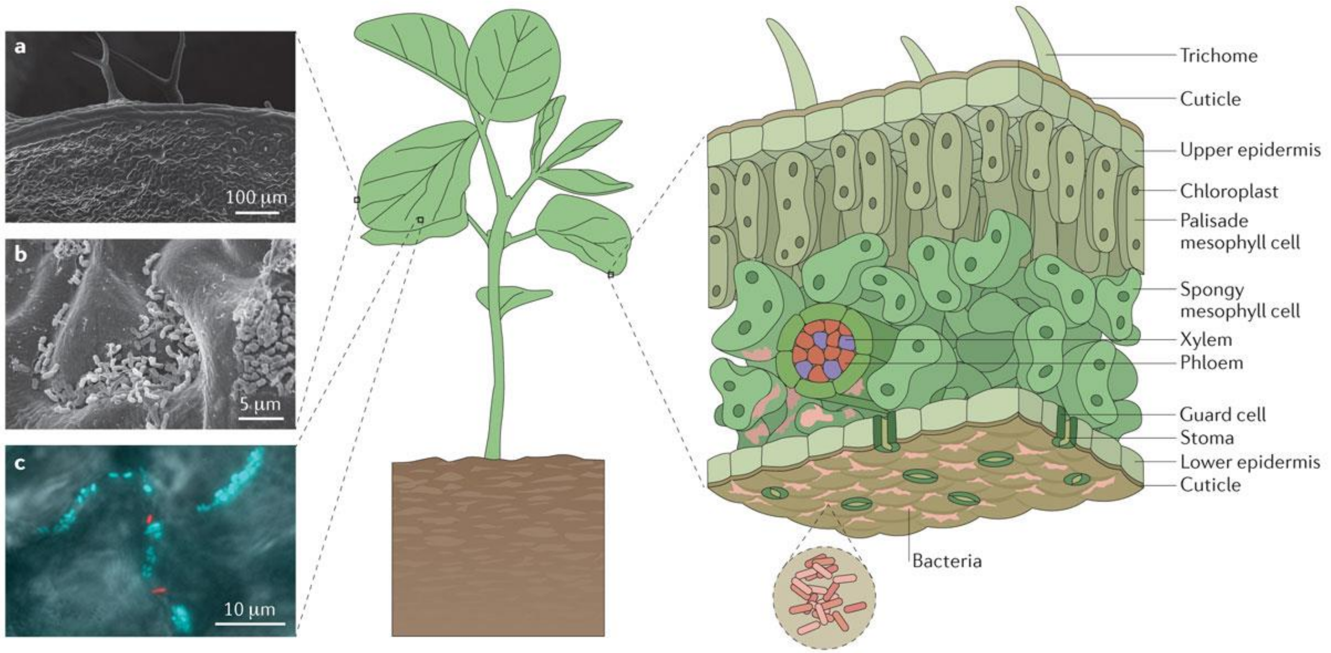
The plant microbiome is a key determinant of plant health and productivity and has received substantial attention in recent years

Manipulation of the plant microbiome has the potential to reduce the incidence of plant disease], increase agricultural production, reduce chemical inputs and reduce emissions of greenhouse gases], resulting in more sustainable agricultural practices.
Virtually all tissues of a plant host a microbial community. Here, we focus on the rhizosphere, phyllosphere (plant aerial surfaces) and endosphere (internal tissues).

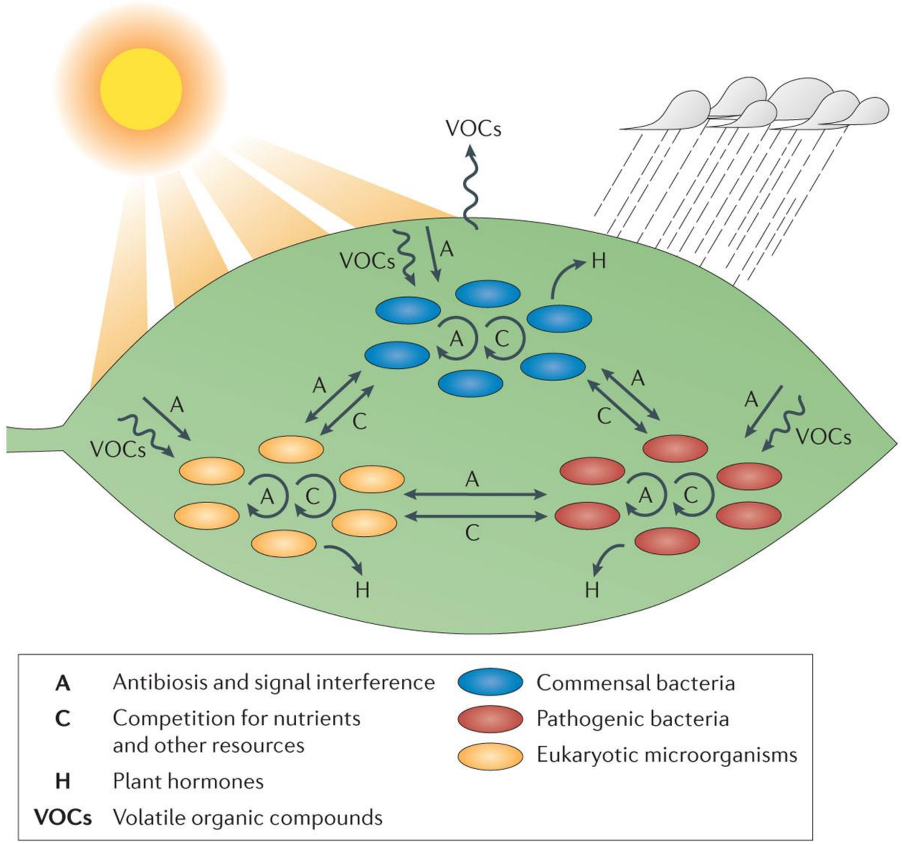
The phyllosphere environment The phyllosphere, or aerial surface of a plant, is considered relatively nutrient poor compared with the rhizosphere. Microbial colonization of leaves is not homogenous but is affected by leaf structures such as veins, hairs and stomata. Leaf surfaces are colonized by up to 10^7 microbes per cm^2. The phyllosphere is a much more dynamic environment than the rhizosphere, with resident microbes subjected to large fluxes in temperature, moisture and radiation throughout the day and night. These abiotic factors also indirectly affect the phyllosphere microbiome through changes in plant metabolism. Precipitation and wind in particular are thought to contribute to the temporal variability in resident phyllosphere microbes-



Emigration
Large
populations
Bacterial genes
housekeeping genes ↓
hrp regulon ↓↓
gac regulon ↓↓
ice ??
Immigration
Pioneer
population
gacS ↓↓
salA ↓↓
high probability
Lesions
Environment
intense rain ↑↑
cool, wet ↑ ?
dry, hot ↓
Survive
adverse
weather?
Habitat, community
susceptible plant ↑
high immigration rate ↑
resistant plant ↓
early reproductive stage ↓
established bacterial community ↓
low probability
Small
populations
processes
occurring
on leaves
seedborne
bacteria
bacteria on
newly
emerged
primary leaves
bacterial growth on preemergent seedlings

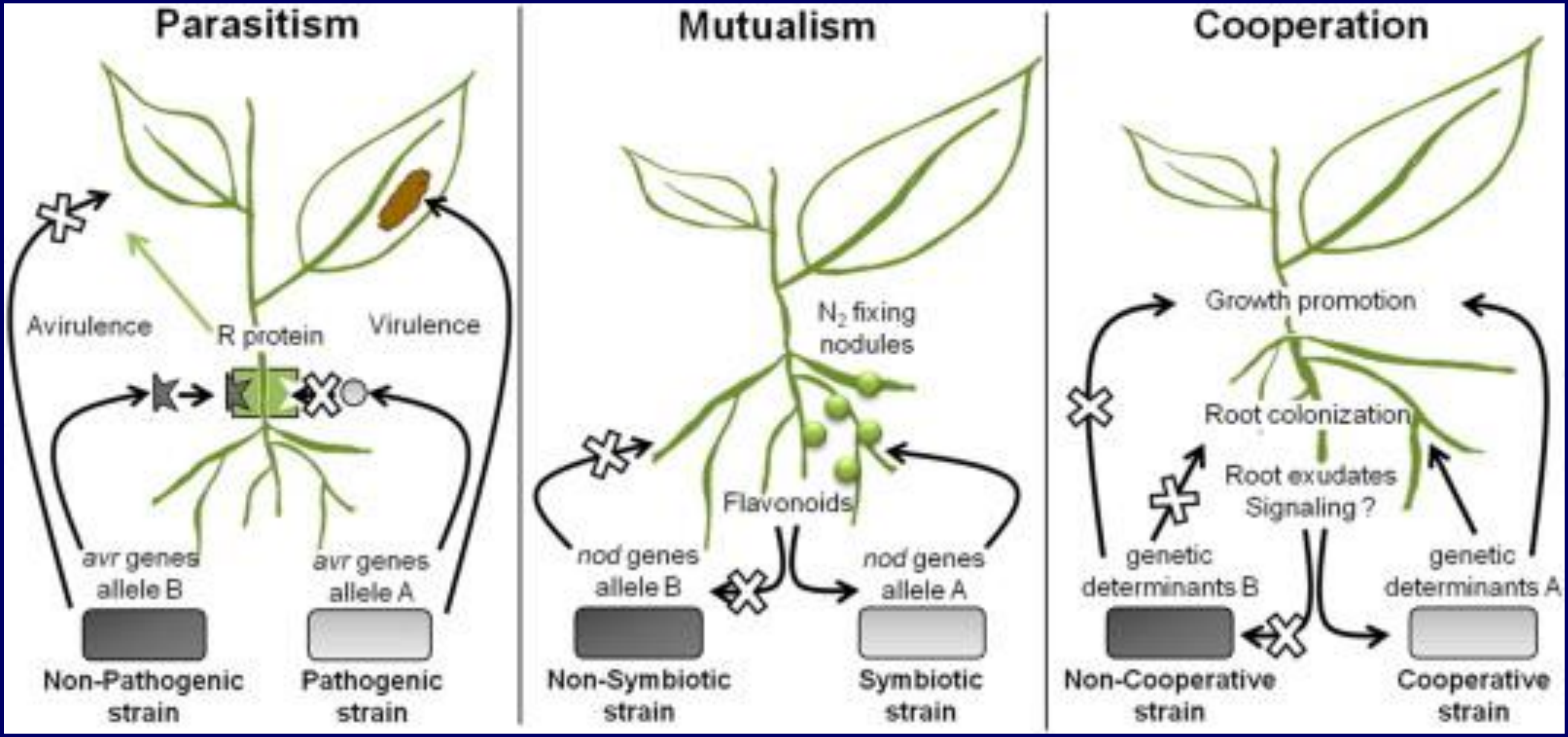


Nature Reviews | Microbiology

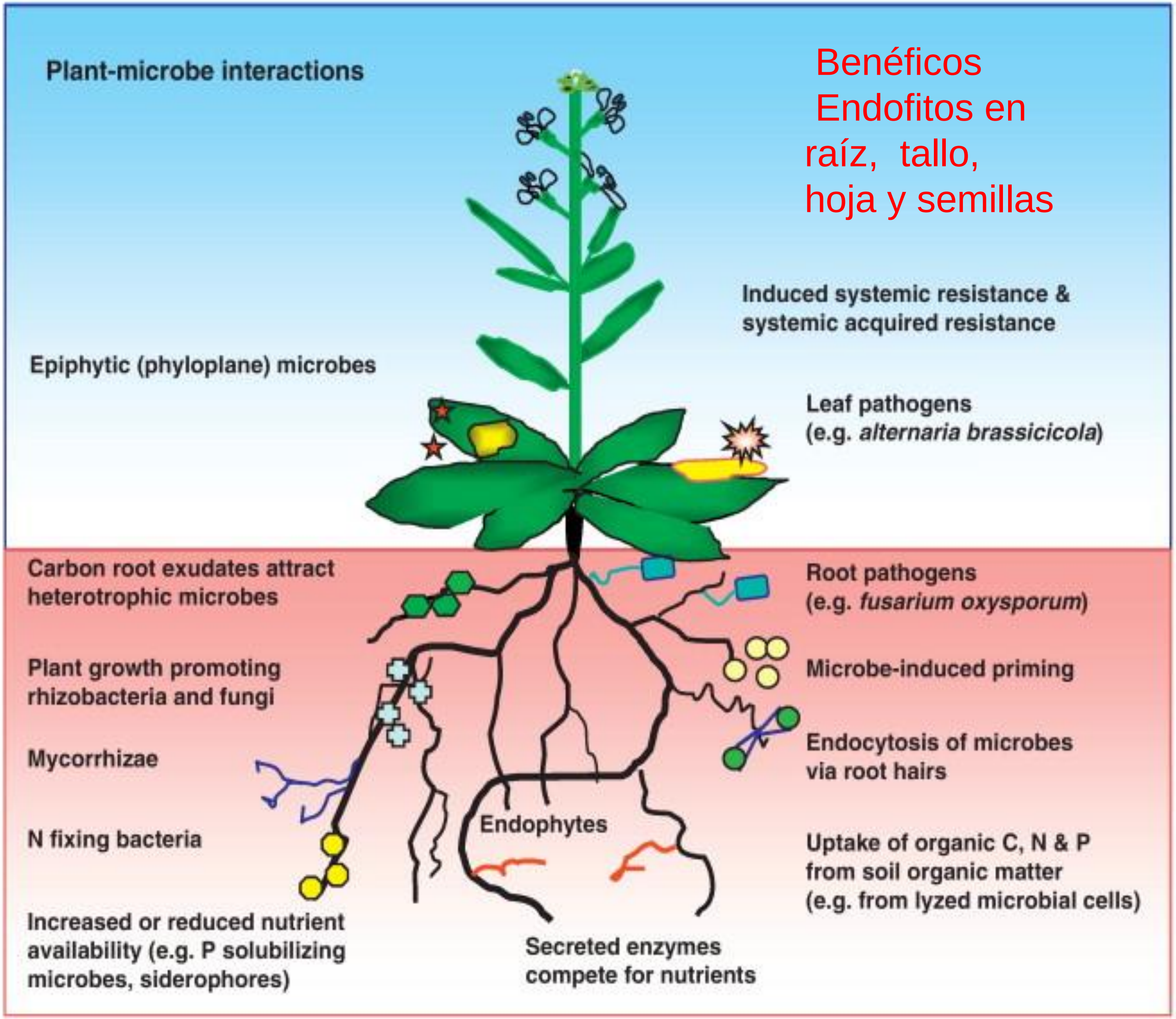


Nature Reviews | Microbiology

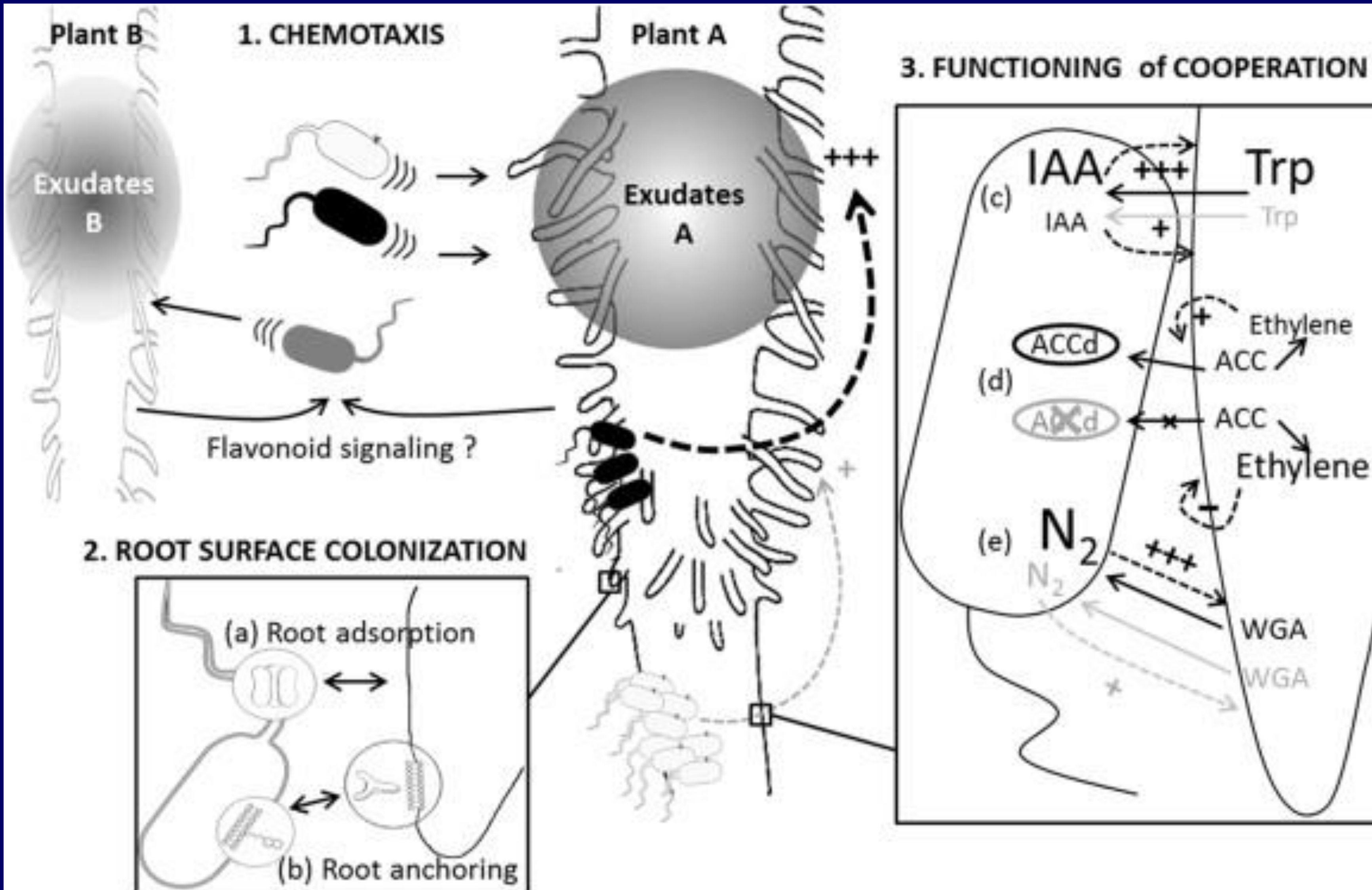
Microorganismos que nos interesan ¿cuáles y porque?.



Which specificity in cooperation between phytostimulating rhizobacteria and plants? Benoıˆt Drogue et al 2012

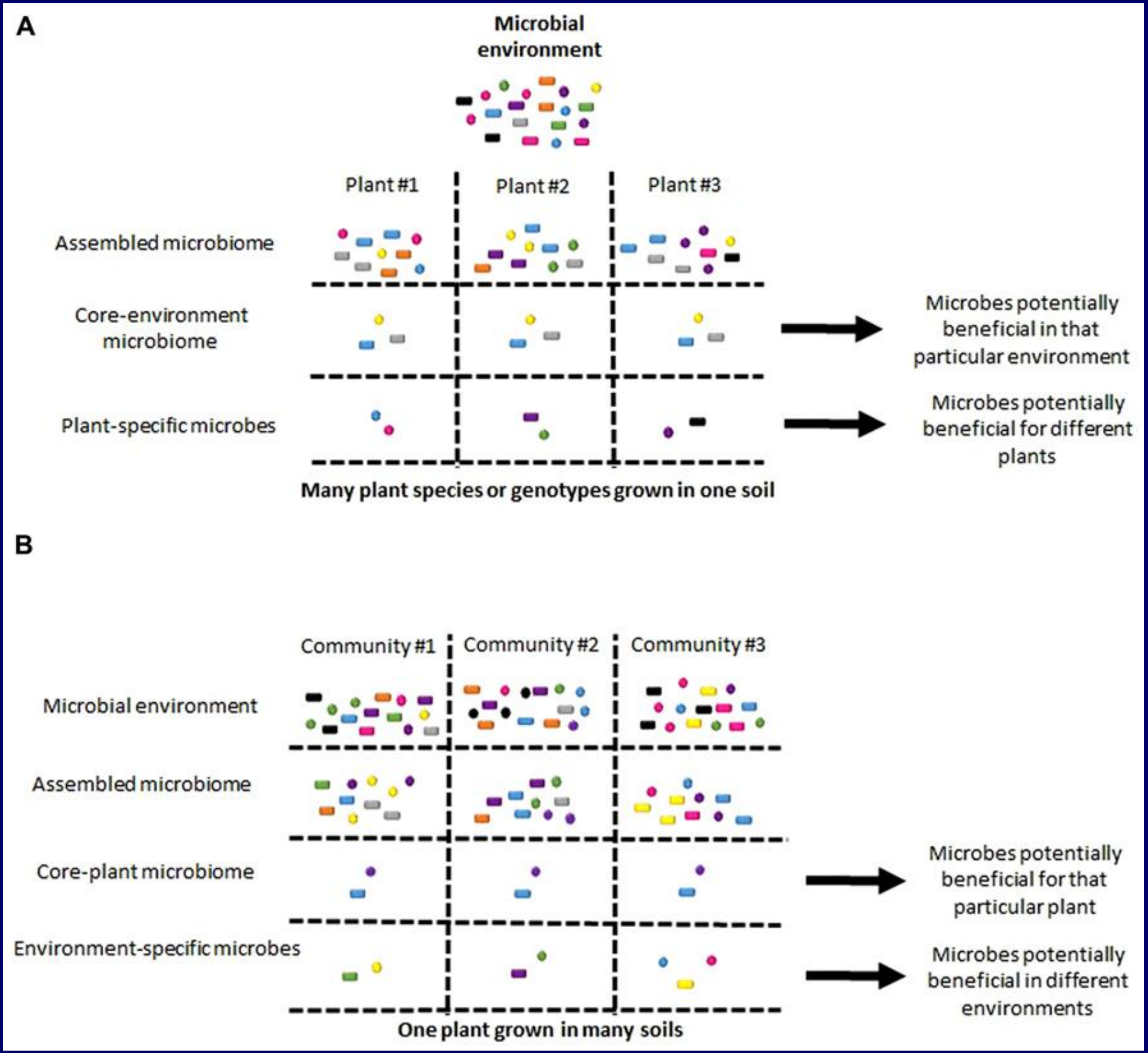


TRENDS in Biotechnology



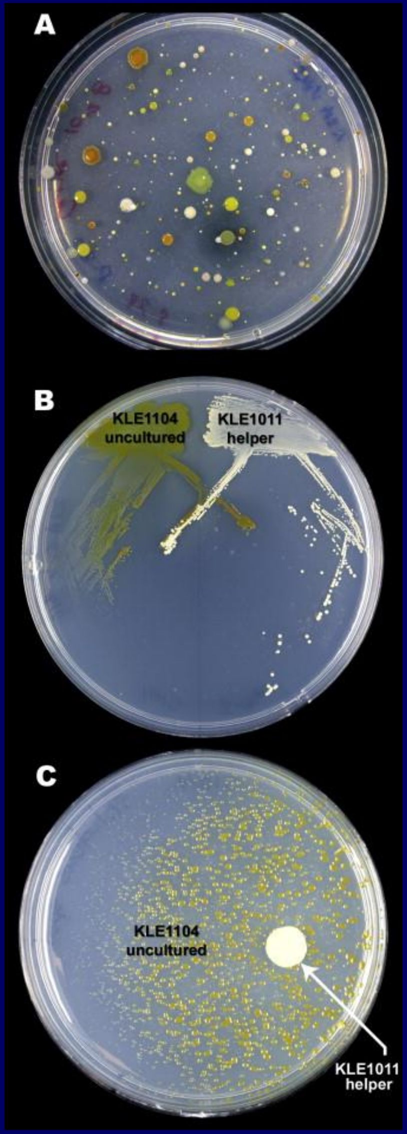
Plant B
1. CHEMOTAXIS
Plant A
3. FUNCTIONING of COOPERATION
Exudates B
Exudates A
+++
IAA
+++
Trp
(c)
IAA
Trp
+
Ethylene
ACCd
ACC
(d)
ACC
Ethylene
Flavonoid signaling ?
+
(e)
N_2
+++
N_2
2. ROOT SURFACE COLONIZATION
(a) Root adsorption
WGA
WGA
+
(b) Root anchoring

The potential for give and take in plant–microbiome relationships ***Sarah L. Lebeis. 2014***



De todos esto mo son
No cultivables el 99% ???
Cultivables solo 1%

Estos se emplean   para Bioinsumos

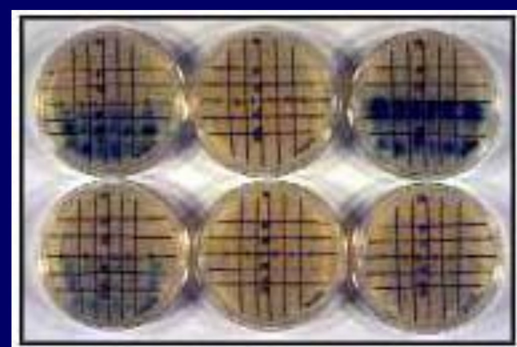


PGPR/PGPB/PGPM

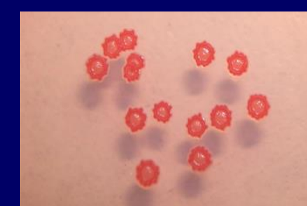
Según Kloepper (1978) una bacteria PGPR es aquella altamente eficiente para aumentar el crecimiento de plantas y aumentar la tolerancia a otros m.o que causan enfermedades.
Según Bashan (2005) Plant growth-promoting bacteria (PGPB) son definidas como bacterias de vida libre en el suelo, rizósfera, rizoplano y filósfera que bajo algunas condiciones son benéficas para las plantas.
Según definiciones actuales PGPM para incluir a todos los MO

Grupo 1: Afectan el metabolismo de las plantas por proveer sustancias que son usualmente escasas. Fijadores de N_2, solubilizadores de P y hierro, productores de fitohormonas.

SOLUBILIZACIÓN DE FOSFATOS



PRODUCCIÓN DE FITOHORMONAS



FBN

Grupo 2: Referido como PGPB Biocontroladoras que indirectamente promueven el crecimiento por prevenir los efectos deletéreos de fitopatógenos. Ellos producen sustancias que matan o inhiben a otros microbios pero no a plantas.

AMILASAS

PRODUCCIÓN DE SIDEROFOROS

BIOCONTROL

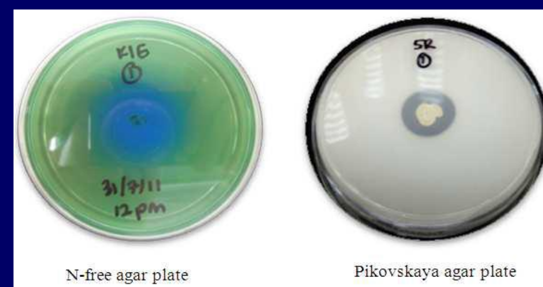
1. AISLAMIENTO DESDE PARTES DE LA PLANTA RAICES, HOJAS, NODULOS , SEMILLA Y SUELO

Fuente

Aislamiento- Colonia Pura










Es fundamental identificar el microorganismo a seleccionar al nivel de género y especie previo a los estudios de selección para su empleo como inoculante..

Las especies patógenas o cercanas no pueden ni deben ser utilizadas para estos fines.

The ISME Journal (2007) 1, 620–631

www.nature.com/ismej

ORIGINAL ARTICLE

Differential interaction of *Salmonella enterica* serovars with lettuce cultivars and plant-microbe factors influencing the colonization efficiency

Michel M Klerks[1], Eelco Franz[2], Marga van Gent-Pe
Ariena HC van Bruggen[2]
[1]Wageningen University and Research Centre, Plant Research
Netherlands and [2]Biological Farming Systems Group, Wagen
Wageningen, The Netherlands

Ecotoxicology and Environmental Safety

Volume 104, June 2014, Pages 285–293

Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress

- Faisal Islam[a], Tahira Yasmeen[a], <img alt="Corresponding author contact information" src="http://origin-cdn.els-cdn.com/sd/entities/REcor.gif">, <img src="http://origin-cdn.els-cdn.com/sd/entities/REemail.gif" alt="E-mail the corresponding author">,

Se ha demostrado que determinados microorganismos utilizados como **insumos microbiológicos** (inoculantes de leguminosas, promotores del crecimiento vegetal) intervienen positivamente en procesos involucrados en producciones agropecuarias sustentables.

GÉNERO	ESPECIES RECONOCIDAS	HABITAT	MECANISMOS INDIRECTOS
Gluconacetobacter	*diazotrophicus*	endófito	FBN, PCP
Azoarcus		endófito	FBN
Azorhizobium	*caulinodans*	endófito	FBN, PCP, solubilizador de P
Azospirillum	*amazonense, brasilense, irakense,lipoferum,*	Rizósfera, endófito	FBN, PCP
Azotobacter	*armeniacus,chroococcum, vinelandii*	Rizósfera,	FBN, fitohormonas, solubilizador de P
Bacillus	*amylililoliquefaciens;subtilis*	Rizósfera,	PCP, control biologico
Bradyrhizobium	*japonicum*	Rizósfera endófito	FBN
Herbaspirillum	*seropedicae*	Rizósfera, endófito	FBN, PCP
Paenobacillus	*polymixa*	Rizosfera, endófito	Solubilizador de P, PCP
Pseudomonas	*fluorescens; aurantiaca; putida*	Rizósfera	Solubilizador de P
Rhizobium	leguminosarum biovar trifolii,	endófito	FBN, PCP, solubilizador

ESPECIFICOS: Azospirillum brasilense, Pseudomonas fluorescens, Rhizobium, Bacillus, Trichoderma, Penicillium billae etc.

MULTIESPECIES: Azospirillum con Pseudomonas. Bradyrhizobium y Azospirillum , más complejos Algas con PGPR, Paenobacillus con Azospirillum

INOCULACION FOLIAR

Productos o formulados microbianos

..Biofertilizantes

..Bioestimulantes

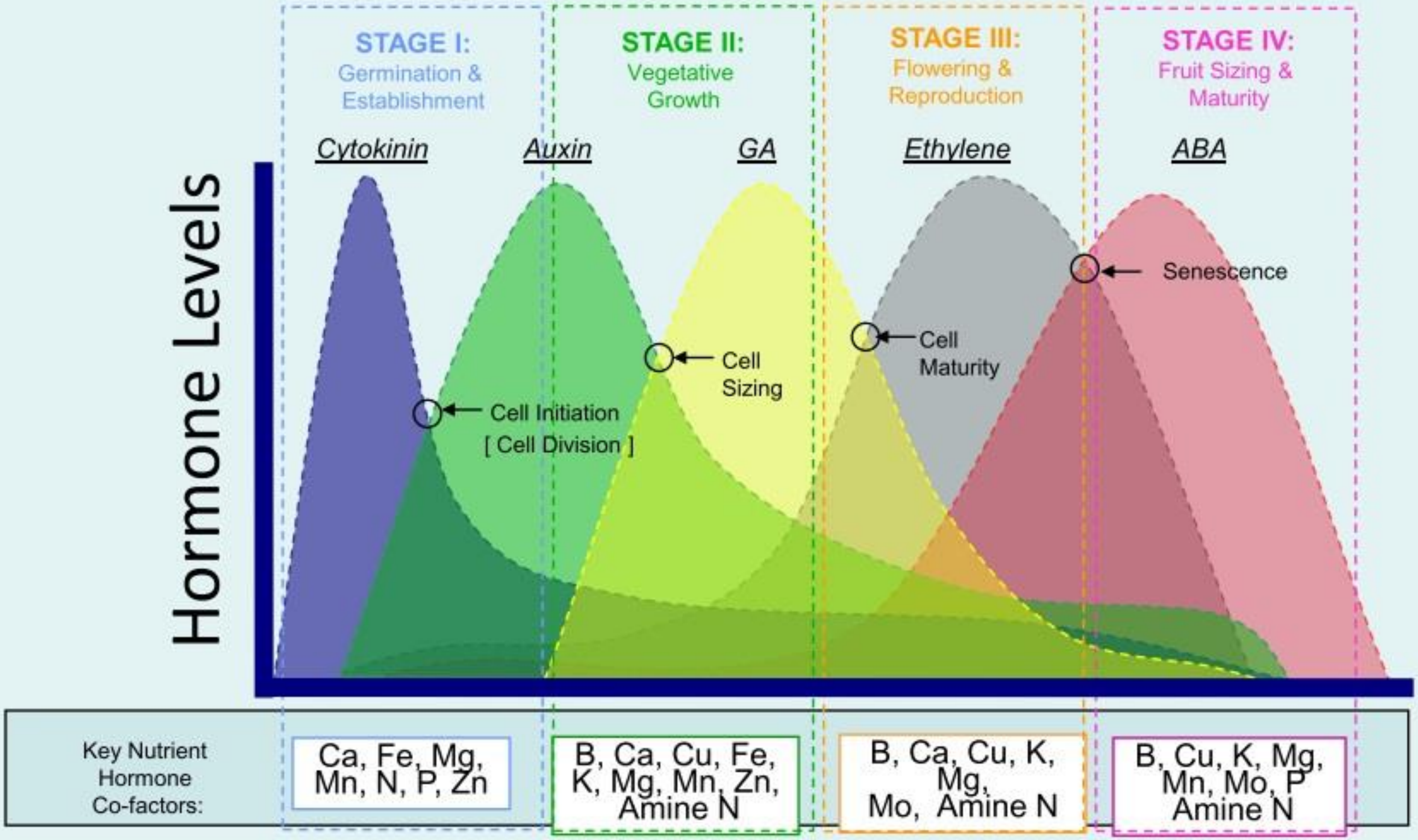
..Biocontrol

..Biofortificación

..Biosanitización

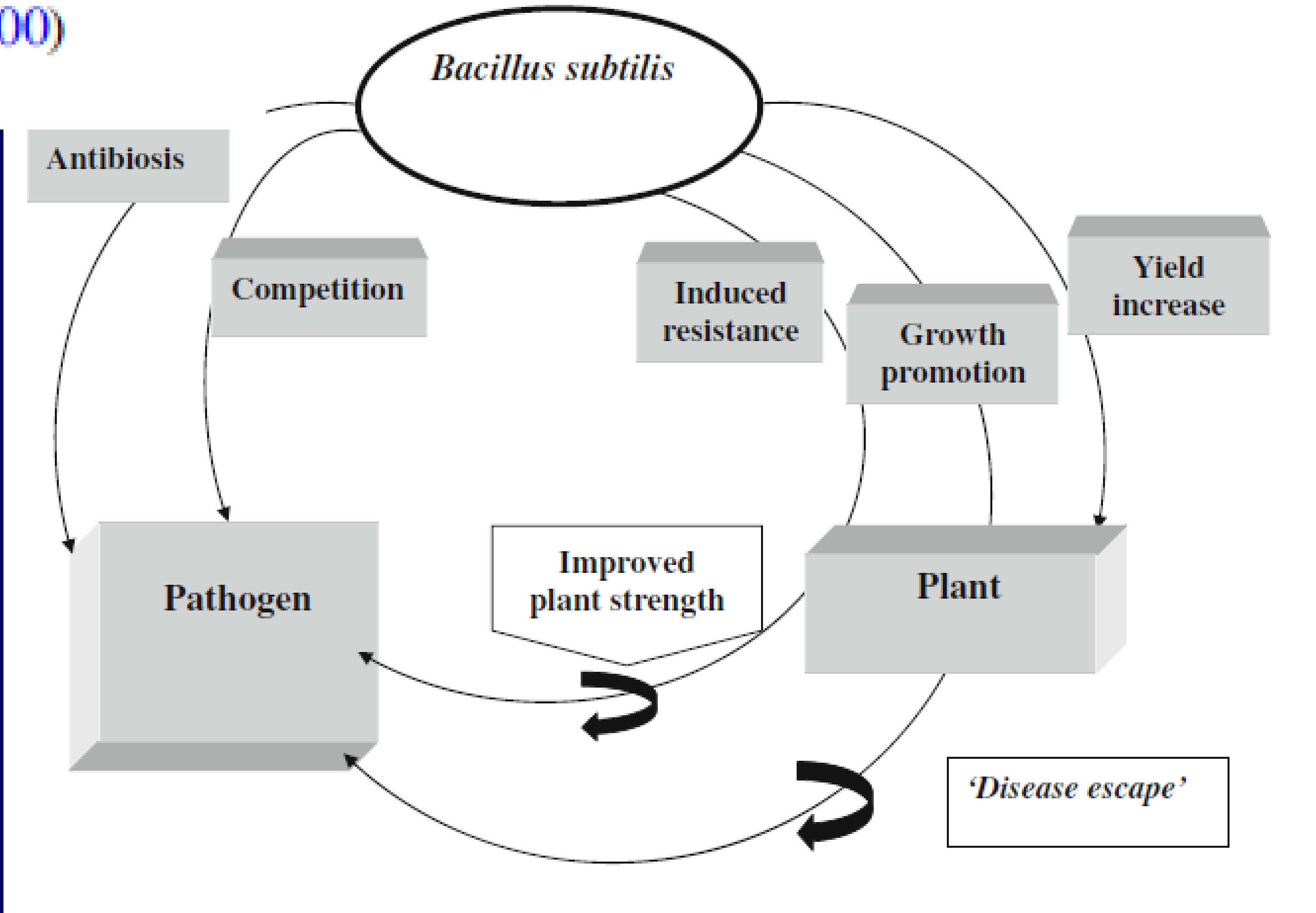
HORMONAS VEGETALES			
	AUXINAS.	Meristemos de yemas apicales. Embriones de semillas, hojas jóvenes.	Estimula la elongación de los tallos. Dominancia apical. Fototropismo y geotropismo. Diferenciación vascular. Inhibición de la abscisión. Estimulación de la síntesis de etileno. Estimula el desarrollo de los frutos. Induce raíces adventicias en esquejes.
	CITOKINI NAS	Sintetizada en raíces y transportada a otros órganos.	Estimula la división celular. Invierte la dominancia apical. Crecimiento del tallo. Retraso de la senescencia foliar.
	GIBERELINAS	Yemas apicales y raíces (meristemos), hojas jóvenes, embriones.	Estimulan la floración en plantas de día largo y en bienales. Elongación del brote. Regula la producción de enzimas hidrolíticas en semillas.
	ETILENO	Frutos en maduración. Nudos de tallos. Hojas y flores senescentes.	Estimula la maduración de los frutos (especialmente los climatéricos). Induce senescencia en hojas y flores, así como la abscisión.
	ÁCIDO ABSCÍ-SICO	Hojas, tallos y frutos verdes	Cierre de estomas. Abscisión y latencia.

Plant Hormone Balance and Cycles



Any imbalance in these hormone cycles at any time can irreversibly reduce genetic expression

Fig. 1 Modes of action of *Bacillus subtilis* strain, FZB24 promting plant growth (Adapted from Kilian et al. 2000)



Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture

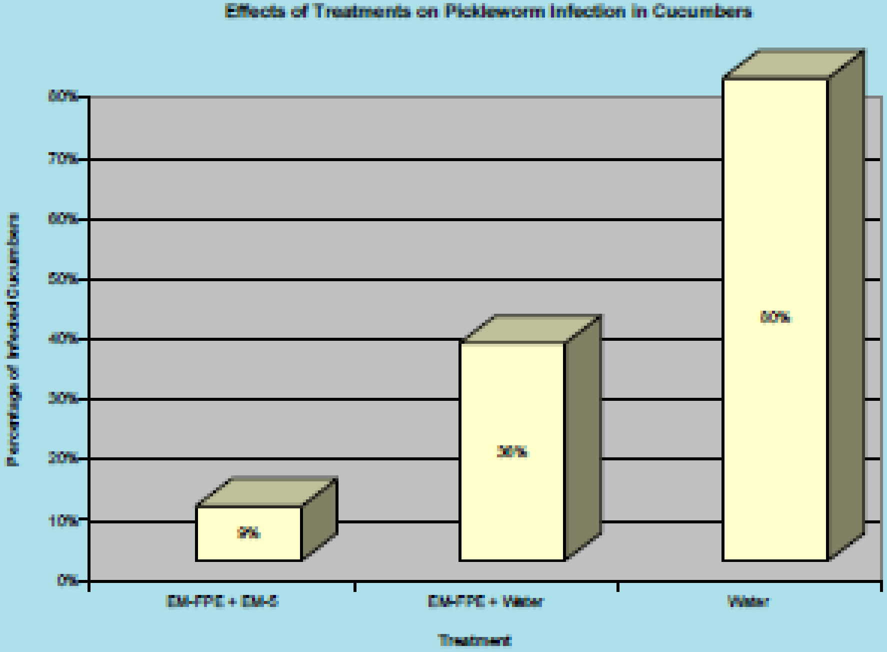
World J Microbiol Biotechnol (2012) 28:1327–1350

Ejemplos en:
CONTROL BIOLOGICO

Efficient Microbes (EM)® for Pest Management in Organic Cucumber Plants in the Humid Tropics

Presented by Matthew Wood, SCD LLC

Cucumber plants from treatments receiving only water.
Note the dead leaves with leaf blight.



As seen in the following figure, the cucumbers that received EM-FPE + EM-5 had very low incidence of pickleworm infection, followed by EM-FPE + Water. Cucumbers that received only water had much higher pickleworm infections than treatments receiving EM inoculants. All of these differences are significantly different using Duncan's Multiple Range Test.

Foliar application of Bacillus subtilis AUBS1 reduces sheath blight and triggers defense mechanisms in rice. J. Jayaraj et al 2004

Table 1. Effect of foliar application of *Bacillus subtilis* AUBS1 in control of rice sheath blight and on total biomass production under greenhouse conditions

Treatments	Disease index (%)*		Biomass (g/plant)	
	Trial 1	Trial 2	Trial 1	Trial 2
B. subtilis	18.8 b	24.5 c	14.2 a	13.5 a
Carbendazim	15.3 c	20.5 b	12.3 b	13.2 a
Control	33.5 a	40.8 a	8.7 c	10.2 b

Table 2. Survival of *Bacillus subtilis* AUBS1 on rice leaves after foliar application under greenhouse conditions

Days after application	Population of *B. subtilis* (CFU/g of leaf)
5	5.2×10^5 a
10	15.0×10^4 b
20	5.8×10^4 c
30	2.1×10^3 d

The bacterial suspension at a concentration of 2×10^7 CFU/ml was sprayed onto 60-day-old rice plants. Phyllosphere population of *B. subtilis* in the treated rice leaves at different time intervals was estimated by a dilution plate method using NA medium supplemented with ampicillin (50 µg/ml) and erythromycin (25 µg/ml). Each value is the mean of six replicates.
Data followed by the same letter in a row do not differ significantly from each other according to Duncan's multiple range test (DMRT).
Log transformation of the data was carried out prior to analysis by DMRT

Effects of Foliar Application of *Bacillus subtilis* Osu-142 on the Yield, Growth and Control of Shot-Hole Disease (Coryneum blight) of Apricot. A. Esitken et al 2002

Table 1. The effect of *Bacillus* OSU-142 on average fruit weight, yield, TSS, acidity, shoot length and diameter, and shot-hole disease incidence and severity in apricot cv. '*Hacıhaliloğlu*' in 2000.

Treatment	Fruit weight (g)	Yield (kg/tree)	Total soluble solid (%)	Acidity (%)	Shoot length (cm)	Shoot diameter (cm)	Disease incidence (%)	Disease severity
Control	27.43	31.6 b	22.10	0.25	22.74 b	0.38 b	10.33 a	9.16 a
A	30.12	41.0 a	21.80	0.28	28.61 a	0.49 a	5.00 b	2.63 c
B	28.39	33.2 b	21.70	0.28	24.35 b	0.47 a	7.67 ab	4.72 bc
C	28.52	33.0 b	22.06	0.28	23.66 b	0.43 ab	10.00 a	7.61 ab
SED	NS	1.99***	NS	NS	3.86**	0.07**	3.35*	4.15**

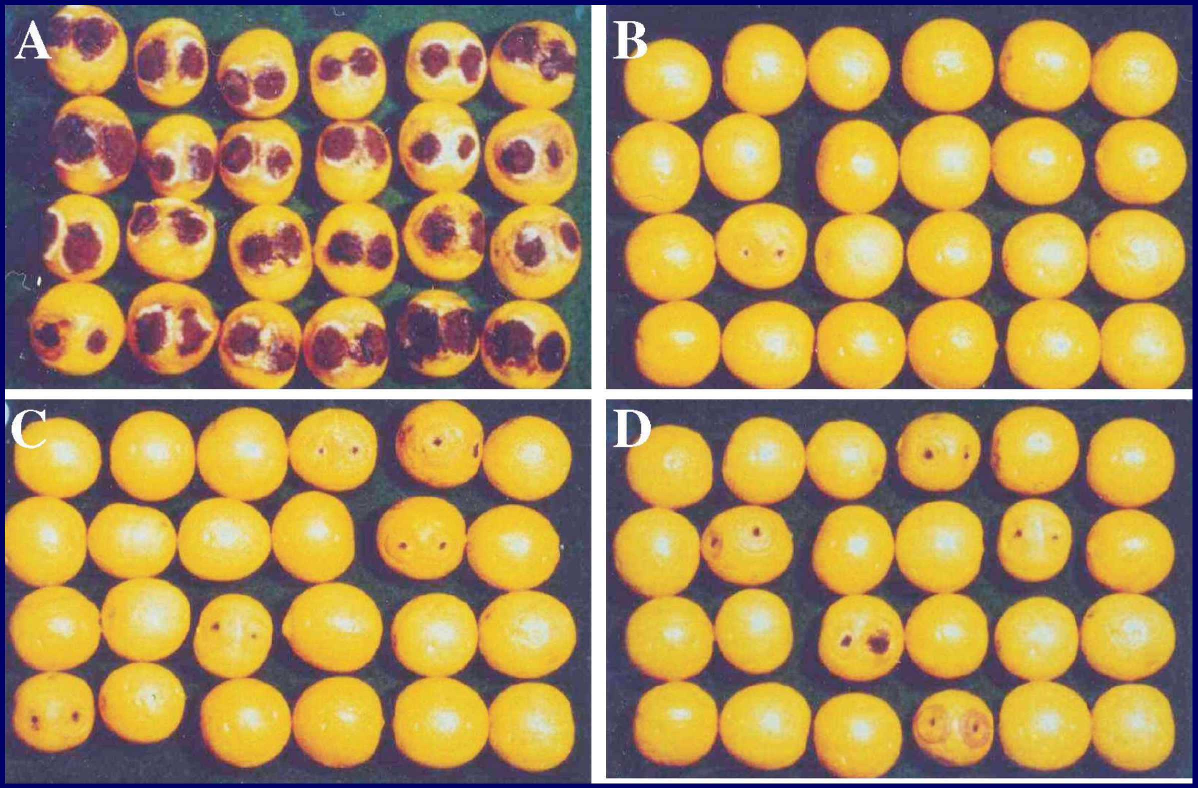
Table 2. The effect of *Bacillus* OSU-142 on average fruit weight, yield, TSS, acidity, shoot length and diameter, and shot-hole disease incidence and severity in apricot cv. '*Hacıhaliloğlu*' in 2001.

Treatment	Fruit weight (g)	Yield (kg/tree)	Total soluble solid (%)	Acidity (%)	Shoot length (cm)	Shoot diameter (cm)	Disease incidence (%)	Disease severity
Control	26.61	30.83 b	21.73	0.27	19.11 b	0.357 b	29.00	5.81 a
OSU-142	28.49	58.50 a	20.00	0.27	25.24 a	0.475 a	24.67	3.43 b
SED	NS	11.46***	NS	NS	4.56**	0.096**	NS	1.83*

NS = Not Significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

Whole cells of *Bacillus subtilis* AF 1 proved more effective than cell-free and chitinase-based formulations in iological control of citrus fruit rot and groundnut rust K. Manjula, G. Krishna Kishore, and A.R. Podile

Fig. 5. Control of soft rot of lemons caused by *A. niger* with midlog-phase cells, CCF, and purified NAGase of *B. subtilis* AF 1. Wound sites on lemons were inoculated with 5 µL of cell suspension (10^8 cells·mL^{-1}), CCF (1.0 mg total protein·mL^{-1}), or NAGase (0.1 mg total protein·mL^{-1}) with phosphate buffer as the control followed by inoculation with 5 µL of *A. niger* spore suspension (10^5 spores·mL^{-1}). (A) control; (B) midlog-phase cells; (C) CCF; (D) purified NAGase.



Ejemplos en:
PROMOCIÓN DIRECTA

Potential for Augmentation of Fruit Quality by Foliar Application of Bacilli Spores on Apple Tree. Choong-Min Ryu, et al 2011

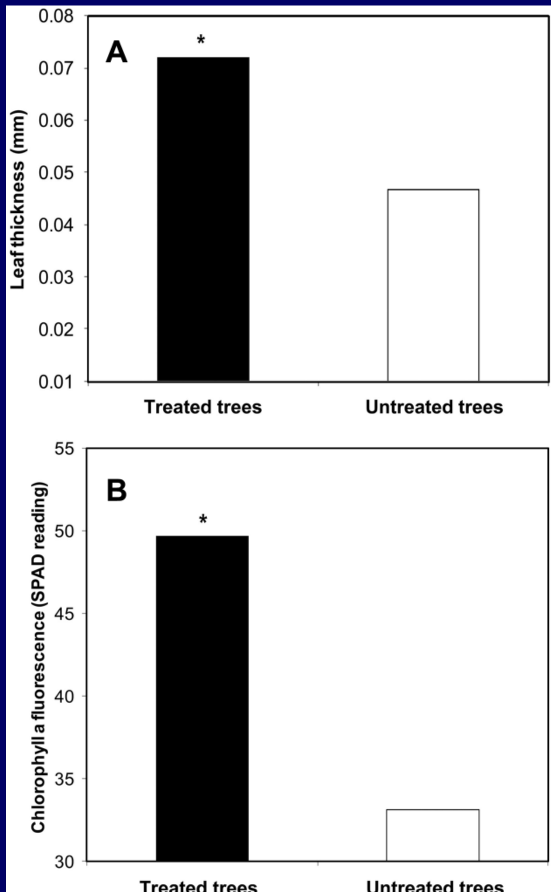


Fig. 2. Leaf growth promotion by foliar application of bacilli spore. The leaf growth parameters including leaf thickness and chlorophyll contents were measured 3 months after the initial application of the biopreparation. (A) Enhancement of leaf thickness by the biopreparation. (B) Enhancement of chlorophyll a fluorescence by the biopreparation. The mean values of five replicates are shown. Asterisks indicate statistically significant differences compared to untreated control plants ($P = 0.05$).

Fig. 1. Effect of bacilli spore preparation on apple growth. The biopreparation at 10^7 CFU/g spray was applied to the foliar parts of apple trees cv. Fuji from May 16 to October 27, 2009, every two weeks through the use of an automatic spray machine. (A) Representative photograph taken 6 months after the first foliar application of the biopreparation. (B) Representative photograph of the control plant taken around the same date.

XXIX CONGRESSO NACIONAL DE MILHO E SORGO - Águas de Lindóia - 26 a 30 de Agosto de 2012

Inoculação com *Azospirillum brasilense* Via Foliar Associada à Doses de Nitrogênio em Cobertura na Cultura do Milho

José Roberto Portugal[1*], Orivaldo Arf[1], Walter Vagaes Longui[1], Douglas de Castilho Gitti[1], Marla Karyne Felippi Barbieri[1], Alex Rangel Gonzaga[1] e Donário Silva Teixeira[1]

[1] Universidade Estadual Paulista – UNESP - Ilha Solteira, SP.* jrp_agro@yahoo.com.br

Tabela 3. Valores médios de população final de plantas (PF), número de fileiras de grãos por espiga (FG), diâmetro (DE) e comprimento de espiga (CE), massa de mil grãos (MMG) e produtividade (PROD) de milho em função da inoculação via foliar com *A. brasilense* e aplicação de nitrogênio em cobertura. Selvíria (MS), 2011/12.

Tratamentos		PF (plantas ha^{-1})	FG – n° –	DE ——— mm	CE ———	MMG — g —	PROD kg ha^{-1}
Inoculação (I)							
Presença		43595 a	17,47	49,50	17,88	314,00	6.751 a
Ausência		40972 b	17,61	49,97	17,75	314,62	5.883 b
Doses de N (D)							
0 kg ha^{-1}		43518	17,52	49,44	178,00	306,19	6.273
30 kg ha^{-1}		41666	17,47	49,25	176,12	312,95	6.353
60 kg ha^{-1}		41512	17,62	49,96	179,87	319,44	6.172
90 kg ha^{-1}		42438	17,55	50,29	178,54	318,65	6.470
Valor de F[(1)]	I	4,90*	0,51 NS	2,72 NS	1,92 NS	0,02 NS	8,07**
	D	0,60 NS	0,11 NS	2,71 NS	2,83 NS	2,20 NS	0,17 NS
	IxD	2,60 NS	0,14 NS	1,48 NS	3,55 NS	0,26 NS	0,70 NS
CV (%)		7,93	3,10	1,65	1,47	3,72	13,69

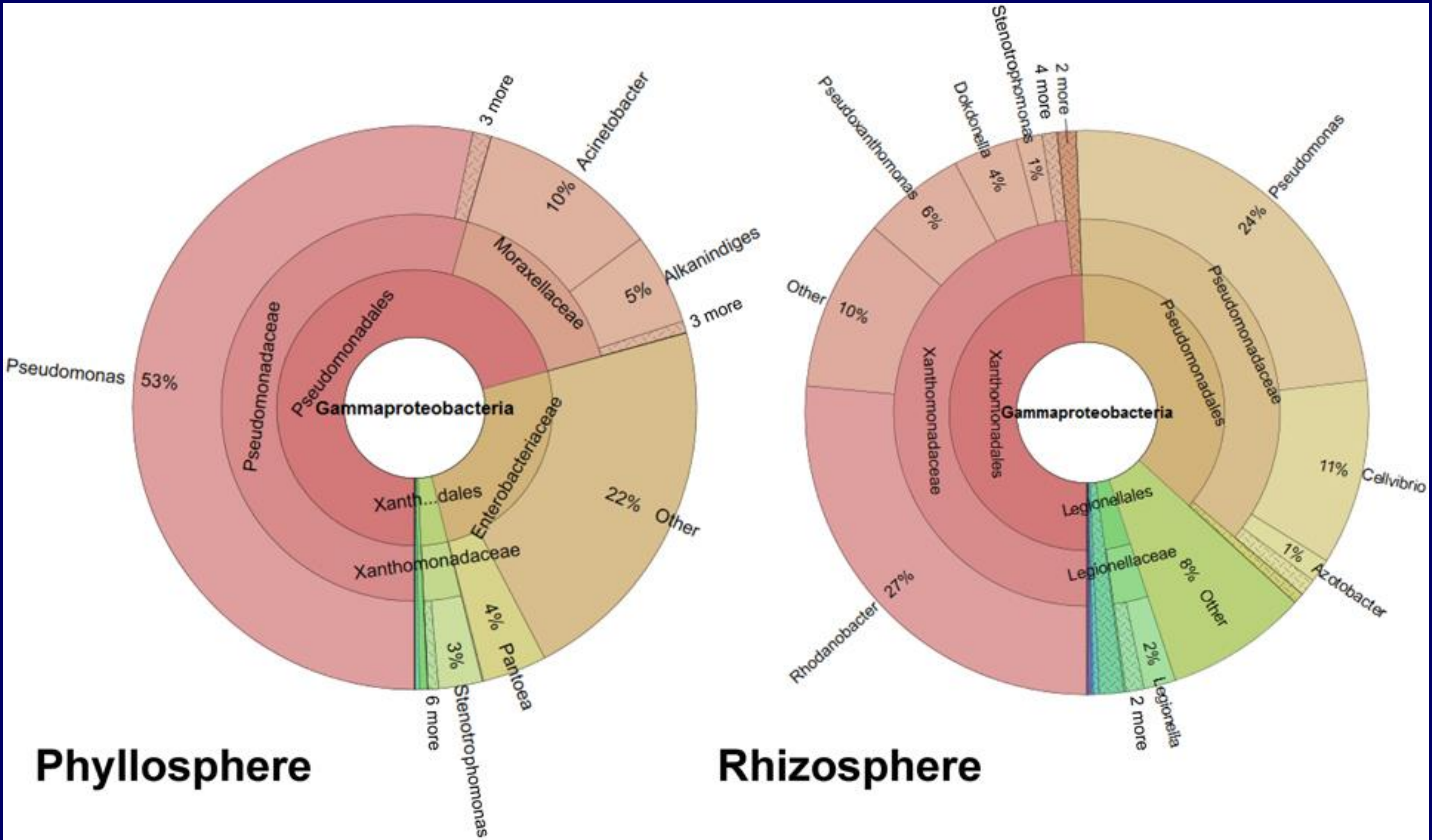
Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry A. Esitken et al. / Scientia Horticulturae 110 (2006) 324–327. The results of the present study suggested that Pseudomonas BA-8 and Bacillus OSU-142 alone or in combination have a great potential to increase the yield, growth and nutrition of sweet cherry plant.

Biocontrol activity of *Trichoderma viride* and *Pseudomonas fluorescens* against *Phytophthora infestans* under greenhouse conditions Ephrem Debebe Zegeye *et al* . Journal of Agricultural Technology 2011, Vol. 7(6): 1589-1602
This study revealed that the foliar application of *T. viride*-ES1 has good potential in controlling the late blight disease of potato.

Foliar Colonization and Growth Promotion of Red Pepper (Capsicum annuum L.) by Methylobacterium oryzae CBMB20. Minkyoung Lee et al J. Appl. Biol. Chem. 54(2), 120-125 (2011). Comparative results of inoculation methods clearly demonstrated that soil+foliar inoculation of M. oryzae CBMB20 lead to the highest biomass accumulation and nutrient uptake which may be due to its efficient colonization in the red pepper rhizosphere and phyllosphere.

EFECTOS SOBRE EL MICROBIOMA

The impacto the pathogen *Rhizoctonia solani* and its beneficial counterpater *Bacillus amyloliquefaciens* on the indigenous lettuce microbiome ***ArminErlacher1,2, MassimilianoCardinale1,2, RitaGrosch3, MartinGrube2 and GabrieleBerg1****



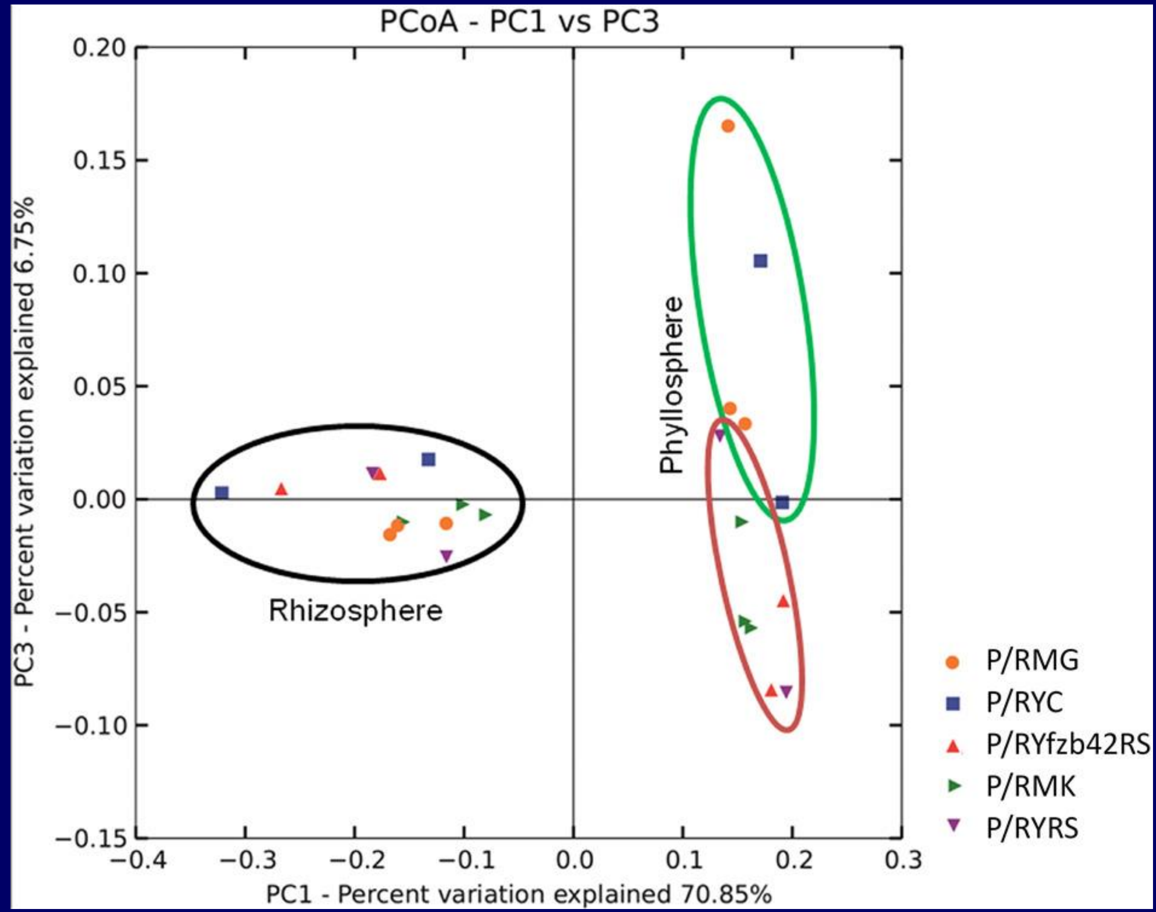
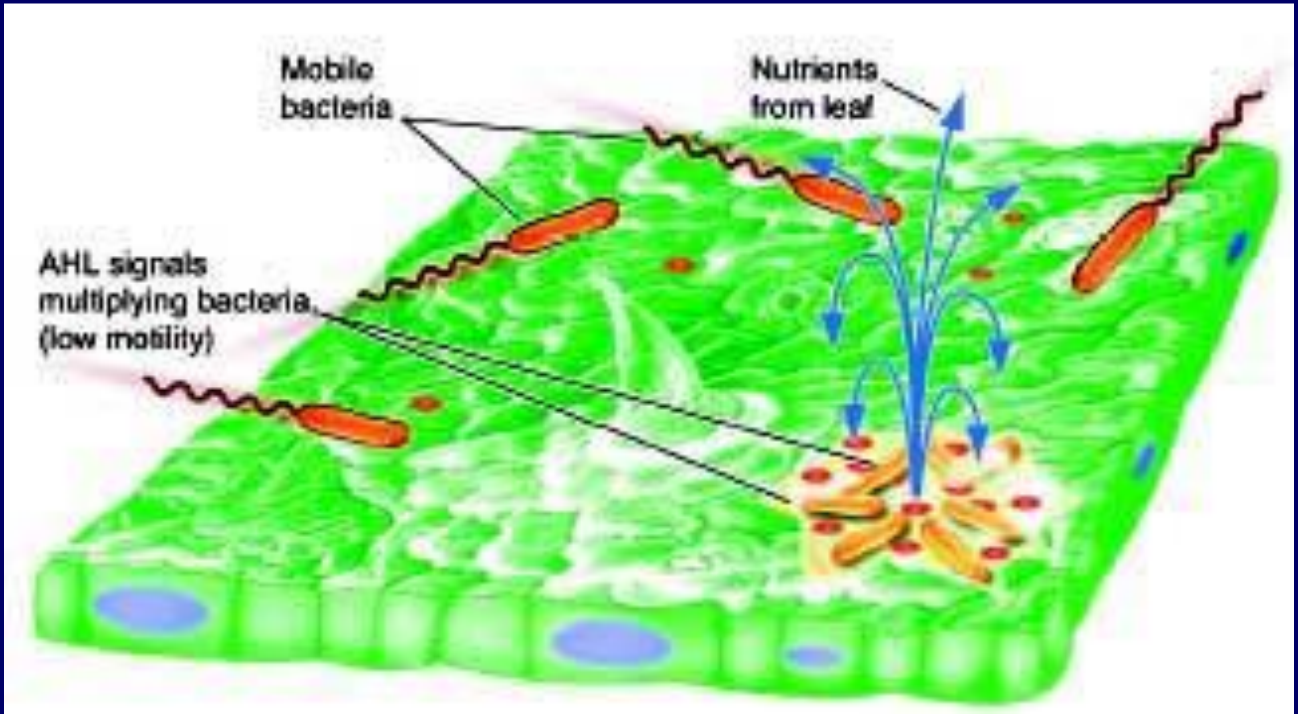


FIGURE 3 | Beta diversity metrics of bacterial 16S rRNA genes reveal distinctly clustered Gammaproteobacteria communities structured between healthy plants and plants affect by *Rhizoctonia solani*. Beta diversity community clustering is observed for phylogenetic beta diversity metrics (weighted UniFrac). In the panel, each point corresponds to a sample from either the lettuce rhizosphere (black) or the phyllosphere (green and red). Red—samples inoculated with *R. solani;* green—untreated control group. The percentage of variation explained by the plotted principal coordinates is indicated on the axes.



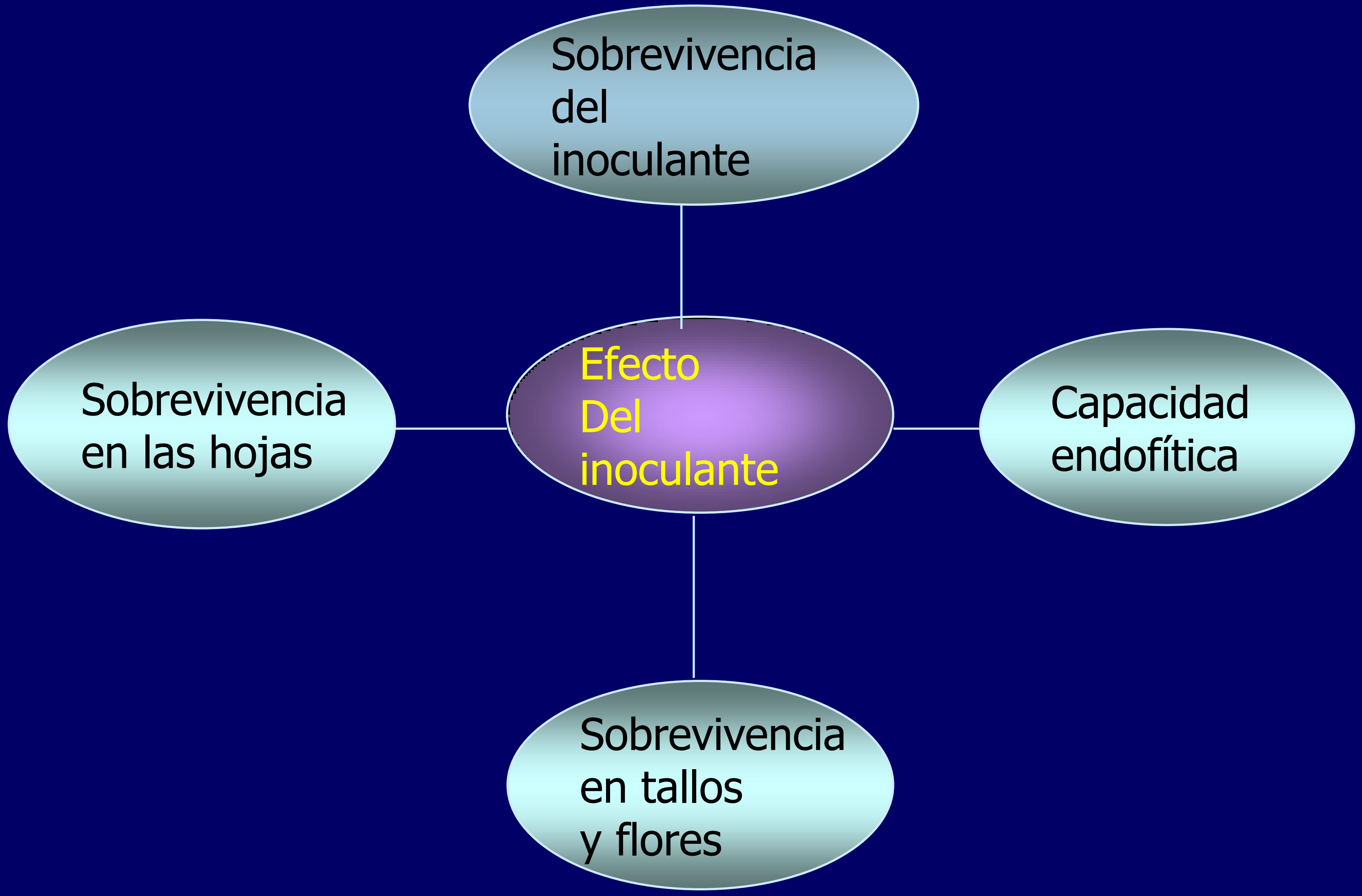
Steven E. Lindow
Ecology of Plant-Associated Microorganisms

How bacteria on plants benefit from cooperating with each other to ensure their survival or to interact with plants. We have found that a large percentage of the cells of pathogens such occur in aggregates on leaves. n-(3-oxo-hexanoyl)-L-homoserine lactone (AHL) is blocked by mutation, the epiphytic survival of mutants under stressful conditions on leaves was greatly reduced. Since the production of AHL quorum sensing molecules by this species is required for stress tolerance on leaves, the disruption of intercellular signaling on leaves could be an effective strategy of minimizing microbial colonization of leaves.

Estudios en Argentina o uso

Aplicación foliar solo se encontraron productos comerciales para el caso del género *Bacillus* registrados en los que se menciona aplicación foliar en todos los cultivos prácticamente. En algunos casos trigo, cebada, maiz, soja se han determinado efectos positivos similares a los observados en la bibliografía.

***Pseudomonas* se han realizado estudios de biocontrol para enfermedades de arroz no hay productos comerciales.**


Sobrevivencia
del
inoculante
Sobrevivencia
en las hojas
Efecto
Del
inoculante
Capacidad
endofítica
Sobrevivencia
en tallos
y flores

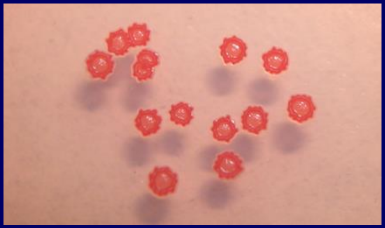
FORMULACION DE MICROORGANISMOS BENEFICIOSOS

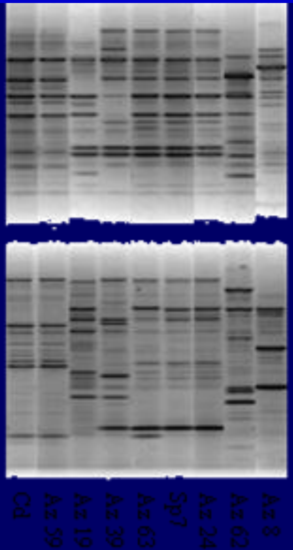
ESTADO	SOLIDO Polvo Granulado	LIQUIDO Oleoso Acuoso Polímero
METODO DE PREPARACION	SECADO - (Spray, Liofilizado, Aire) IMPREGNADO - Sobre sólido seco	- Cultivo - Cultivo mezclado con otro soporte liquido - Cultivo impregnado en soporte sólido y mezclado con otro soporte líquido
METODO DE APLICACION	SEMILLAS - Preinoculación - Polvo sobre semillas a la siembra SUELO - En surco	SEMILLAS - Preinoculación - Líquido sobre semillas a la siembra SUELO - En surco **PARA FOLIAR ??????**

JLBoiardi 2007

Control de calidad PGPM – Protocolos validados - REDCAI

Identificación de cepas presentes





Número en hojas, flores, tallos etc.

Número por g/ml de inoculante – Recién comienza

Actividad Biológica

- ✓ **¿En las hojas los microbios del inoculante persisten?**

- ✓ **¿Hay estandares de calidad de los inoculantes espécificos para aplicación foliar ?**
- ✓ **¿La coinoculación es un tecnología completa?**

- ✓ **¿Los microbios promotores en formulados inoculantes toleran condiciones ambientales adversas?**

- ✓ **¿Las plantas favorecen la promoción o todas las variedades son iguales en estos aspectos?**

Prospectivas:

-

En un contexto de necesidad de aumentar la productividad con altos costos de fertilización química el empleo de microorganismos promotores del crecimiento vegetal adquiere relevancia y tiene amplias posibilidades de insertarse en el sistema de producción actual y futuro.

Para que su empleo tenga credibilidad en el sector productivo no deberían acelerarse los tiempos de desarrollo y de evaluación de los inoculantes elaborados con bacterias solas o combinadas.

Muchas gracias!!!!!!!