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MINISTERIO DE CIENCIA E INNOVACIÓN

CSIC
Consejo Superior de Investigaciones Científicas

Estación Experimental del Zaidín

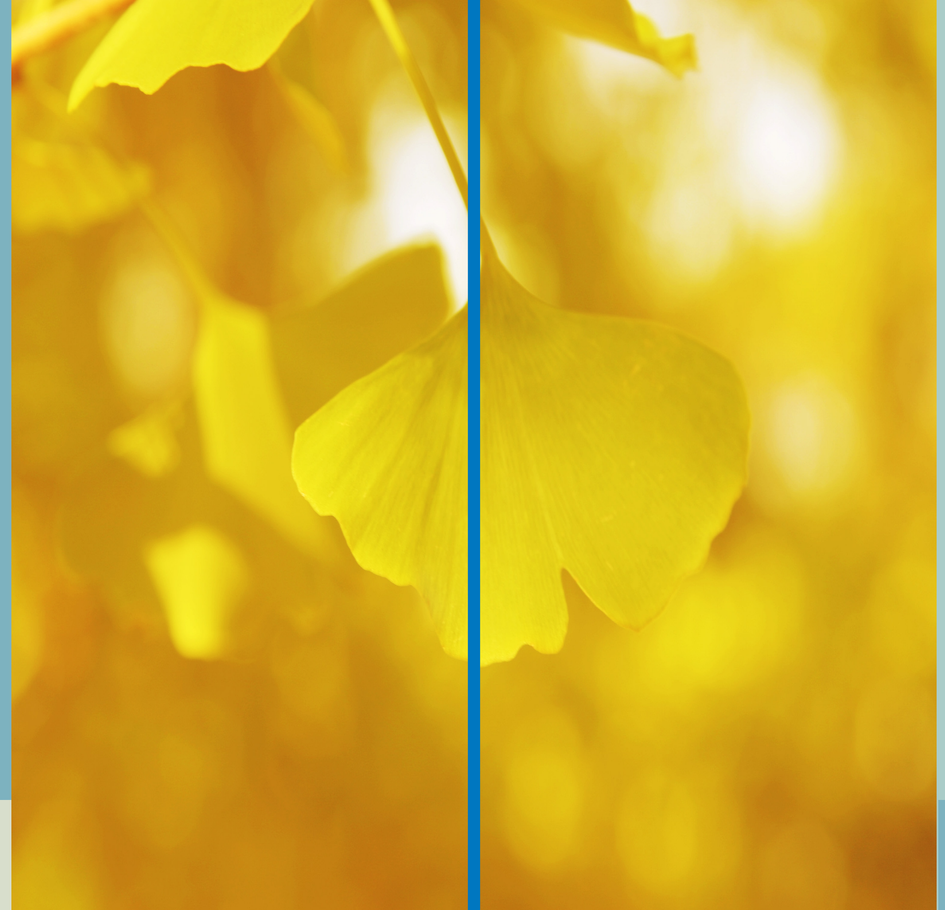
The Estación Experimental del Zaidín (EEZ) is a research Centre of the Spanish Council for Scientific Research (CSIC) Agency. The EEZ is currently the largest Research Centre of the CSIC Agency within the Area of Agricultural Sciences; hence the EEZ represents a leading Centre of the institution able to tackle the challenges of the Area with an innovative character. The EEZ is a Centre of reference in Europe that carries cutting edge research, especially in the fields of bioremediation, biotransformation of organic wastes and plant protection, simbiotic interacions between plants and microorganisms, and signalling, stress and development in plants. In the area of Animal Nutrition, the main research activities include protein and energy metabolism, nutritional requirements, quality of the products and ruminal metabolism.

The multidisciplinary character of the EEZ's research integrating different areas of knowledge places the Centre at an excellent position to significantly contribute to the development of sustainable agriculture and environmental protection.

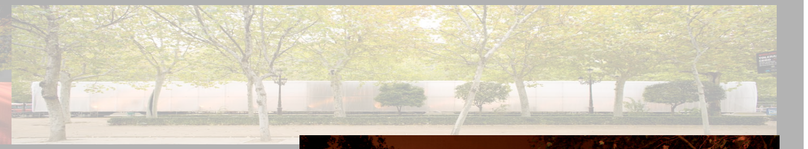
The integration of the EEZ's research activity in the local and regional scenario contributes to draw attention to the centre of the corresponding national and regional government departments and productive sectors in the Mediterranean region.

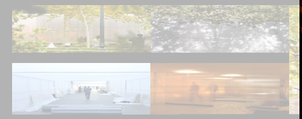
The microbial culture collections of Plant-Growth-Promoting-Microorganisms, Pseudomonas and Arbuscular Mycorrhizal fungi are of great value to the different activities at EEZ, and are also available for the use by external academic and industrial groups.

The Services will provide support to the research activities carried out at the Centre. The services are supervised by designed staff-scientists. These services are: Information Technologies, Knowledge Transfer, Science Outreach, Library, Radiochemistry laboratory, Greenhouses and Plant Growth Chambers, Scientific Instrumentation, Microscopy and DNA Sequencing.



SCIENCE OUTREACH





RESEARCH LINES AND SUBLINES

Biology and Biotechnology of Plant-Microbe Interactions

- Biofertilization and Biodegradation by Rhizospheric Fungi
- Ecology, Molecular Biology and Biotechnology of Mycorrhizas
- Molecular Plant-Bacteria-Environment Interactions
- Structure, Dynamics and Function of Rhizobacterial Genomes

Bioremediation and Biological Protection of Agricultural Systems

- Bioremediation, Rhizoremediation, and Molecular Biology of Pseudomonas of Interest in Plant Protection
- Biotransformation of Organic Wastes, Protection of Soil and Agricultural Crops

Signalling, Stress and Development in Plants

- Antioxidants and Cell Signaling by ROS and RNS
- Ion Homeostasis and Membrane Transporters
- Plant Reproductive Biology
- Redox Regulation and Chloroplast Metabolism under Adverse Environmental Factors

Mediterranean Pastures and Sylvopastoral Systems

Metabolism of Nutrients and Energy: Productive and Health Beneficial Aspects

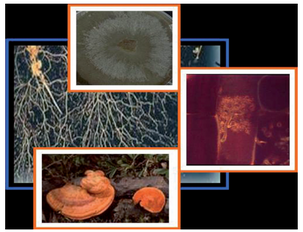
- Metabolic Responses to Nutrients Supply. Biological Activity of Specific Compounds
- Metabolism and Nutrients Utilization in Ruminants

Biology and Biotechnology of Plant-Microbe Interactions

Biofertilization and Biodegradation by Rhizospheric Fungi

Endophyte and saprobe fungi are considered the most important among rhizospheric fungi by their biofertilization and/or biodegradation abilities. Among endophyte fungi, arbuscular mycorrhiza fungi are considered the most important because their effect on plant growth by the formation of the mutualistic mycorrhizal symbiosis. Besides, the use of other fungal endophytes with biofertilization and biodegradation abilities such as dart septate fungi (DSE), ericoids fungi and some saprobe fungi allow us a more extended study on biofertilization and biodegradation processes carried out by fungi in the rhizosphere and open more expectatives of practical application of this fungal research.

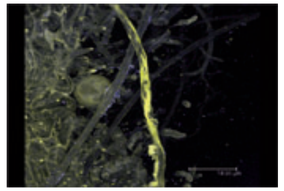
The use of the different fungal endophytes together with new plant cultures (including those with potentiality to obtain biodiesel), in presence of agro-industrial residues, will allow us to manage their biofertilizer and biodegradative abilities. Moreover, the possible sinergistic and antagonic interactions between these fungi and other rizosphere inhabitants, such as symbiotic bacteria of the genus *Rhizobium* and plant root endophytes such as *Orobanche* and *Striga*, and their common processes of root colonization and signaling and in their interactions with crop plants are also studied.



Rhizosphere fungi

Ecology, Molecular Biology and Biotechnology of Mycorrhizas

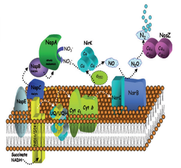
The mission of the sub-line is to carry out basic and strategic research aimed at promoting knowledge on the arbuscular mycorrhizal (AM) symbiosis, and its application, following a multidisciplinary approach. The knowledge on AM functioning should increase due to the impact of AM symbioses in sustainable agriculture (as key factor for the sustainable production of healthy foods, representing a drastic reduction of agrochemicals inputs) and in the improvement of environmental quality (by contributing to establish and/or maintain a diverse and stable plant cover and alleviating both biotic and abiotic stresses). The current research lines will be pursued and developed by integrating the forthcoming knowledge on plant and fungal genome sequencing. This team is well prepared for technology transfer (to both nursery and inoculum producer companies). Since a “spin off” company has been founded by members of this sub-line, applied biotechnology studies on AM and ectomycorrhiza will be implemented. This will support inoculum production and applications, and commercial developments.



Co-inoculation of *Azospirillum* and mycorrhiza in rhizosphere

Molecular Plant-Bacteria-Environment Interactions

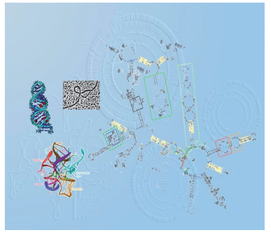
Legume Roots/*Rhizobium* Nodules



Denitrification pathway in *Bradyrhizobium japonicum*

Biotechnology represents a major sector in the agribusiness and will become crucial in the future considering current world-wide energy and environmental problems. Many agrobiotechnological approaches will deal at obtaining plant varieties resistant to biotic and abiotic stresses, but also with making use of natural biological resources and processes, such as interactions with microbes for plant protection and nutrition. Plant-associated bacteria may be beneficial or pathogenic. Understanding the mechanisms of both types of interactions as well as the subtle differences that determine the different outcomes can be exploited to optimise beneficial interactions as well as to limit the negative effects of pathogens. Likewise, the outcome of plant-bacteria associations depend on the individual capacities of interacting partners and their relationships with the environment. Particularly, beneficial microbes such as rhizobia for legumes, can provide their partners with novel capacities to optimise plant growth and nutrition in constrained, growth-limiting environments. The progressive replacement of agrochemicals with microbial inoculants in agriculture shall certainly contribute to alleviate current energy limitations and environmental pollution while maintaining reasonable crop productivities.

Structure, Dynamics and Function of Rhizobacterial Genomes



Sinorhizobium meliloti group II intron catalytic domain and structure

This sub-line is focused in the study of the genomes of rhizosphere colonizing bacteria, through the following approaches:

- Genetic and functional diversity of bacteria interacting with plants. The study of the genetic repertoire of rhizobacteria will be approached with the aid of novel high throughput sequencing technologies. First, suppression subtractive hybridization, multiple strain sequencing and genome comparisons will be conducted to characterize the genome of the nitrogen-fixing endosymbiont Sinorhizobium meliloti. Second, metagenomes from the rhizosphere of plants of agronomic and forestry interest will be characterized to gain insights on the functional relationship between metabolic and bacterial diversity in this ecological niche.
- RNome and riboregulation of gene expression. Computational comparative genomics and high throughput shut-gun cloning approaches will be undertaken to asses the structure and -proteobacterial RNomes. sRNAs will be functionally characterized as key components of regulatory networks operating in common adaptive responses of proteobacteria for the interactions with their eukaryotic hosts.
- Mobilome: basic and applied aspects of bacterial group II introns. Bacterial group II introns are highly efficient retroelements with modifiable specificity for DNA target sites. The mobility features of the S. meliloti group II intron RmInt1 will be implemented into new insertional mutagenesis protocols for the generation of mutant libraries of diverse rhizobacteria. Its possible application for gene knockdown in plants will be also explored.

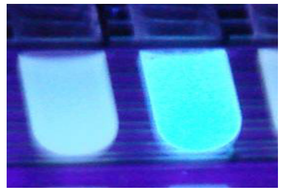
Bioremediation and Biological Protection of Agricultural Systems

Bioremediation, Rhizoremediation, and Molecular Biology of Pseudomonas of Interest in Plant Protection

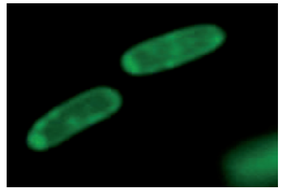
This sub-line focuses on the mechanisms underlying the key roles bacteria play in environmental recovery and plant health, and their biotechnological exploitation.

Research in biodegradation has been oriented towards the elimination of toluene, TNT and lindane. *Pseudomonas* was the microorganism of choice for most studies of this sub-line what has allowed gathering in-depth knowledge on the physiology and molecular biology of this model bacterium. The mutant collection assembled by the Pseudomonas Reference Culture Collection (PRCC) has been a valuable tool for the development of this research line. The research activitivies of the PRCC are directed to the construction of a collection of mutants in all ORFs of the *P. putida* KT2440. The work on the XylR/XylS and TodS/TodT regulators of two different toluene degradation pathways and TtgR and TtgV regulators of solvent efflux pump genes has been part of the aims of this sub-line in the biodegradation and transcriptional regulation fields. Biodegradation studies have been expanded to the removal of other contaminants such as PAHs and chloroaromatics by soil bacteria in aerobiosis and anaerobiosis.

From the point of view of plant protection, the study of *Pseudomonas* is particularly attractive. Thus, *P. fluorescens* and *P. putida* colonize the root surface and surrounding soil area (rhizosphere), promoting plant growth, mobilizing nutrients or protecting plants from harmful organisms through the induction of systemic resistance, or by direct mechanisms affecting survival of the pathogen. The molecular mechanisms involved in bacterial establishment and survival on plant surfaces and the role of signaling processes remain to be fully explored. Research is aimed at gaining insight into the bacterial genes, proteins and metabolites that play a role in competitive fitness, colonization, infection and defence.



Siderophore production by *Pseudomonas*



Cardiolipin fluorescence microscopy in bacterial membranes

Sustainable olive orchard management

Vermicomposting with *Eisenia fetida* earthworms

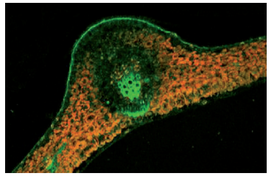
Biotransformation of Organic Wastes, Protection of Soil and Agricultural Crops

The overall goal of this research subline is to contribute to soil and crop protection through the use of low-cost bioremediation technologies and the promotion of sustainable development of agricultural systems by means of ecological alternatives. This general objective is approached from a triple perspective: (i) the development of the bioremediation processes, mainly vermicomposting, that favour the biotransformation, recycling and recovery of organic wastes, as well as the development of low-cost technologies for the prevention and protection of soil against organic contaminants; (ii) the development of an integrated management of pests in agroecosystems; and (iii) the development of methods to evaluate and to maintain the biodiversity in sustainable agrosystems.

Signalling, Stress and Development in Plants

Antioxidants and Cell Signaling by ROS and RNS

This sub-line studies the function of different reactive oxygen and nitrogen species (ROS y RNS) in the cell signal transduction pathways and expression of antioxidative systems during plant development and fruit ripening, and in the response to abiotic and biotic stresses, using physiological, biochemical, molecular and cellular approaches. Different plant species are used (pea, pepper, olive and Arabidopsis) which are subjected to abiotic stresses, mainly by cadmium, the xenobiotic 2,4-D (2,4-dichlorophenoxyacetic acid) and high solar radiation, and to biotic stress produced by the pathogen Pseudomonas syringae. The ROS and RNS studied include superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), nitric oxide (NO), S-nitrosoglutathione (GSNO), and peroxynitrite ($ONOO^-$). In plants, like in microorganisms and animals, these signal molecules seem to be involved in the cell communication systems (cross-talk), in the expression of specific genes of defence, and in the activation of the process of programmed cell death (PCD).

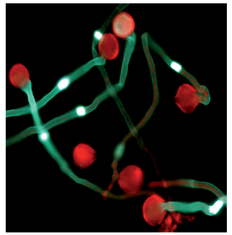


Localization of H_2O_2 in pea leaves

Ion Homeostasis and Membrane Transporters

This sub-line uses an integrated approach to study the biochemical and molecular mechanisms of ion homeostasis in plants in order to develop biotechnological applications to improve salt tolerance and mineral nutrition efficiency. The identification and function of transport systems of Na^+, K^+ and H^+ and their regulatory proteins are investigated in model systems (yeast and *Arabidopsis*) and crop plants (tomato). For this purpose we use in vitro and in planta functional analysis. In vitro approaches imply membrane protein purification and reconstitution in liposomes, whereas in planta analysis is mainly based on gene overexpression and silencing (RNAi) experiments.

Evaluation of the biotechnological potential in real conditions

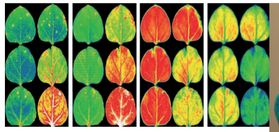


In-vitro olive pollen germination

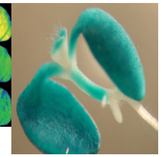
Plant Reproductive Biology

State of the art unanswered questions about plant reproduction implies a series of complex cellular interactions involving a continuous exchange of signals between the pollen and the pistil. Processes in this topic concern to male and female gametogenesis, compatibility, pollen-stigma interaction and mechanisms of gamete attraction and fusion, and fertilization mechanisms, among others. True comprehension of events such as signal transduction pathways among cells, and how asymmetries are established among and within single plant cells are aims of this sub-line. Cellular, molecular and proteomic approaches to determine the function of gene products (including allergens) in the pollen grain and the pistil are developed.

The success of reproduction is also an interesting topic under an agronomical perspective. Thus, during the next 50 years we will need to produce more and better quality food on an ever-decreasing amount of cultivatable lands. In this context, the knowledge of the genes and processes underlying plant reproduction will be essential to ensure a sustainable and affordable supply of food. Therefore, the major challenge for plant biologists and breeders will be to engineer new higher-yielding crop varieties without decreasing their quality by using these basic discoveries.



Multi-colour fluorescence imaging in tobacco leaves



Detection of market genes in *Arabidopsis* plants

Redox Regulation and Chloroplast Metabolism under Adverse Environmental Factors

This research sub-line focuses on the study of plant processes involved in redox signalling and efficient carbon assimilation, as well as on plant tolerance mechanisms to biotic and abiotic stresses. These studies combine physiological, biochemical, biophysical and molecular approaches using plants of agronomic interest such as pea and strawberry, besides other model plants like tobacco and *Arabidopsis*. The use of molecular, proteomic, crystallographic, immunological and imaging techniques (fluorescence, thermography, etc) allow us to decipher the molecular regulation mechanisms of complex processes in plants including carbon metabolism, key antioxidant systems and the response of the host plant to pathogen infection).

Metabolism of Nutrients and Energy: Productive and Health Beneficial Aspects

Metabolic Responses to Nutrients Supply. Biological Activity of Specific Compounds

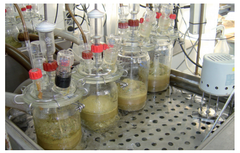
Two research groups are involved in this sub-line: one is devoted to studies on animal (single-stomached) nutrition, and the other to human nutrition. Work is firstly focused on nutrient and energy utilization, and to identify specific events and compounds involved in the metabolic response to food intake. In farm animal studies, attention is chiefly paid to pigs and poultry, and particularly to local breeds. The main task is the assessment of their balanced nutrition within sustainable, environmentally friendly animal production systems. Some specific objectives are: 1) defining animal nutrient and energy requirements for specific physiological functions; 2) evaluation of feed resources; 3) contributing to the development of experimental models to examine nutrition-health interactions in humans.

The study of the mechanisms of action and biological effects in productive (swine, poultry) and laboratory (rat) animals and in humans of bioactive substances is the second main topic within this sub-line. Some specific problems addressed are: 1) to determine the nutritional value of chemically well defined, purified fractions (proteins, carbohydrates, lipids) from feed- or foodstuffs; 2) the local (intestine) and/or systemic mechanisms of action of purified compounds such as protease inhibitors, Maillard reaction products, prebiotics, etc.; 3) the potential usefulness of dietary compounds as functional feed or food ingredients on gut microbiota, protein and lipid metabolism, cholesterolemia, oxidative stress, etc. together with the mechanisms involved.

Animal Nutrition Institute (IFNA)

Iberian pigs in a mountain agro-ecosystem

Goats in the IFNA facilities



Simulation systems of ruminal fermentation

Metabolism and Nutrients Utilization in Ruminants

The activity deals with nutritional studies in small ruminants (sheep and goats), in three main areas: (i) nutritive evaluation of conventional and non-conventional feedstuffs and products quality, (ii) study of the fermentation and microbial ecology in the rumen and the interaction of the microbes with the host immune system and (iii) environmental impact of ruminants production.

The study of the rumen fermentation and the microbial ecosystem currently represents the most important activity within this subline, given the key role that the rumen ecosystem plays in the nutrition of the ruminant. A wide range of in vitro techniques to study the rumen metabolism are currently used, which contributes to minimize the use of experimental animals. We are optimizing and developing new molecular tools which, combined with classical culture techniques, will contribute to better understand the interactions between microbes and of microbes with the host, especially with its immune system. In response to the increasing public concern on the environmental impact of animal production, we try to increase digestion efficiency and understanding the role of ruminal microorganisms on the fibre degradation, lipid metabolism and methane production by applying genomic technologies in order to maximize the use of lignocellulosic by-products and decrease methane and nitrogen emissions by ruminants.

Mediterranean Pastures and Sylvopastoral Systems

The general purpose of this research line is to increase the knowledge and understanding of pasture resources and silvopastoral systems in the most arid and extreme region of the Iberian Peninsula, the Southeast. Despite being one of the most significant biogeographical regions in Europe, it has hardly been studied and, therefore, it requires specific forest and rangeland research.

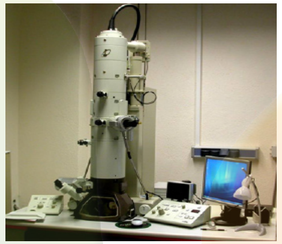
The investigation of this line includes the study of the characteristics of these systems and resources: their natural components, nutritional value of forage species, rangelands' carrying capacity and management alternatives. Consequently, this research line widely contributes to a better knowledge of those resources and has also helped enhancing the value of many previously despised natural areas and agro-ecosystems of the Iberian southeast.

Firebrake as part of sustainable sylvopastoral management

SERVICES

Greenhouses and Growth Chambers

Library



Confocal and Transmission Electron Microscopy

DNA sequencing

Scientific Instrumentation Service