Beneficial microorganisms: the best partner to improve plant adaptative capacity

Beatriz Ramos-Solano*1, Ana García-Villaraco 1, Enrique Gutierrez-Albanchez2, Estrella Galicia 1, Jose A. Lucas 1, M.Belen Montero-Palmero 1, and F. Javier Gutierrez-Mañero 1

1 Facultad de Farmacia, Universidad San Pablo-CEU Universities; Ctra. Boadilla del Monte km 5,300, Madrid 28668, Spain.
2 Biobab R&D. Bajada a Vargas 1, Agüimes, 35240 Las Palmas, Spain
* Corresponding author: bramsol@ceu.es
Abstract:
Currently, the world is facing a high population increase as well as climate change involving global warming, water shortage which limits agronomic productivity, necessary to achieve food security for the growing population. As sessile organisms unable to run away from danger, plants are endowed with sophisticated mechanisms to overcome all stressing situations for survival, involving an enormous amount of chemical molecules, specific for each situation. In addition, they establish intimate relationships with beneficial microorganisms creating the plant microbiome. Within this microbiome are beneficial bacteria, known as Plant Growth Promoting Rhizobacteria (PGPR), which represent a great tool to boost plant fitness in different aspects, as they are able to trigger multiple targets simultaneously.

The present work describes the physiological mechanisms involved in plant adaptation to water stress, nutrient absorption, and adaptative responses to biotic stress and how bioeffectors are able to modulate these responses, focusing on the mechanisms involved in plant adaptation to water stress (salinity and water shortage), plant innate immunity and general mechanisms involved in plant protection to pathogen outbreaks. A few examples in Solanum lycopersicum, Olea europea and Rubus sp illustrate effects of PGPR increasing plant adaptative capacity.

Keywords: beneficial bacteria, adaptation, water stress, food security
The world population is expected to triple between 1950 (2.5 billion) and 2050 (10 billion).


The rate of human population growth is greater than the rate of increase of food supply --> will lead to famine.

https://www.google.es/search?hl=es&authuser=0&biw=965&bih=575&tbm=isch&sa=1&ei=5otZW_jwLtGPlwSF7voDg&q=human+population+through+time&oq=human+population&gs_l=img.1.1.0j4i0j30k16.13399.16365.0.19489.16.10.6.6.0.101.719.9j1.10.0....0...1c.1.64.img..0.16.757...35i39k1j0i67k1.0.ided1OdXEdO#imgrc=KlQ2z6QbkeEoYM:
Globally, more than one billion people per year are chronically hungry

More than two billion people per year are chronically anemic due to iron deficiency
Scarcity of foods is not the only problem.....
Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. (FAO)

A major objective of plant science is to increase food production; current estimates indicate that we need to increase production by 70% in the next 40 years.
MILESTONES IN BIOTECNOLOGY ENABLING POPULATION GROWTH

INCREASES IN GLOBAL POPULATION ALWAYS ASSOCIATED TO AN INCREASED CAPACITY TO PRODUCE FOOD

Agriculture
BIOTECHNOLOGY
MENDEL'S LAWS
GREEN REVOLUTION

2000 Arabidopsis thaliana GENOME
2003 HUMAN GENOME
After a long period of strict abiotic concept of agriculture ………
SOIL IS A COMPLEX SYSTEM, AN ECOSYSTEM HOLDS AN ENORMOUS DIVERSITY OF ORGANISMS MICROORGANISMS ESTABLISH COMPLEX INTERACTIONS AMONG THEM AND WITH THE PLANTS THAT LIVE THEREIN

DIFFERENT SOILS, DIFFERENT PROBLEMS, DIFFERENT MICROBIOMES

- Microbial density: above $10^{11}$ per gram of soil.
- Highest diversity: more than $10^4$ different species per gram of soil
A MILESTONE IN THE BIOTIC COMPONENT OF THE SOIL

THE RIZOSPHERE

Lorentz Hiltner (1904)

EFFECT

Rhizosphere

Bacteria

Soil

MICROORGANISMS

SOIL

PLANTS

THE PLANT-MICROBE INTERACTION

Lynch (1990)

ECOSYSTEM
SOIL PLANT PATHOGENS

COMMENSALISTS, no direct effect on plant pathogen

PHOTOSYNTHESIS

ENERGY INPUT

AERIAL PLANT PATHOGENS

SELECTION

BENEFICIAL MICROORGANISMS

MICROBIOME SELECTION

SELECTION

SOIL PLANT PATHOGENS
Metabolic changes are triggered in roots and appear in leaves.

**A) INVOLVING PLANT METABOLISM**

Metabolic changes trigger secondary metabolism.

**B) AFFECTING EXTERNAL FACTORS:**

Nutrient mobilization and control of other microorganisms...

**MULTIPLE MECHANISMS SIMULTANEOUSLY TRIGGERED BY BENEFICIAL MICROORGANISMS**

- **EPIDERMIS IN A PLANT CELL**
  - Cell wall
  - Cytoplasm

- **Microbiome**

- **ELICITOR: A MOLECULE RELEASED BY BACTERIA**

- **Receptor**
  - Hormone or environmental stimulus

- **Signal transduction**
  - Signal transduction by second messengers
  - Gene activation

- **Response**
  - Activation of cellular responses

- **How do microbes improve plant fitness?**

**PLANT DEFENSE**

**BIOTIC STRESS**

- Intracellular Signal molecules (SA, JA/Et)
- Genetic expression changes
- Defense Molecules (PRs, PDFs, Phytoalexins)
- Systemic Signal molecules (MeSA, PiP, etc.)

**PTI: PATHOGEN TRIGGERED IMMUNITY**

**ETI: EFFECTOR TRIGGERED IMMUNITY**

**HOW DO MICROBES IMPROVE PLANT FITNESS?**
**BEYOND PLANT DEFENSE**

**PRIMING PHASE**
- ROS /gene activation/others

**POST CHALLENGED PRIMING STATE**
- Phytoalexin synthesis/PR proteins/other

**MORE ROBUST DEFENSE**
- More robust defense

**HOW DO MICROBES IMPROVE PLANT FITNESS?**

**HIGHER QUALITY OF PLANT FOODS DUE TO MORE BIOACTIVES (SECONDARY METABOLISM)**

- Higher quality of plant foods due to more bioactives (secondary metabolism)

**DEFENSE RESPONSE**
- Elicitation with Bioeffectors or elicitors
- Pathogen attack

**TIME**
- Elicited plant
- Non-elicited plant
PLANT MECHANISMS FOR ADAPTATION TO WATER STRESS

HOW DO MICROBES IMPROVE PLANT FITNESS?

PLANT MECHANISMS FOR ADAPTATION TO WATER STRESS

ABIOТИC STRESS (DROUGHT /SALINITY)

NECROSIS

PHOTOSYNTHESIS ARREST

GROWTH ARREST STOMATAL CLOSURE

ROS

PHOTOSYNTHESIS ARREST

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

OSMOTIC BALANCE CONTROL MECHANISMS

HKT

NHX

SOS1

MAPK PROTEINS Ca

+ ROS SCAVENGING + OSMOLYTE SYNTHESIS

HORMONAL MECHANISMS

GROWTH ARREST STOMATAL CLOSURE

PHOTOSYNTHESIS ARREST

ROS

ANTIOXIDANT MECHANISMS

HORMONAL MECHANISMS

ION FLUX CONTROL MECHANISMS

PREVENTING STOMATAL CLOSURE AND GROWTH ARREST, TARGETTING ANY OF THESE POINTS
HERVIBORES

FUNGI

LIGHT

$T^a$

BACTERIA

Fungi

PLANT MICROBIOME

MICROORGANISMS

ENVIRONMENTAL FACTORS

SECONDARY METABOLISM

ADAPTATION

METABOLITES INVOLVED IN:
PLANT DEFENSE
PLANT ADAPTATION TO STRESS

ENORMOUS METABOLIC DIVERSITY

THOUSANDS OF DIFFERENT MOLECULES

EVOLUTIVE STRATEGY TO ENSURE ADAPTATION

AIM: FIGHT PATHOGENS- HERVIBORES-
TEMPERATURE- SALINITY-WATER STRESS-
COMUNICATION

SURVIVAL
AGRONOMIC GOALS

MANIPULATING PLANT PHYSIOLOGY TO

▪ Lower water and fertilizer demand
▪ Improve nutrient content
▪ Improve drought tolerance
▪ Improve tolerance to other stresses
▪ Enhance pathogen resistance

A) GENETIC MANIPULATION
SINGLE TARGETS

B) BENEFICIAL MICROORGANISMS :
MULTIPLE TARGETS

PGPB / ELICITORS

OLEA EUROPEA

DECREASE IN WATER INPUT (~25%)

LOWER WATER DEMAND (BY 25%)

INCREASED HARVEST YIELD UP TO 20% ON WATER LIMITED PLANTS

ENHANCED C FIXATION

Bacillus sp.
Fresh weight (20 fruits) g

+80%
LOWERING CHEMICAL INPUTS
IMPROVING NUTRIENT CONTENTS IN TOMATO (Fe)

P6 CN | CONTROL WITH Fe

Fe (mg/Kg)

0 20 40 60 80 100 120 140 160 180 200

+16%*
Pseudomonas sp.
**DIFFERENT STRATEGIES TO IMPROVE PLANT ADAPTATION TO HIGH ABIOTIC STRESS (DROUGHT AND TEMPERATURE):**
- PHOTOSYNTHETIC PIGMENTS
- ANTIOXIDANTS (ENZYME AND NON-ENZYME)

### IMPROVE DROUGHT TOLERANCE & RESISTANCE TO OTHER STRESSES

**CONTROL ROS BALANCE**

<table>
<thead>
<tr>
<th>PIGMENTS</th>
<th>ANTIOXIDANTS</th>
</tr>
</thead>
</table>
| No pigment variation  
(2 strains) | K8  | H47  | —   | —   |
| Lower pigment contents  
(8 strains) | G7   | L44  | —   | L24  |
F3H (Flavonol-3-hydroxylase) PLAYS A PIVOTAL ROLE ON FLAVONOID METABOLISM IMPROVING ADAPTATION TO BIOTIC STRESS IN BLACKBERRY  

**MILDEW OUTBREAK**

- **VEGETATIVE**: SEPTEMBER
- **FLOWERING**: NOVEMBER
- **FRUITING**: FEBRUARY

**TRANSCRIPTOME**

qPCR  
TARGETED METABOLOMICS

<table>
<thead>
<tr>
<th></th>
<th>Flowers/m2</th>
<th>Production (Kg/plant)</th>
<th>Evolution of disease (%affected surface average)</th>
<th>Relative disease index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>237.95 ± 2.28 (a)</td>
<td>6.2 ± 0.22 (a)</td>
<td>15% (b)</td>
<td>100 ± 1.05 (b)</td>
</tr>
<tr>
<td><strong>QV15</strong></td>
<td>323.5 ± 1.77 (b)</td>
<td>6.4 ± 0.09 (a)</td>
<td>5% (a)</td>
<td>12.02 ± 0.36 (a)</td>
</tr>
</tbody>
</table>

_Bacillus amyloliquefaciens_  
PATENT P201730818
Control overexpressed genes: 173
Commonly expressed genes: 28,586
QV15 overexpressed genes: 367

- Photosystems I and II
- Chlorophyll biosynthesis
- SOD, APX
- Glutathione –S-transferase

Defence related:
- ser/thr kinases
- Fbox/FBD/LRR, ubiquitin ligases

Active vacular transport
Sugar metabolism

Active Defence:
- glucanases, chitinases
- GDSL Esterases/lipases
- Flavonoid synthesis
- Monoterpene synthesis
- Aufsatz kinase
- Fbox/FBD/LRR
- Ubiquitin ligases
- Active vacular transport
- Sugar metabolism

Active Flavonoid biosynthesis:
- Phenylpropanoid metabolism
- Flavonoid anthocyanin biosynthesis
- Sugar metabolism

Bacillus QV15

Settled Defense

More Photosynthetic pigments
Less destruction and higher synthesis

Lower SOD and APX activity
Less stress
Flavonol-anthocyanin pathway under the influence of *B. amyloliquefaciens*

- **Early Flavonol-Anthocyanin Pathway**
  - Phenylalanine → Trans-Cinnamic Acid → Cumaril 4 Hydroxylase → 4-Coumarate Coa Lyase → 4-Cumaryl-Coa → Chalcone Synthase → Naringenin Chalcone → Chalcone Isomerase → Naringenin → F3H
  - Naringenin Chalcone → Chalcone Isomerase → Narigenin Chalcone

- **Late Flavonol Pathway**
  - Phenylalanine → Trans-Cinnamic Acid → Cumaril 4 Hydroxylase → 4-Coumarate Coa Lyase → 4-Cumaryl-Coa → Chalcone Synthase → Naringenin Chalcone → Chalcone Isomerase → Narigenin
  - Narigenin → Flavonol 3′ Hydroxylase → Flavonol Synthase → F3′H
  - Flavonol 3′ Hydroxylase → Flavonol Synthase → Quercetin Synthase → DFR
  - Leucoanthocyanin Reductase → (+)-Catechin

- **Late Anthocyanin Pathway**
  - Cyanidin → Anthocyanin Synthase → Cyanidin
  - Cyanidin O-Glucosyl Transferase → Cyanidin O-Arabinoside

- **Catechin Pathway**
  - Cyanidin → Anthocyanin Reductase → (-)-Epicatechin

- Protection: 80%

- Bioactives enhanced, yield increased

https://doi.org/10.1371/journal.pone.0232626
EXPLORE WHAT NATURE DID FOR YOU

TAKE HOME MESSAGE

BENEFICIAL MICROORGANISMS SELECTED BY PLANTS & SORTED BY MEN

BIOFERTILIZERS

TRIGGER MANY TARGETS

IMPROVE PLANT FITNESS

ROS CONTROL

SUSTAINABILITY

FOOD SECURITY

ADVERSE CONDITIONS: DRY AND POOR SOILS

USE LESS WATER
USE LESS CHEMICAL FERTILIZERS
INCREASE YIELD IN SUSTAINABLE CONDITIONS

PRODUCTION IN DRY AREAS
USE OF LOCAL SOIL NUTRIENTS
INCREASED YIELD TO ALLOW LOCAL PRODUCTION
Acknowledgments

Project founded by Ministerio de Economía y competitividad:
Projects of I+D+I, government research program, innovation and development orientated to society challenges. Reference: AGL-2013-45189-R
Founding, Ministerio de Economía y Competitividad: BES-2014-069990

https://www.biotecnologia-vegetal-microbioma-ceu.es/
@powering plants
@plantaMicrobioma