Biotechnology for the challenges of the 21st century

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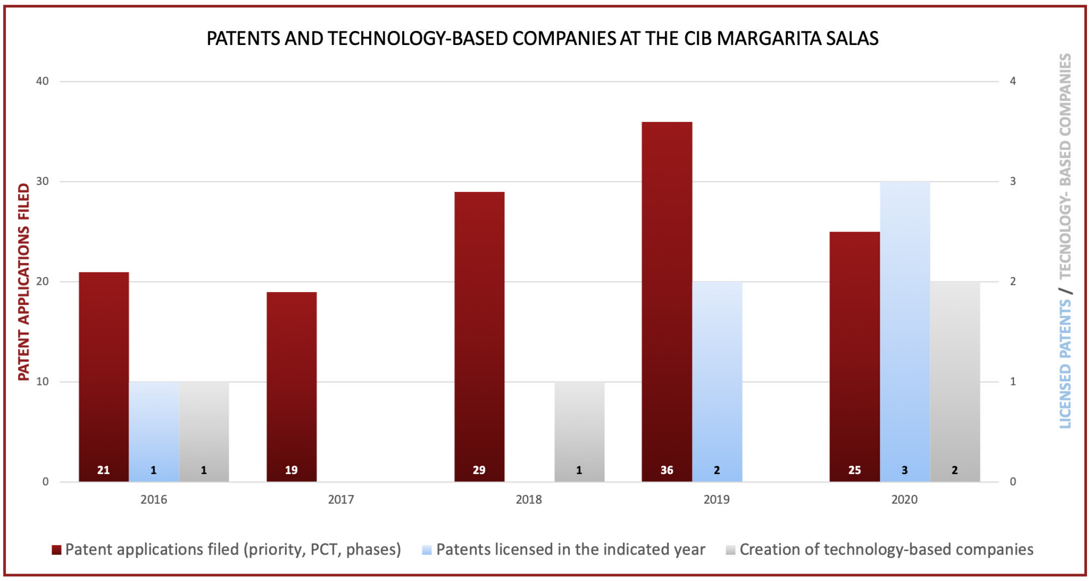
In this current pandemic, we are all aware of the importance and enormous utility of biotechnology, the discipline that produced the modern techniques used to develop the long-awaited COVID-19 vaccine. Not only has biotechnology produced vaccines in record time, but this field will be crucial to respond to the complex challenges that lie ahead, as laid out by the United Nations in 2015 in its Sustainable Development Goals (SDG), the aim of which is to eradicate poverty, protect the planet, and ensure prosperity for all. These ambitious goals entail profound transformations of our production systems, as well as measures to combat hunger, achieve food security and better nutrition, promote sustainable agriculture, combat climate change, and keep terrestrial and marine ecosystems safe from pollution, among others. Global well-being and quality of life depends on being able to achieve these objectives.

In the broad sense, biotechnology is any technological application that uses biological systems, living organisms, or parts/derivatives thereof to create or modify products and processes for specific uses. For centuries humanity has used biotechnological processes to make products such as beer, wine, cheese, and bread, all of which are produced by the action of live microorganisms. However, in recent decades biotechnology has undergone spectacular advances, increasingly contributing to fundamental daily activities, from pharmaceutical development to food production to the treatment of contaminants. In the Department of Microbial and Plant Biotechnology at the CIB Margarita Salas, 13 research groups are seeking to understand how plants, arthropods, and microorganisms interact and respond to their environment in order to develop biotechnological applications for agricultural, environmental, and industrial sectors. Our aim is to improve the resilience of plants to pathogens and new environmental conditions associated with climate change, develop novel strategies for the control of pests and diseases, accelerate breeding of crops, and harness the potential of microbiological systems for bioremediation of pollutants and the production of chemicals, biofuels, and biopolymers (plastics) from biomass or industrial waste, all via sustainable and circular economy systems. The transfer of technology and knowledge to productive sectors is a key objective of the department's research groups, as well as groups from other departments that participate in health-related biotechnology projects.

In this new issue of our newsletter, some of these projects and the latest achievements in these areas are described in more detail. We also present an interview with Prof. Auxiliadora Prieto, coordinator of the SusPlast Interdisciplinary Thematic Platform of the CSIC for sustainable plastics for a circular economy.

We depend on biotechnology to address the imminent challenges of the 21st century. However, we must not ignore the importance of expanding basic knowledge about biological structures and their dynamics, interrelations, and functions at the molecular, cellular, and whole-organism levels, knowledge to which we at the CIB Margarita Salas contribute through our research in **Biology for Global Well-being.**

A few numbers



Knowledge generation is the primary activity at the CIB Margarita Salas. Our researchers address important scientific questions relating to the structure and function of molecules, cells, and organisms. Following the scientific process, we share our findings with the global research community via specialized publications and scientific conferences. But the process does not end here. The knowledge generated acquires an additional dimension when it is transferred in the form of products and services to society, from where our funding comes. To facilitate this process, we have a Strategic Transfer Unit that is directly dependent on CIB Management, and also forms part of the Marketing Unit of the CSIC's Deputy Vice-presidency of Knowledge Transfer.

Thus, in the 2016–2020 period, the CIB Margarita Salas has filed 30 patent applications, which are necessary (although not sufficient) to ensure the development of products that reach the broader public. Subsequent potential steps include the licensing of patents to companies and the creation of companies by the researchers themselves in order to harness the knowledge generated. During this period, 6 patents have been licensed and 4 technology-based companies have been created. All of this is proof of our commitment to society and determination to apply knowledge obtained in the field of **Biology for Global Well-being**.

The CIB Margarita Salas, committed to the Sustainable Development Goals

Begoña García Sastre
Journalist (supported by a contract from the *Fondo de Garantía Juvenil*)

Sustainable development is defined as development capable of fulfilling present needs without compromising the ability of future generations to fulfil their own needs, guaranteeing a balance between economic growth, care for the environment, and social well-being. This concept was first described in 1987 in the Brundtland Report, which was prepared by different nations for the UN and warned of the negative environmental consequences of economic development and globalization. However, this term goes beyond the environment; it is about tackling human development by transforming economies and, above all, reducing social inequalities.

Many of the challenges facing today's society can only be addressed by promoting sustainable development from a global perspective, supported by three fundamental pillars: care for the planet, economic prosperity, and social well-being. This requires combined efforts to build a better future by all players in society: governments and public institutions, social entities, the private sector, and individuals all over the world.

With this objective of joining forces, in September 2015 all UN member states, in an unprecedented international agreement, approved the 17 Sustainable Development Goals (SDG) of the 2030 Agenda for Sustainable Development, which proposes to achieve those goals within 15 years. This agreement represents a strong commitment to redirect our steps towards a better future for the next generations around the planet.

The 17 Sustainable Development Goals are:

1. No poverty.
2. Zero hunger.
3. Good health and well-being.
4. Quality education.
5. Gender equality.
6. Clean water and sanitation.
7. Affordable and clean energy.
8. Decent work and economic growth.
9. Industry, innovation and infrastructure.
10. Reduced inequalities.
11. Sustainable cities and communities.
12. Responsible consumption and production.
13. Climate action.
14. Life below water.
15. Life on land.
16. Peace, justice and strong institutions.
17. Partnerships for the goals.

These are not stand-alone objectives: they are transversal and interact with one other. Therefore, they must all be addressed together to achieve the sustainable future that we seek. Objectives have been assigned to each goal that are measured using different indicators. While progress is being made in many areas, in general the measures aimed at achieving the objectives are not yet advancing with the requisite speed or on the necessary scale. Therefore, 2020 was selected as the beginning of a decade of highly ambitious actions. World leaders promised to mobilize funding and strengthen institutions to achieve the 2030 targets within the available time.

Research and innovation play fundamental roles in achieving these objectives. At the CIB Margarita Salas these goals are ever present, and are embedded in many of the center's lines of research. Moreover, the multidisciplinary nature of the CIB Margarita Salas means that research may simultaneously contribute to multiple SDGs. This Newsletter highlights some of the research lines focused on biotechnology, an area closely related to SDGs aimed at protecting the environment and modifying habits that have caused so much environmental damage over the years. Several key lines of research at the CIB Margarita Salas share some of these goals. For example, in line with goal number 2, ZERO HUNGER, several groups are studying pest control and sustainable agriculture. Protecting crops will prevent food losses and ensure that more people can be fed. Furthermore, if we manage to improve plant resistance to stressful situations such as drought, we will be able to grow crops in remote areas of the planet where nothing has grown to date. There are also many groups at the CIB Margarita Salas conducting biotechnological research that can contribute to achieving other SDGs related to the environment, including numbers 7, AFFORDABLE AND CLEAN ENERGY; 11, SUSTAINABLE CITIES AND COMMUNITIES; 12, RESPONSIBLE PRODUCTION AND CONSUMPTION; 13, CLIMATE ACTION; and 15, LIFE ON LAND; Several research lines seek to exploit plant biomass to improve industrial processes or manufacture biofuels. Others are attempting to harness the residues produced by certain industries to manufacture other products that until now were made using highly polluting processes. Yet others are researching bioplastics as an alternative to plastic derived from the highly polluting petrochemical industry. Research groups are also searching for biotechnological tools that can eliminate pollutants from the soil, air, and water, while others are focused on making plants more resistant to hostile environments in order to combat desertification. These are not the only SDGs addressed by researchers at the CIB Margarita Salas. Other important goals include number 3 (GOOD HEALTH AND WELLBEING), to which several groups are highly committed. Indeed, biomedicine is the other major branch of research that forms the backbone of the center's research activity. There are very promising basic scientific research lines that seek to understand the molecular bases of certain diseases and the search for therapeutic targets to facilitate the discovery of drugs and treatments for neurodegenerative and rare diseases and other alterations that threaten global health, such as cancer and COVID-19.

Another SDG to which the CIB Margarita Salas is highly committed is number 5, GENDER EQUALITY, fostering the visibility, advancement, and promotion of women in science. In February 2021, the equality commission was created in order to promote an integrated approach to gender at the CIB, and to implement measures to achieve the transversal principle of equality between men and women.

The effort to make the SDGs a reality in 2030 must be shared by all, both individuals and institutions, and the CIB Margarita Salas, aware of its role and commitment to society, puts its biological research at the service of a better future for global well-being. This strategy is key to solving the main challenges facing human, animal, and planetary health, in addition to converting basic research into industrial developments in these sectors to contribute to a more sustainable and healthy way of life.

SUSTAINABLE DEVELOPMENT GOALS

Auxi Prieto: "The environment, after our passage through it, must remain exactly as it was"

Carmen Fernández Alonso
PhD in Chemistry at CIB Margarita Salas

Environmental sustainability and environmental protection are key challenges within the Sustainable Development Goals. In this context, the problem of pollution generated by the massive use of plastic materials and their accumulation in the natural environment is cause for great concern about the risk to the environment and to human and animal health. The implementation of more sustainable processes is therefore an important goal, and bio-based plastics (bioplastics) represent an alternative to plastics derived from the petrochemical industry, thanks to their potential biodegradability and their production from renewable sources, such as biomass derived from waste.

For this issue of the Newsletter, we interviewed Prof. Auxiliadora Prieto, principal investigator of the Polymer Biotechnology group of the CIB Margarita Salas. Her laboratory focuses on the production of polymers of biotechnological interest such as bacterial nanocellulose, and bacterial polyesters such as PHA, one of the most promising bioplastics owing to its biodegradability. She is also the coordinator of the SusPlast Interdisciplinary Thematic Platform of the CSIC. The platform deals with the development of research activities and innovative processes, in addition to socio-educational strategies, in the context of plastics production and recycling, in order to implement plastic management processes based on a circular economy. In 2020, Auxi Prieto joined a group of experts coordinated by SAPEA (Science Advice for Policy by European Academies) to prepare a report on the biodegradability of plastics in the environment and to evaluate the problem of plastic pollution based on the latest scientific data.

Q | You studied pharmacy, but your research is in the field of microbiology and biotechnology. What led you to make that leap from your studies to your current job? What attracted you to biotechnology?

A | I studied pharmacy because I liked biology and chemistry and I liked understanding at the most basic level the molecular mechanisms that underlie life. Also, I had a very practical approach to science and wanted to be able to create something tangible that could be transferred to industry or society. When I was studying - I started my degree in 1985 - there was no such thing as a biotechnology degree, and pharmacy was what best suited my interests.

When I started studying microbiology in university I said to myself, "this is my thing!". The concept of the cell as a factory, of industrial microbiology, of using a living organism to produce something and transfer the chemical capacities of a catalyst to a cell, was something I found fascinating. When I finished my pharmacy studies I joined the CIB to do my doctoral thesis in biotechnology under the direction of Prof. José Luis García. I was very attracted to his research and his approach to it, as he came from industry and was working in areas related to genetic engineering and microbiology with industrial applications. With José Luis I started working in the field of environmental biotechnology and wrote my doctoral thesis on metabolic pathways for the degradation of aromatic compounds.

Q | When did you start working in bioplastics production? What does your research group do specifically, within this field?

A | I started in 1996 when I went to the Institute of Biotechnology of the Swiss Federal Institute of Technology (ETH) in Zurich, to Prof. Bernard Witholt's group. This was one of the first centres in Europe devoted entirely to biotechnology. I wanted to get more familiar with other aspects of environmental biotechnology and Prof. Witholt was one of the pioneers of bacterial bioplastic production. In fact, it was him who discovered the production of bioplastics in Pseudomonas. I joined his group as an EMBO postdoc. There, I trained in this field, I learned how bioprocess engineering could contribute to biotechnology, and I established a line of research studying and controlling the regulation of gene expression to produce bioplastics. This particular approach to projects, which differed from what my fellow engineers were doing, was what led to the establishment of the polymer biotechnology group (POLYBIO) that I currently lead at the CIB Margarita Salas.

My group uses a variety of tools, ranging from genetic and metabolic engineering to synthetic and systems biology, to create products that cover a broad spectrum of bacterial biopolymers, including bacterial cellulose, polyesters, and bio-based monomers that are subsequently used to generate bioplastics.

Q | What characteristics do bioplastics have? Are they all biodegradable?

A | This is a term that tends to cause some confusion. Generally, it refers to a plastic material generated through biotechnological processes, although in truth they can also be produced through chemical procedures. And the prefix "bio" can refer to either bio-based or biodegradable. Bio-based materials are polymers made from renewable sources such as potato starch or plant biomass. Most are biodegradable, but this is not always the case. For example, polyethylene can be produced from bioethanol, but is not biodegradable. Furthermore, there are bioplastics that are generated from fossil sources, such as polycaprolactone generated from petroleum derivatives, but which are also totally biodegradable. So this is a pretty broad concept. To avoid confusion, a bioplastic is a plastic that can be biodegradable and can be generated from renewable sources, but it does not have to simultaneously fulfil both criteria.

Q | What are the main applications of bioplastics?

A | Those in which a conventional plastic is not easily recyclable. For example, PET is easily recycled, so it might not make much sense to replace it with a biodegradable plastic, although it would be nonetheless interesting to produce it from renewable sources. Instead of thinking about the applications, we should think about the origin of the plastic itself. Ideally, the carbon footprint of the manufacturing process for any chemical compound would be zero. If a fossil fuel is used, the consequent mobilization of CO_2 means that these requirements are not met. Another factor that will determine if the application is suitable for bioplastics is whether it will be contaminated with organic material, as is the case with food packaging. Having to clean this product after it is mixed with other materials makes things more complicated. It would therefore be more appropriate to treat it in composting plants, where both food waste and these bioplastics can be processed, usually by bacterial fermentation. Another application is in the environment, from which plastics cannot be removed. This occurs with material used for agriculture, which is very difficult to recycle from a chemical point of view because it has to be collected and treated. If no other management strategy can be used, I think that a biodegradable or compostable plastic would be suitable.

Biodegradable bacterial plastics

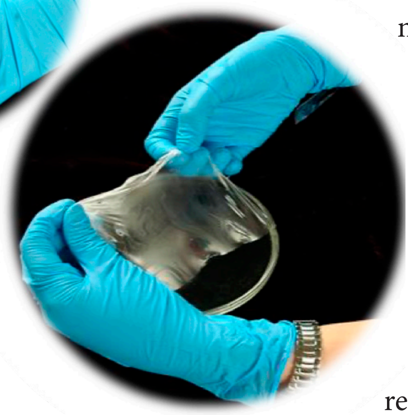
Q | What are the main advantages and disadvantages of bioplastics compared with other alternatives?

A | Let's start with the bad first, and save the best for last. The main disadvantage is that the mechanical and thermal properties that we can currently achieve with these materials are not always as good as those of plastics produced by the petrochemical industry. Polymer chemistry is very powerful right now, as it has advanced over many years and can produce excellent materials. Bio-based materials have been on the market for much less time. The greatest challenge is complying with biodegradability requirements while simultaneously ensuring that the material can be processed and has the properties necessary for the final application. Another disadvantage is the production capacity. Because bioplastics are produced from renewable sources, we need waste, biomass. This entails adequate management of organic waste, something we are currently lacking. Each European citizen produces around 1 kg of garbage per day. If we were able to manage this effectively, we would have enough organic matter to transform it into bioproducts. But this is not currently possible as we lack the necessary processing plants and capacity. Supplying a company with enough bioplastic so that it can test and process it is also an odyssey, as we do not have commercial suppliers of these materials, and specific projects have to be carried out to develop bioplastics for a given application. This hinders and delays technology transfer to industry.

The advantages of using bioplastics primarily relate to the environment. For example, avoiding microplastic pollution, which has proven extremely recalcitrant. It is true that bioplastics can also yield microplastics if not properly managed, or as a consequence of leaks in industrial composting plants. However, these are bioplastics that persist for weeks or, in the worst-case scenario, 50 years (and not hundreds of years like conventional plastic). There is no comparison. On the other hand, we must take into account the origin of bioplastics. In the context of the circular economy and especially in terms of CO_2 emissions, they differ greatly to conventional plastics.

Q | Are there bioplastics already on the market?

A | There are already many production plants, and materials are being generated right now for specific applications. My "frustration" is that there could be so much more done, because the technology exists. There is a great demand to comply with European regulations for the production of this type of material, but, as I mentioned before, a manufacturer needs "virgin" plastic to be able to develop it, process it, and generate the appropriate application. And this is where we are today, but we cannot produce it in sufficient quantities to supply all the companies that could potentially develop it. This is the bottleneck that we need to work on. There are many initiatives to build production plants and generate enough material. Many companies are trying to do this themselves. In this sense, demand is outpacing development: the technology exists, but the intermediate stage is where we are falling short. And this is because of insufficient prior investment. Development is dependent on regulations being implemented first. When regulations are in place, everything starts to happen. If we had thought about this when the technology became available, or if there had been enough investment to generate the virgin material necessary to perform tests, we would be ready right now. Now we have to make up for lost time ... We will succeed, but it will take time...



Q | Moving on from legislation, how can we help as citizens? What can I do at home to help manage waste in a more rational way?

A | It comes back to the 3 Rs: reduce, reuse, and recycle. The first thing to ask ourselves is whether we really need a given object. For example, if we go to the pharmacy or any store with a suitable bag, we don't need a plastic bag for our purchase. However, in the case of food packaging, plastic is sometimes necessary. This was evident during the pandemic. Even I, who generally try to avoid plastic, bought everything in packaging. Food packaging is necessary to prevent infection and ensure optimal food preservation. But when the goal is simply aesthetic, its use can be avoided.

Thus, it is always necessary to reduce as much as possible, and to always, always recycle! We cannot say to ourselves "I don't feel like it, it's a pain to separate the rubbish": we should always recycle, and follow the regulations set out by the local municipality and waste management unit, making sure we are doing it correctly. There is no other way.

I believe that we cannot live without plastics and I believe that they are very useful and we must continue to use them, but we must use them appropriately. The same goes for bioplastics. They are beginning to reach the market and it is important that we know how to manage them. It is not good enough to just use biodegradable plastics. We need to know how to properly manage them. In this context, authorities should already be thinking about how to do this. It is very easy to say "everything for composting goes in the brown container", but there are plastics that have enormous added value and that can be recycled.

Right now, only that which has commercial value is recycled. There are many things that are burned or not recycled properly because there is no economic benefit. Public authorities need to be on top of this issue, and need to identify the advantages from the environmental perspective, with a view towards the future. We need to determine what is recyclable, what is not, what is revaluable, what can we convert, what is pyrolysable. I am totally in favour of pyrolysis because the gas that is produced is usable. It does not matter if the carbon is in a solid material or is in gas form, as gas can be used later. What you cannot do is to release it into the atmosphere. There are materials that cannot be revalued or recycled, but that can be burned to generate energy, and if you recover the gas and reuse it, you continue to contribute to a circular economy. Incinerators have a terrible reputation because the gas they produce is not reused. In Copenhagen they have a wonderful gasification and pyrolysis plant where they are reusing all the gas produced (syngas or CO_2) for other purposes. Gas is a raw material. Syngas or CO_2 can be used to feed bacteria that can produce bioplastics. Furthermore, methane and syngas can be used in millions of chemical reactions to produce other compounds. What we have to keep in mind is that "nothing is thrown away". Ideally, everything, whether chemical or biological in origin, should be kept within the carbon cycle of the biosphere.

And we cannot forget the concept of multidisciplinarity. In ecology, there is a tendency to say that everything organic is wonderful, but "organic" makes no sense in the absence of chemistry. Chemistry must be combined with biotechnology to truly achieve its full potential and ensure that the generated product is of high quality. It makes no sense to produce bioplastic for a container only to have it break before you even get it home…

Q | You have recently been part of the group of experts that advised SAPEA on the preparation of an evidence report on plastic biodegradability. What aspects of this report would you like to highlight?

A | I think it is important to emphasize that the environment, after our passage through it, must remain exactly as it was. Biodiversity and environmental climatic conditions must be preserved. There are some very interesting environmental applications for bioplastics: for example, mulch covers or fishing nets. In these cases, plastic is useful, as these items cannot be made any other way. But what we have to keep in mind is that when we finish using that material, the environment must remain as it was. It is necessary to generate certification procedures for materials for specific uses. The use of a biodegradable material in the soil is not the same as in the sea, or in a river. Biotic and abiotic conditions are different. Marine microorganisms are different from those in the soil: their enzymatic degradative capacity is not the same. Therefore, there must be specific certifications for each habitat.

Also, there must be an educational commitment. People must be taught the difference between compostability and biodegradability. Something compostable is something that you control: a container of food for example. If it is compostable it has to be managed, degraded in a confined system with controlled temperature, pH, microorganisms, etc. In the environment, this process would be uncontrolled, and would vary depending on the habitat in question. Materials should be certified and labelled so that members of the public know what to do with it, and where to dispose of it. If it can be composted it will go in a specific container, and if not, this should be clearly indicated. We don't have to know how a fishing net is recycled or managed, but a fisherman does. If I buy a material to make a fishing net that is similar to nylon, but is of organic origin or is biodegradable, I have to know if I can use it in the river, if I can use it in the sea, and the manufacturer has to also specify how it should be disposed of after. It doesn't have to be complicated: just as there are symbols indicating whether a product is recyclable, there should be symbols to indicate where it can be used and under what conditions, and in which container it should be disposed of.

Although these regulations exist, they need to be much more demanding. The approach used nowadays is known as "green washing": the seller tags a product with the words "bio" or "organic", and this is considered sufficient. We have to demand regulations that prevent this and oblige producers to comply with a series of requirements.

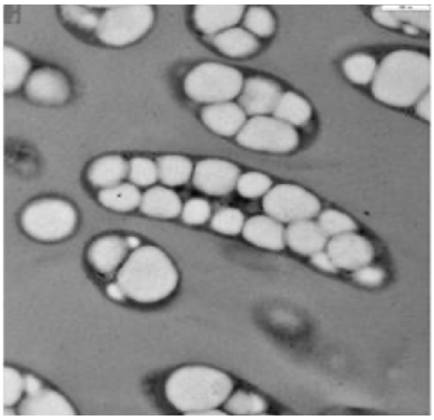
Q | You are the coordinator of the SusPlast Interdisciplinary Thematic Platform (ITP) of the CSIC. What are the main objectives and challenges faced by this platform?

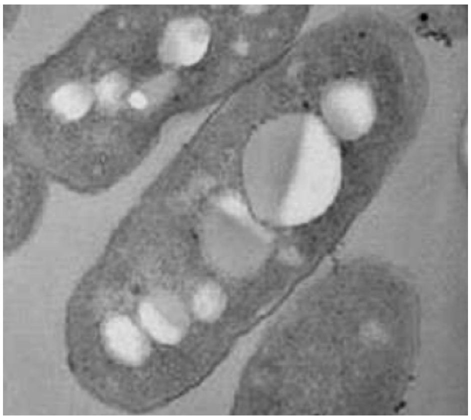
A | The main objective is to organize ourselves as scientists in order to be able to provide specific solutions to global problems within a short time frame. The current pandemic has highlighted this need. The CSIC has enormous capacity in terms of the sustainable management of plastics from a mechanical, biotechnological, and chemical points of view. The SusPlast platform is multidisciplinary in this sense. It organizes all the technological resources of the CSIC to provide services to all kinds of projects related to the management and production of plastics, following the principles of the circular economy. For example, from an environmental point of view, by studying marine life and the impact that plastics can have in this environment. Or from an industrial point of view, by examining how we make plastic production more sustainable or design materials that can be degraded or recycled in one way or another. The platform seeks to take the CSIC's knowledge and technology in these areas and transfer it to industry, where it can be implemented as soon as possible.

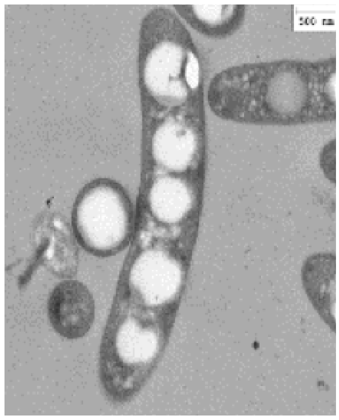
The current challenge is to take advantage of the economic recovery funds that are being provided to generate projects and infrastructure, and to attract specialized personnel and trained technologists to meet society's demands by reducing pollution and promoting recycling. And all this must be done with the urgency that climate change warrants.

Q | Do companies or other research groups from outside the CSIC participate in the SusPlast ITP?

A | We have always felt that if there is a technology that is unavailable to us, we will be able to count on other


Cupriavidus necator


Pseudomonas putida



Rhodospirillum rubrum

Bioplastic-producing bacteria

universities. We do not want to close that door. But at the moment all the groups involved are from the CSIC, since we have not yet encountered any need that we cannot fulfil ourselves. From the point of view of academic research, there are only the CSIC groups: 33 in total, from 18 different centres and institutes. The platform also includes private technology centres and more than 20 private entities.

What we do is provide technical advice to the industrial sector and create contacts: when a company wants to solve a specific problem, it contacts us and we incorporate it into the platform and disseminate its needs among the participants so that the company makes contact with the appropriate group, or vice versa. Right now, many research projects are being established as part of national and international public calls or private contracts put out by companies with specific technological needs. The platform is a showcase of the technological capacity of the CSIC in this area.

SusPlast

Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy

Space Biology: Searching for the keys to adaptation to extra-terrestrial environments for exploration and colonization of the solar system

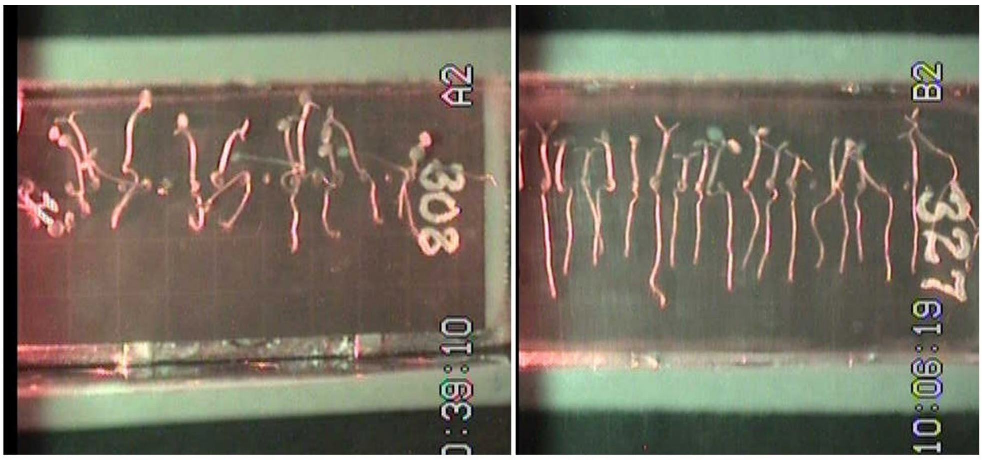
Raúl Herranz
PhD in Biochemistry at the CIB Margarita Salas

The priorities of the scientific strategies of the Spanish National Research Council (CSIC) have varied over time. Certain thematic areas have expanded in response to specific needs to export scientific knowledge to the productive sector, while at other times the strategy has been reoriented towards the formation of large platforms to meet the needs of technological modernization and mitigate climate emergencies (e.g. the SusPlast Interdisciplinary Thematic Platform [ITP]) or to be better prepared to address health-related threats (e.g. Global Health ITP).

Nonetheless, the CIB Margarita Salas (CIB-CSIC) maintains its commitment to multidisciplinarity, and includes many unique research groups that can respond to tomorrow's scientific needs. The CSIC is undertaking the necessary preparation with the 2030 Challenges. One of these groups, "Nucleolus, cell proliferation and microgravity in plants", has been actively working for more than two decades on research projects aligned with Challenge 12: Space, Colonization, and Exploration. Space research by definition seeks answers to questions of global importance: technologies in orbit already improve people's lives by facilitating the connectivity on which the Internet of Things is based. These technologies also allow us to improve our responses to climate catastrophes and optimize the increasingly scarce resources of our planet. In the coming decades, investment in space research will be critical to ensure the success of colonization and resource exploitation goals on the Moon and Mars.

Although it has been more than half a century since the first man stepped on the Moon, this feat occurred in a specific international political context and entailed significant risk: short-term survival off our planet had only been proven possible a few years earlier. The entire subsequent space program was a successful international collaboration, particularly the shuttle flights and the International Space Station (ISS), on which humans have been continuously present for 20 years, providing insight into the important cumulative physiological changes that living beings undergo when exposed to space flight conditions.

The main environmental factor responsible for these alterations is microgravity, a term that we associate with a lack of weight. Similar to the weightlessness experienced in free fall, this is one challenge that astronauts traveling in an orbiting spacecraft must face. If in a spacecraft in orbit the effective force of gravity is very close to 0 g (where g corresponds to Earth's gravity), on the



Effects of microgravity on plant growth

microgravity, confirming alterations in animal development in space as well as increases in energy metabolism and the response to stress. These three very different experiments, using different model systems, all confirmed that there are essential molecular mechanisms (cell proliferation, mitochondrial activity, aging) that are greatly affected by the space environment, even in experiments lasting only a few days. They also demonstrate that biological systems are capable of adapting to

Moon the gravity remains 0.17 g and on Mars reaches 0.38 g. Therefore, long-term stays can cause serious illness in space colonies. Many of these alterations at the level of the musculoskeletal, metabolic, immune, and neuronal systems are associated with aging on Earth, but can appear earlier and progress faster in orbit. Therefore, the life support systems required for long-term stays on the Moon or even manned trips to Mars will need to be largely autonomous (closed) in order to be sustainable. These systems go beyond the mere maintenance of atmospheric pressure and shielding from cosmic radiation, and include nutrition (oxygen and food) and the recycling of biological waste. Plants play a key role in these life support systems by providing moisture, oxygen, and food to astronauts and by recycling CO_2. The Melissa Project, an initiative of the European Space Agency (ESA) with significant Spanish participation, is a good example of a life support system designed for human space exploration.

Although the Spatial Biology activities at the CIB Margarita Salas began earlier, October 2003 was the turning point at which the group led by Dr. Javier Medina began to specifically focus on these objectives. In collaboration with Professor Roberto Marco's research group from the Autonomous University of Madrid, three experiments with animal (*Drosophila*) and plant (*Arabidopsis*) model systems travelled to the ISS in the "Cervantes Mission", with the participation of the Spanish astronaut Pedro Duque. In the AGING experiment, we confirmed altered behaviour in insects living under microgravity, effects that were associated with an increase in energy metabolism and aging. In the ROOT experiment we showed for the first time that microgravity uncouples the rates of growth (decrease) and division (increase) of root meristematic cells (which, like mammalian stem cells, are the source of new cells for plant development). The GENE experiment revealed dysregulation of a spectacular number of genes in insects that developed in this environment, novel to terrestrial organisms, from an evolutionary point of view. On our planet, although life has been able to evolve under extreme conditions of temperature, acidity, and humidity, gravity has remained constant since the beginning of life on Earth.

Another conclusion of the space experiments of the "Cervantes" mission was the difficulty of recovering biological samples in quantity and quality enough to perform analyses using common laboratory techniques. To work around this problem, studies using microgravity simulation tools on Earth have been carried out within the framework of the ESA programs in the most advanced laboratories in Europe (European Space Research and Technology Center [ESTEC], German Aerospace Center [DLR], EFML [European Magnetic Field Laboratory], University of Toulouse, University of Nottingham) and in simpler simulators in our center. In our laboratory, simulation experiments have allowed us to delve into the alterations in plant cell division that occur in microgravity, identifying some of the epigenetic mechanisms involved and the specific phases of the cell cycle that are altered. The experimental approach that we have used, combining microscopy, flow cytometry, and omics tools to perform analyses at the whole genome level, would have been unfeasible if we had relied only on samples acquired in space.

Our line of research in Space Biology at the CIB Margarita Salas reached its zenith in the last 5 years with the Seedling Growth experiment, a combined effort of the North American (NASA) and European (ESA) space agencies carried out over three missions to the ISS. Thousands of seedlings have been exposed to this environment, including mutant and reporter plants. Analysis of these plants in our laboratory is helping us unravel the molecular mechanisms that control adaptation to the extra-terrestrial environment. We have also been able to control environmental conditions with great precision, thanks to the European Modular Cultivation

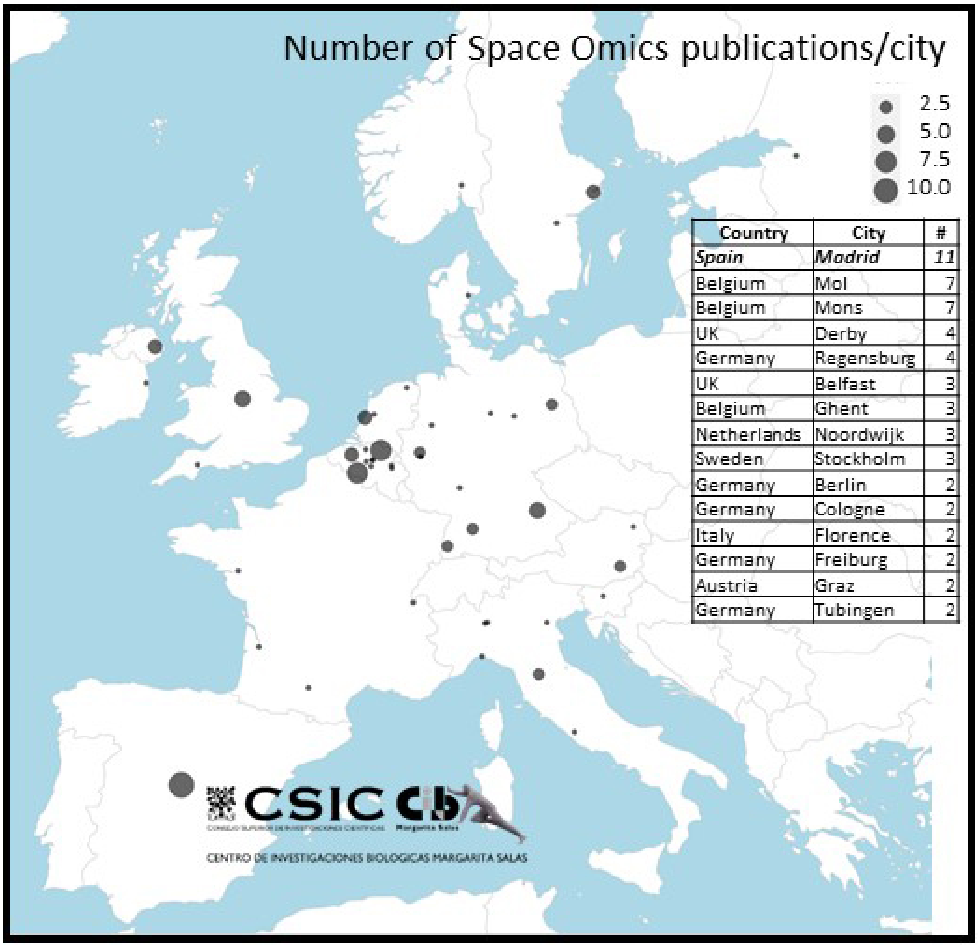
System (EMCS), which has allowed us to modulate the type of lighting as a possible countermeasure to the lack of gravity, and to replicate the gravity on the Moon or Mars using centrifugation. The results obtained, which are also supported by the findings of experiments with lunar/Martian gravity simulation tools, will allow us to identify the different types of plant genes affected by distinct environmental elements. The international collaborative effort led by Dr. Medina at the European level, despite limited national funding due to the effects of the 2008 economic crisis, has achieved unanimous international recognition from the main scientific agencies and societies for space research (ELGRA, ASGSR, COSPAR and NASA), and has received several prestigious international awards.

Our group is currently concluding the analysis of the results of Seedling Growth project, and preparing to participate in new space experiments, following a hold on calls by the ESA. Meanwhile, we are maintaining and strengthening our international collaborations, mainly focusing on simulation projects studying plant growth in a Martian colony environment, in collaboration with several French research groups and with Dr. González Pastor of the Center for Astrobiology (INTA-CSIC). At the CIB Margarita Salas we are also leading efforts at the European level in omics publications, and I am the coordinator of the Space Omics Topical Team project, funded by the ESA. Through this project we manage connections within the entire community of European bioinformatics and space biologists working in any biological system in order to identify synergies and common elements relating to adaptation to extra-terrestrial environments. Recently, we coordinated the European contributions to a set of publications on Space Biology in different journals of the Cell Press group, in collaboration with our American colleagues working in the GENELAB project.

We continue to investigate whether the adaptation of living beings to space is dependent of specific sets of genes or on reaching new transcriptional states at the whole genome level that facilitate biological function in such an extraordinary environment. From insects to plants, to astronauts and the beneficial or pathogenic microbes that accompany them, all living beings will have to be exposed to suboptimal environmental conditions outside our planet that combine and interact to induce an adaptive response, which we must then identify, understand, and hopefully be able to modulate. Given the global importance of this field, and the international cooperation around the ISS, we expect great progress in our scientific discipline in the coming years. This progress must be the result of coordinated action at the European level to exploit existing -omics data and conduct increasingly better controlled experiments, both in space and in simulated conditions on Earth.

All this will lay the foundations to ensure that space exploration and colonization efforts of the 21st century can be carried out fulfilling all the necessary life support requirements of space colonists.

Space Omics
An esa funded Topical Team



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UK	Derby	4
Germany	Regensburg	4
UK	Belfast	3
Belgium	Ghent	3
Netherlands	Noordwijk	3
Sweden	Stockholm	3
Germany	Berlin	2
Germany	Cologne	2
Italy	Florence	2
Germany	Freiburg	2
Austria	Graz	2
Germany	Tubingen	2

Map depicting the geographical distribution of Space Omics publications in Europe

The use of biotechnology in pest control: insect-resistant crops in the European Union

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Pests are one of the main causes of yield losses in crops, accounting for around 20% of losses globally, although this percentage is considerably higher in certain crops and geographic areas. It is therefore essential to apply measures to control them. Pest control in agriculture is carried out within the paradigm of integrated pest management (IPM), which takes advantage of all available options to control the damage caused by pests using approaches that entail the minimum possible risk for humans, products, and the environment. The concept of the IPM has been accepted and incorporated into the public policies of the EU. In Spain, alternative approaches to chemical control have been promoted since 2012 as part of a framework of action established to ensure sustainable use of plant protection products. The use of biological, cultural, physical, and biotechnological methods is thus promoted over chemical methods, as well as application of the most specific products possible. Nonetheless, effective control is not always achieved, and therefore it is sometimes necessary to resort to chemical insecticides. This implies the annual release of millions of tons of these products throughout the world, with consequent harmful effects, mainly for health and the environment.

Transgenic plants are those that have been genetically modified (GM) through the use of biotechnological tools, conferring them certain advantages, which can be agronomic, environmental, nutritional, economic, or a combination thereof. GM crops were planted for the first time in 1996 and their use has grown exponentially since then, owing to specific features that make them more competitive than non-GM counterparts. In 2019 alone a total of 190.4 million hectares of GM crops were planted in 29 countries. However, the global situation differs from that of Europe, where around 100,000 hectares of GM crops are cultivated (0.05% of the global surface area planted with GM crops). This situation looks unlikely to change in the short term, in part due to the strict EU legislation governing cultivation of new GM varieties.

Since their introduction, GM plants have suffered significant public rejection in many European countries. This has created a contradictory situation, as dozens of ingredients and food and feed additives derived from GM plants grown in other countries are approved in the EU. One possible reason why consumers reject GM crops is that they do not perceive any direct benefits to their well-being, nor the possible negative consequences (economic, environmental, food, etc.) associated with other preferred alternatives. On the other hand, citizens may not be fully aware of the scientific risk assessment to which these organisms are subjected. As with uses of biotechnology applied to any branch, a given GM crop will not receive authorization until it has undergone meticulous studies to guarantee the safety of the new product. These studies also continue after authorization has been granted in order to protect animal and human health and the environment against possible harmful effects.

One issue that should be clarified is that GM crops are not the definitive solution to agronomic, economic, food, or environmental problems, but are one more resource to turn to in order to mitigate these problems. Moreover, GM plants do not all share the same characteristics, risks, or benefit, and therefore evaluations to regulate their commercialization are made on a case-by-case basis. It has already been demonstrated in different countries that insect resistant crops are a powerful IPM tool that can significantly improve the control of certain pests. An illustrative example published in January 2021 in PNAS reported the eradication of a devastating cotton pest in the southwestern United States and northern Mexico, where it arrived in the 1920s from Asia[1]. Its eradication ended the damage caused to cotton and the use of insecticides to control it, with consequent economic, environmental, and social benefits. This milestone was achieved thanks to the use of different strategies, most notably the use of GM cotton that produces insecticidal proteins against this pest.

Achievements like this are not common, but they show how a plant transformed using biotechnological methods can be very useful if used correctly and in the right place. From the agronomic point of view, the more alternatives there are to control a pest, the better, as this slows down the development of resistance, one of the main problems caused by the massive use of insecticides.

Currently in the EU only one GM plant is commercially grown, MON 810 maize or Bt maize. This corn expresses an insecticidal protein that is toxic to two of its main pests, the corn borers *Sesamia nonagrioides* and *Ostrinia nubilalis*. After hatching, the larvae of the borers penetrate the stalk, where they spend their entire larval stage, making it very difficult to combat them using other methods. Bt maize controls these pests effectively and specifically, and is one of the IPM methods used for corn cultivation in Spain[2].

For more than two decades, our group at the CIB, Applied Entomology to Agriculture and Health, has sought to provide fundamental knowledge and applied tools for the sustainable use of Bt maize. Within this research line we have focused on two possible environmental effects of this crop. First, we have evaluated the impact of Bt maize on different groups of non-target arthropods that live on the crop, and have found no significant negative effects on any of them. Second, since 1998 we have monitored the evolution of resistance of corn borers to the insecticidal protein expressed by Bt maize. The development of resistance is the greatest threat to long-term crop sustainability. If resistance develops, the crop's pest control efficacy is lost. After more than 20 years of cultivation, results obtained in the field indicate that there are no signs of resistance of either of the two borer species to the insecticidal protein expressed in MON 810 maize, although we have developed a model of the evolution of resistance in S. nonagrioides that predicts the emergence of resistant populations in 2047–2050[3].

The responsibility to ensure effective GM crops with sustained efficacy must be shared by all parties involved. First, the competent authorities must evaluate the tech-

The corn borer *Ostrinia nubilalis* feeding inside a corn plant.
Image: Pablo Jalón.

nical information relating to the modified plant, and later regulate and control the experimental release or the commercialization of the crops. Responsibility also lies with permit applicants, who must provide all necessary information, including data relating to possible risks that may arise, the conditions under which this could occur, how they can be avoided, and how to respond in cases in which undesirable effects, whether expected or unpredictable, are detected. Finally, responsibility must be borne by farmers, who have benefited most from the cultivation of transgenic plants in Europe. In planting these crops farmers must commit to comply with the indications for each type of crop to ensure that they are used safely and without long term risks (mainly agronomic and environmental). Our group continues to pursue this line of research, and collaborates with national and EU authorities, providing knowledge and recommendations to ensure the sustainability of Bt maize over time.

1 Tabashnik et al., 2021. Transgenic cotton and sterile insect releases synergize eradication of pink bollworm a century after it invaded the United States. PNAS, 118 (1) e2019115118; DOI: 10.1073/pnas.2019115118.

2 https://www.mapa.gob.es/es/agricultura/temas/sanidad-vegetal/guiamaiz_tcm30-57958.pdf

3 Farinós, G.P.; Ortego, F. 2019. Resistencia de las plagas al maíz Bt: estado actual y planes de seguimiento en España. Boletín SEEA, 4: 52-57. https://drive.google.com/file/d/1Bkep8aDfXuusw8Rc68wl8Xk-SQ1xTb_t/view

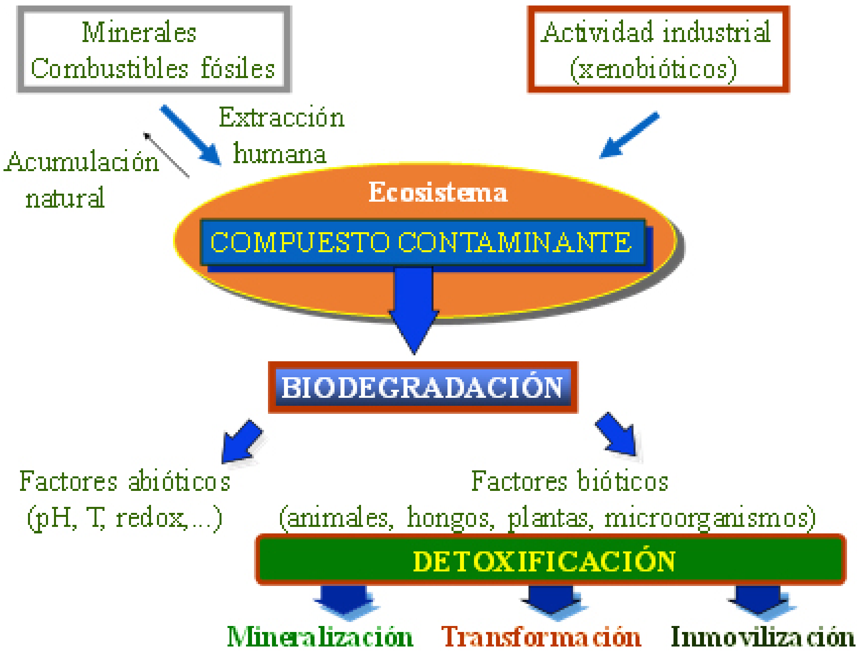
Biotechnological applications for the degradation of pollutants

José Luis García López
CSIC Research Professor at the CIB Margarita Salas

Land, air, and sea

Very broadly speaking, there are three basic scenarios that result in exposure of our planet's inhabitants to pollutants. The first is via the air, which can contain different types of suspended particles as well as toxic gases. The second is water, which can contain pollutants in solution as well as all kinds of solid elements, from micro to macro elements, in suspension. The third is the soil. Here the situation is more complex, as pollutants can exist at different levels of bioavailability depending on the physical-chemical (abiotic) parameters of the soil (e.g. humidity, pH, temperature, redox status, etc.). In reality, many pollutants are exchanged between the air, water, and soil, depending on the environmental conditions. For example, sulfur and nitrogen oxides (SO_X, NO_X) can transition from the atmosphere to the soil and water, forming so-called acid rain[6]. Similarly, a pesticide in the soil can be washed into an aquifer or stream by heavy rain. A contaminating metal in water can pass into the soil if the water is used for irrigation. The steroids we consume become part of urban effluent via different routes[3,4].

Biotechnology has developed multiple tools to eliminate different types of pollutants, although not all are equally efficient. In general, the most difficult pollutants to biotechnologically degrade are the so-called xenobiotics, i.e. products that are not part of living beings and are generally chemically synthesized. These compounds are highly recalcitrant to biodegradation because, a priori, they do not form part of any metabolic process that has had sufficient time to evolve. A well-known example is petroleum-derived plastics, which create problems both as large fragments and as microparticles. Although enzymes are being developed that can degrade some of these plastics, this is a problem that is closer to being resolved using proper waste management strategies than using biotechnological treatments.



Polluting compounds in the environment undergo different processes of physical-chemical degradation (abiotic) or biodegradation (biotic) mediated by different organisms. Organisms can convert the pollutant into CO_2 and H_2O (mineralization), transform it into another, less toxic compound, or simply immobilize it by reducing its bioavailability.

Every pollutant poses a distinct biotechnological challenge

There are two main large groups of pollutants: inorganic pollutants and organic pollutants. The latter group comprises pollutants that contain carbon. Both groups include gaseous compounds such as H_2S, SO_X, NO_X, CO, CH_4, and chlorofluorocarbons (CFCs), and even CO_2 and O_3, which can be toxic at high concentrations. Depending on the ambient temperature some compounds can be found in gaseous form. These include volatile organic compounds (VOC), including many aromatic compounds (e.g. toluene, benzene, styrene, etc.), which are used as solvents for a broad range of applications[5].

To eliminate gaseous pollutants, biotechnology offers different solutions that generally involve the development of biofilters (bioreactors, digesters) containing consortia of cultured microorganisms that mineralize or transform the pollutant into a less toxic substance. Biofilters have also been used for water purification. In a way, sludge in water treatment systems operates under the same principle as biofilter technologies, although the two systems differ.

Inorganic pollutants (mainly metals) can also be eliminated using biotechnological procedures. Toxic metals are removed by changing their ions to oxidation states that are less toxic or bioavailable. This involves decreasing solubility, sometimes simply by converting them to their insoluble metallic form or by methylating and volatilizing them. These soil or water decontamination processes can be carried out using microorganisms, but also using plants (phytoremediation). Plants alone or in combination with the rhizosphere are capable of eliminating metals and other pollutants from the soil. Biofilters have been used to remove metals from waste water, after which the metal accumulated in the biofilter can be easily removed.

Bioremediation

The greatest environmental problems occur when pollutants are accidentally released into the environment, requiring *in situ* cleaning of the contaminated area (bioremediation). Well-known cases include accidental spills of oil or industrial waste (e.g. lindane). In these cases, biostimulation techniques can be used. This involves stimulating the development of the native microbiota with nutrients to eliminate the pollutant. An alternative approach is bioaugmentation, which involves inoculation of specificmicroorganisms to selectively eliminate the pollutant. *Ex-situ* bioremediation procedures such as landfarming (bio-farming) can also be used. This method consists of delimiting an area of land where contaminated soil or sludge is transported and treated in a controlled manner. Biopiles (mountains of contaminated soil) function as large open-air reactors, analogous to composting piles. These processes are usually combined with humidification and ventilation systems to promote degradation. Sometimes degrading enzymes are added to the soil via pipelines in order to remove the pollutant, but these processes are very expensive.

Biodegradation and molecular biology

The traditional method of studying biodegradation of pollutants generally involves isolation of one or more microorganisms capable of mineralizing, biotransforming, or at least immobilizing the target pollutant. As only a small fraction of microbes in the environment can be cultured using conventional culture methods, it is not always easy to isolate a degrading microorganism in a pure culture.

For many years our Environmental Biotechnology group has sought to better understand the processes of bacterial degradation of pollutants, both in the presence and absence of oxygen (aerobic and anaerobic biodegradation)[1,7,8]. Understanding how these two processes work and are regulated is one of our key goals. One of the basic facts we have learned about biodegradation pathways is that they are built around a small number of highly regulated central pathways that act as a catabolic funnel.

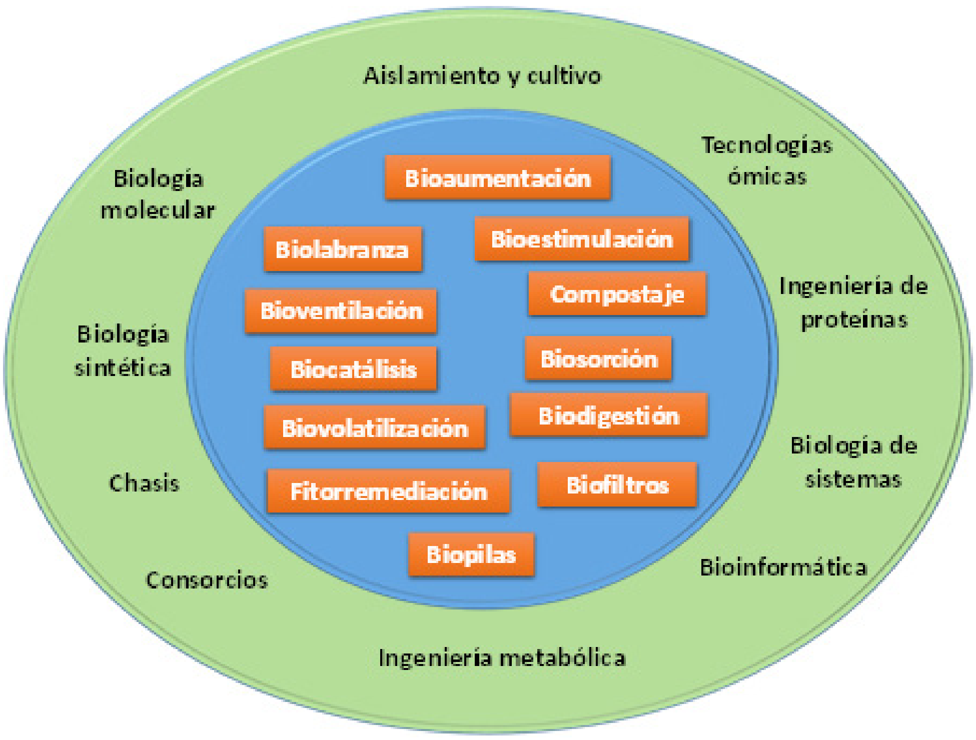
In recent years, molecular biology (omics) techniques have allowed us to progress rapidly, so that once a microorganism capable of biodegrading a pollutant is isolated, we can quickly determine the underlying metabolic pathway. Metabolic engineering technologies, supported by synthetic biology, protein engineering, and metagenomics approaches, allow us to combine genes/enzymes from different organisms to create new routes that, once implemented in microorganisms that function as a chassis, allow degradation of pollutants for which no natural degradation pathway has yet been identified. Increasingly, tailored microbial consortia are also used in such a way that the degradation path is distributed across several chassis.

Currently, genetically modified microorganisms cannot be used in open field bioremediation. Despite exhaustive research in this area, no 100% effective biological or genetic containment systems have been found[2]. However, these microorganisms can be used in contained systems such as biofilters or bioreactors.

Future prospects

The global bioremediation market is expanding rapidly, and is expected to reach 55.6 billion dollars by 2028. Although the estimated value varies greatly between different sources, it nonetheless provides some idea of the magnitude of expected growth. About 30% of this market is accounted for by American companies (e.g. Altogen Labs, Drylet, Green Apple Environmental Technologies, Oil Spill Eater International, Aquatech International, InSitu Remediation Services Limited, Ivey International, PROBIOSPHERE, REGENESIS, Sarva Bio Remed, Sevenson, Environmental Services, Soilutions, Sumas Remediation Services, Xylem). Some companies that are active in Spain and focused on soil remediation use bioremediation techniques to some degree (e.g. Hydrolysis, Litoclean, Regenesis, Emgrisa, Geocisa, Insuma, Kepler, Envirotecnics, Invesoil, GMC Ingeniería, Geotecnia 2000, Suez, AMS, Sereco).

The future of pollutant elimination will first require the establishment of laws to reduce their production,



The basic processes that biotechnology applies to eliminate environmental pollutants are supported by a collection of molecular tools that help us to understand how metabolic degradation routes work and how to make these processes more efficient.

consumption, and release into the environment, and the replacement of polluting compounds with less toxic ones, applying the principles of green chemistry and REACH regulations.

Communities are employing novel approaches to purify polluting gases, for example using green areas on the roofs or walls of buildings, or biofilter systems for air purification inside buildings. Microbial consortia that include microalgae or cyanobacteria constitute a very interesting alternative for this purpose, as photosynthetic organisms are self-sustaining.

While the use of bacteria for bioremediation processes has been heavily researched, the same cannot be said for fungi and enzymes. The use of fungi is particularly interesting, as many species secrete strongly oxidizing enzymes (e.g. laccases, peroxidases, etc.) that can help initially modify pollutants, which can later be more easily degraded in the natural ecosystem. Enzymes are essential elements for biodegradation processes. Because they can be produced more economically through efficient fermentation processes, the direct addition of selective enzymes to contaminated sites can be a very useful pre-treatment method to facilitate the subsequent work of autochthonous organisms.

A better understanding of the functioning of natural microbial consortia will mean that tailored consortia containing both bacteria and fungi can be designed to better target specific pollutants, thereby helping to faster reduce contamination when applied to an affected area by bioaugmentation. Big data yielded by powerful -omics technologies (e.g. metabolomics, transcriptomics, proteomics) and modelled using systems biology will help us to better understand how complex niches operate and thereby accelerate biodegradation processes, either by improving consortia, adding essential nutrients, or controlling the abiotic conditions of the environment.

The possibility of using synthetic biology to create genetically modified organisms (GMOs) with novel, à la carte metabolic pathways, combined with modern genetic modification techniques using CRISPR technology, is not currently possible for open field bioremediation, as current legislation on the release of GMOs into the environment is extremely restrictive. However, these technologies can be used to develop contained decontamination systems, which have more limited applications.

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Plant biotechnology in the fight against climate change

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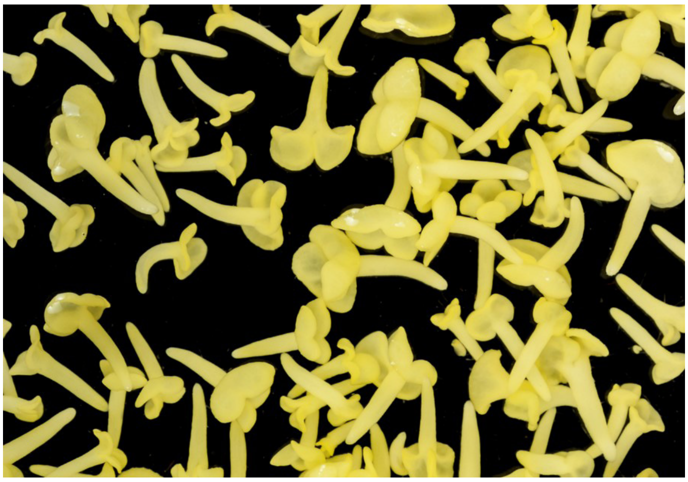
One of the greatest challenges facing humanity is how to produce enough food for the exponentially growing population of our planet. To give an idea of the scale of this challenge, nearly 800 million people currently suffer from hunger, according to the FAO[1]. Unfortunately, changes in environmental conditions caused by climate change, which are already evident in our daily lives, are going to make even harder to successfully resolve this problem. As a consequence of climate change, we are witnessing increases in the duration and frequency of dramatic climatic events such as periods of drought, and extreme temperatures (both high and low)[2]. These stressful situations already severely limit agricultural production, and increases in their frequency or intensity will further exacerbate the negative effects on the production of staple foods. Many laboratories around the world are focused on tackling this problem, and have set themselves the goal of developing biotechnological tools with which to generate new varieties of crops that allow us to increase, or at least maintain, agricultural production in a manner that is sustainable and, above all, respectful of the environment. It is important to highlight that the development of new biotechnological tools will not only help achieve food security on the planet, but will also play a central role in reducing the impact of agriculture on the environment. It is obvious that increasing crop productivity will help reduce deforestation resulting from the expansion of areas of arable land. Production of new varieties that more efficiently capture nutrients such as nitrogen and phosphorus will help reduce pollution caused by the use of fertilizers. Similarly, the development of more water-efficient crops will reduce irrigation needs and consequently alleviate problems associated with forced irrigation.

Two laboratories at the CIB Margarita Salas are tackling the challenge of developing new tools to increase agricultural production in unfavourable environmental conditions. The Pollen Biotechnology of crop plants laboratory, directed by Dr. Pilar S. Testillano and the Plant Molecular Biology laboratory directed by Dr. Julio Salinas, group leader, and Dr. Rafael Catalá.

Dr. Testillano's group studies the regulatory mechanisms of stress-induced cell reprogramming. Research of this process is key for the improvement, propagation, and selection of high quality plants or those that are better adapted to new environmental conditions. *In vitro* plant regeneration systems, based on the induction of cell reprogramming through stress treatments, are essential in modern breeding techniques. These systems allow, on the one hand, the production of large numbers of genetically identical plants from a plant selected for its optimal characteristics, a process known as clonal propagation of elite genotypes (through somatic embryogenesis). They also allow the production of double haploid (DH) plants by embryogenesis of microspores, the precursor cells of pollen. DH plants enable the acceleration of improvement programs. While classical techniques require many generations and numerous crosses and selection processes to produce new varieties, DH plants present new genetic variability (combinations of characteristics, derived from pollen) in homozygosity, meaning that all characteristics are completely fixed, and all within a single generation. Likewise, *in vitro* regeneration systems are essential to regenerate whole plants after genomic editing or transformation. However, *in vitro* regeneration of many agronomic and forest species remains very inefficient. Dr. Testillano's goal is to increase knowledge about the cellular and molecular processes underlying stress-induced cell reprogramming in order to identify new targets and effectors that allow efficient manipulation of regeneration systems *in vitro*. For these studies, rapeseed and barley are used as model species. Subsequently, the researchers evaluate the applicability of their findings to other species of agronomic or forestry interest, such as the cork oak. To do this, they follow a multidisciplinary and integrative approach that includes modern cellular, molecular, physiological, and genomic techniques, among others. The laboratory has several different lines of research. The first consists of the identification of key molecular determinants of plant cell reprogramming. The group has recently characterized (i) the role of key elements that regulate the balance between cell survival and death, including autophagy and cysteine proteases; (ii) the regulation of cell reprogramming by plant hormones, the most important of which are auxins and cytokinins; (iii) the role of various epigenetic mechanisms in cell reprogramming; and (iv) remodelling of the cell wall[3,4,5,6]. Furthermore, Dr. Testillano has started a pioneering line of research involving the screening of small molecules from chemotheques, in collaboration with Dr. Ana Martínez and Dr. Carmen Gil, experts in biological chemistry at the CIB Margarita Salas. This work facilitates the identification of new compounds, initially designed for therapeutic use in biomedicine, that promote plant reprogramming and regeneration. This approach has already led to the licencing of several patents, with others pending. In addition, Dr. Testillano's group believes that it is essential

Cork oak plant with root, stem, and leaves, regenerated *in vitro* by somatic embryogenesis from cells of an adult tree



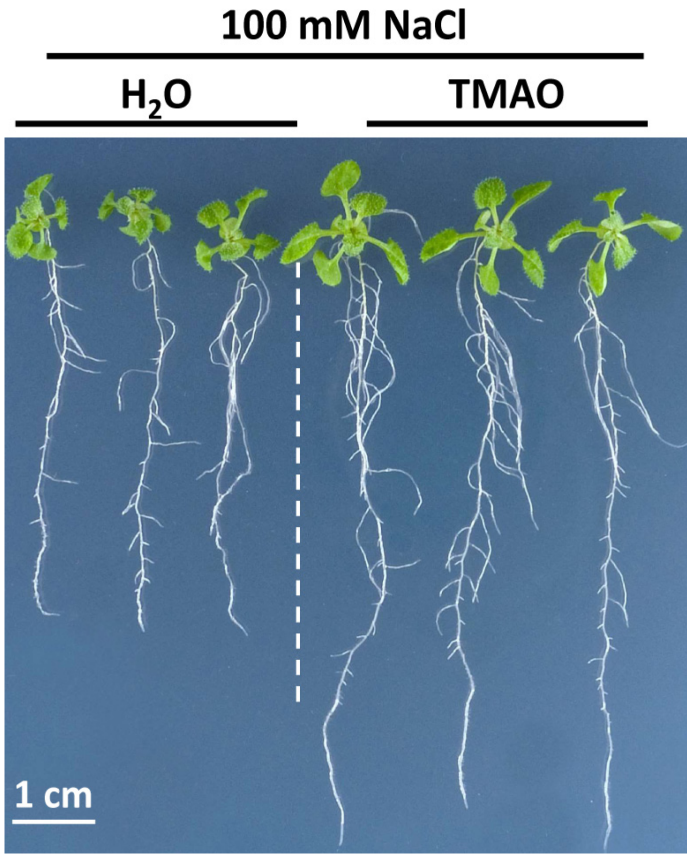
Rapeseed embryos produced *in vitro* from microspores (pollen precursor cells), which after germination will regenerate double-haploid plants

Wild *Arabidopsis* plants (Col-0) and two mutants with alterations in RNA metabolism (rci10-1 and rci10-2) exposed to drought stress.

to transfer the knowledge acquired in their laboratory to the productive sector. This will enable the design of new applications in species of economic and environmental interest through the establishment of contracts and collaborations with companies in the agriculture, forestry, and biotechnology sectors for use by broader society.

In the Plant Molecular Biology laboratory, directed by Dr. Salinas and Dr. Catalá, research is focused on the development of biotechnological strategies that increase the tolerance of crops to adverse environmental conditions such as low temperatures, drought, or high soil salt concentrations. The approach followed by this group is based on one key concept: understanding the molecular mechanisms that control the tolerance of plants to environmental stress is fundamental to develop new tools that can increase the tolerance of crops to these adverse situations. In recent years, the work of the group has focused on the identification and characterization of intermediaries and signalling pathways that control the process of acclimation to low temperatures. This process is an adaptive response acquired by plants throughout evolution that allows them to increase their tolerance to frost after spending a period of exposure to low temperatures (0–4°C). An interesting feature of this process is that in addition to increased tolerance to freezing, acclimated plants also show increased tolerance to other stresses, such as drought and salinity. In fact, research into the regulation of the process of acclimation to low temperatures is also helping to unveil the mechanisms followed by plants to cope with these stressors. Dr. Salinas' initial line of research identified genes from Arabidopsis, the model plant par excellence, expressed in response to cold. The group has been able to identify and characterize important regulators of the plant response to abiotic stress that act at different levels (transcriptional, post-transcriptional, and post-translational)[7,8]. The results obtained have shaped the laboratory's two current lines of research. The first seeks to understand the role of RNA metabolism in controlling the plant's response to abiotic stress. Studies by this group have revealed that modulation of both intron cleavage of the precursor messenger RNAs and the stability of these RNAs play a key role in generating adequate and specific responses to each stressor to which the plant is exposed. The second line of research is based on the identification of a new molecule in plants, TMAO, which until now had only been described in animals. TMAO increases plant tolerance to low temperatures, as well as frost, drought, and salinity[9]. This research focuses on understanding the molecular mechanisms that control TMAO synthesis in plants, and identifying the signalling pathways through which this molecule exerts its protective effects. The biotechnological relevance of TMAO has led to filing of several patents licensed to the company Plant Response Biotech, which has already launched a TMAO-based product that increases tolerance to drought in crops such as corn, tomato, and pepper.

In 2015 the UN identified the key challenges that will be



Arabidopsis seedlings exposed to saline stress (100 mM NaCl) treated with water or TMAO.

faced by humanity in the near future, and promoted the Sustainable Development Goals (SDGs), as described in another report in this newsletter. Seventeen goals were set for the year 2030, with a particular emphasis on the fight against climate change and sustainable development. The tools that plant biotechnology provides us are not only very useful, but in some cases are essential to achieving these goals. In order to achieve food security and improve nutrition and agricultural sustainability, as stated in SDG 2, we must develop more productive crops that provide a greater supply of nutrients and are better adapted to the environment in which they are grown. Similarly, the development of plants with lower water or fertilizer requirements is necessary to achieve sustainable water management in line with SDG 6: clean water and sanitation. These plants will also reduce the pressure of crops on ecosystems by facilitating SDG 15, which focuses on protecting ecosystems. Finally, plant biotechnology is also essential to combat climate change (SDG 13), providing powerful solutions to reduce fossil fuel reliance (e.g. biofuel production) and imaginative strategies such as the development of plants with a greater capacity to fix CO_2.

Humanity faces formidable challenges, and plant biotechnology is our best ally in this fight.

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Biotechnology for the use of cellulose and hemicellulose from plant residues

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Alicia Prieto
Senior CSIC Scientist at the CIB Margarita Salas

Most of the carbon fixed by plants through photosynthesis is found in the walls of plant cells, which represent the most abundant renewable carbon source on Earth. The basic component of the plant wall is lignocellulose, which consists mainly of three polymers: i) cellulose, the most abundant, is a β-1,4-glucose homopolysaccharide; ii) hemicellulose, the next most plentiful, is formed by different branched heteropolysaccharides, the names of which depend on the type of sugars that form it (xylan, mannan, galactan, glucan, glucuronoxylan, galactoglucomannan, etc.); and iii) lignin, which is a heterogeneous and recalcitrant aromatic polymer formed by phenylpropane units that protects the plant from external aggressions.

An important proportion of vegetable biomass is used by the food sector and to obtain healthy compounds. However, for the circular economy model of food production to function properly it is essential to also use the abundant agricultural and agro-industrial waste generated. In this context, so-called lignocellulose biorefineries attempt to establish a sustainable means of using plant biomass, acting like petrochemical refineries but producing chemicals, fuels, and energy from renewable resources.

Soil-dwelling fungi that grow on lignocellulose play an essential role in the decomposition of plant residues and recycling of their components. Therefore, we can exploit their metabolic capacities to convert lignocellulosic residues into products of interest to humans through biotransformation. Some of these fungi can preferentially degrade lignin, leaving accessible cellulose. This occurs naturally in southern Chile, where cows directly ingest the cellulose-rich material produced by decomposition of fallen tree (known as “rotten stick”).

Decaying wood in southern Chile that serves as food for livestock (image courtesy of AT Martínez)

Other fungi preferentially degrade polysaccharides, or have no preferences and degrade everything simultaneously. These differences in degradation patterns are due to the distinct enzymatic systems in these fungi, which specialize in the degradation of different components. Purification and characterization of these lignocellulolytic enzymes and research into their role in degradation processes will allow us to use them as biotechnological tools to extract value from lignocellulosic residues.

In the Biotechnology for Lignocellulosic Biomass group at the CIB Margarita Salas, we use fungi and fungal enzymes to help achieve comprehensive use of plant biomass. We currently have a large battery of purified and characterized enzymes derived from natural isolates or found in fungal genomes, and we work with different heterologous expression systems that allow us to produce these enzymes in larger quantities and modify them using genetic engineering techniques. This way, we can enhance their properties and design taylored enzymes for specific applications.

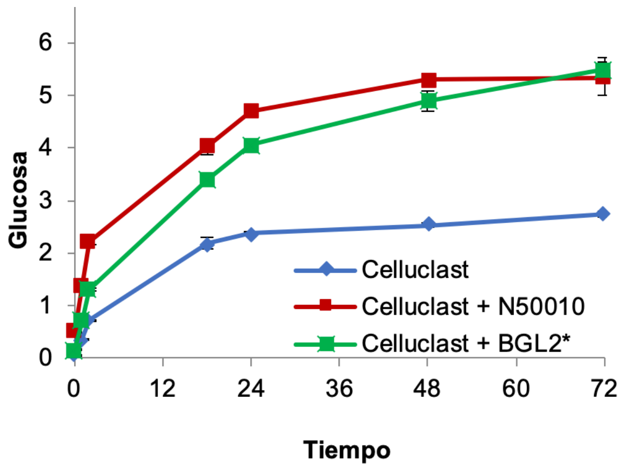
Our research has yielded important findings in recent years, in particular our studies of the enzymatic system of the fungus *Talaromyces amestolkiae*, which is isolated from cereal residues. This fungus produces high levels of all the enzymes involved in the degradation of cellulose and hemicellulose, which we have applied mainly to the enzymatic production of second-generation bioethanol and value-added products from agricultural residues.

Ethanol is a biofuel with properties similar to gasoline that is produced through the ethanolic fermentation of monosaccharides. Its use can enable reductions in dependence on fossil resources and the release of CO_2 into the atmosphere. First-generation ethanol is obtained from easily metabolizable sugars found in sugar cane, beets, and cereal grains. However, controversy over the use of food resources to produce biofuel has led to the development of second-generation ethanol technology, a much more complex process in which fermentable sugar is obtained from lignocellulose polysaccharides (mainly cellulose). First lignin is removed via chemical or physical–chemical processes. Next, in the enzymatic saccharification process, cellulose is hydrolyzed with robust and efficient enzymatic cocktails to produce glucose, which yeasts can then ferment to yield ethanol. In our laboratory, we have demonstrated the efficacy of the lignocellulolytic enzyme cocktail produced by T. amestolkiae for the saccharification of wheat straw and beer bagasse, and as a supplement for addition to commercial cocktails deficient in β-glucosidases.

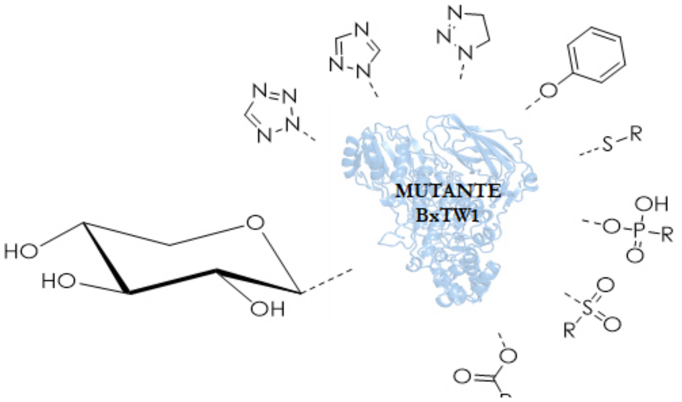
Other characterized enzymes isolated from *T. amestolkiae* are also highly efficient catalysts for other reactions of biotechnological interest. For example, we have obtained xylooligosaccharides by hydrolyzing birch xylan with an endoxylanase from *T. amestolkiae,* and have shown that these oligosaccharides possess prebiotic activity, i.e. they promote the growth of bacteria in the digestive tract that are beneficial to human health but cannot be digested by our bodies.

We have also synthesized bioactive glycosides (compounds with potential health benefits) through reactions catalyzed by *T. amestolkiae* glycosidases by binding a glucose or a xylose to compounds with antioxidant, antitumor, or anti-neurodegenerative properties. These new glycosylated compounds, which include hydroxytyrosol glucoside and epigallocatechin gallate (found in olive oil and green tea, respectively), are more soluble and bioavailable than non-glycosylated compounds, and therefore improve and/or enhance the compound’s properties.

For this we have used native enzymes produced by the fungus, and their recombinant forms (expressed in Pichia pastoris), to obtain higher levels of enzymes and create a system that allows us to manipulate these proteins to enhance their catalytic efficiency.



Saccharification of beer bagasse using a commercial cocktail (Celluclast) and this cocktail supplemented with commercial β-glucosidase (N50010) and the enzyme BGL2 * from *T. amestolkiae* (commercial enzymes were provided by Novozymes)



Schematic depicting the types of compounds that can be glycosylated by the *T. amestolkiae* β-xyloxidase thioglycoligase mutant

Using genetic engineering approaches (directed mutagenesis), we have substituted one of the acidic amino acids of the catalytic center of these enzymes for a neutral amino acid, nullifying the enzyme's hydrolytic capacity and directing all its activity towards synthesis in order to create new types of enzymes (thyoglycoligases and synthases). Moreover, we have already evaluated and immobilized these enzymes (binding them to an inert material, which enables enzyme recovery and recycling) for use in the synthesis of different bioactive glycoconjugates and other products of interest for different industrial sectors.

The aim of this research is to ensure better use of natural resources and extract value from plant waste using biotechnological tools derived from fungi and their enzymes. This allows us to replace traditional chemical processes with others based on the principles of green chemistry. These achievements have been possible thanks to funding received through national and international projects and contracts with private companies, collaborations established with other research groups, and the enthusiastic and invaluable work of the members of our research group.

Endocrine disruptors, another global threat

Jesús del Mazo
CSIC *Ad Honorem* Scientific Researcher at the CIB Margarita Salas

We live in a time when multiple global threats are becoming apparent. The COVID-19 pandemic and climate change and its consequences are some such examples, both of which have caused social alarm. These are real threats that can be tackled by public powers, science, and, in particular, biotechnology. However, there are other, less evident but equally concerning threats that affect an essential aspect in the perpetuation of this planet's species, including our own: reproductive capacity. Life cannot be conceived without the ability to reproduce.

Thousands of synthetic and natural chemical compounds are present in our environment as environmental pollutants, and hundreds of them interfere with hormone-mediated processes, including hormones crucial for the development, differentiation, and function of germ cells and sexual organs. Reproductive health is at stake. These compounds have been detected in the environment since the 1950s owing to their effects on the endocrine system. The term "endocrine disruptors" was coined in the 1990s. Since then, the effects of these compounds on fertility and reproductive health have been demonstrated at the epidemiological level. For example, a recent meta-analysis[1] indicates a cumulative annual decrease of 1% over the last 50 years in sperm quality and quantity in different human populations. Some countries such as Australia (Reproductive Health Australia) are already considering reproductive biology as a national social, health, and economic priority[2].

Endocrine disruptors can affect different functional pathways, cell types, and stages of male or female development. Several features of these compounds complicate evaluation of their adverse effects, including variability in their dose-effect relationship (some compounds have greater effects at low doses than at higher doses), their transgenerational effect (modifications in the genome of exposed individuals that do not alter DNA [epigenetic] but are transmissible to several unexposed generations), and effects induced by exposure to combinations of compounds (with synergistic or antagonistic effects).

The difference in the development of the germ line in male *versus* female mammals underscores the need for specific analyses in both sexes. Evaluation in the embryonic stage is of particular importance in the female line, in which meiosis begins in mammalian oocytes. This implies methodological complexity, which is one of the reasons why the available data are derived mainly from males, and greater research contributions into the effects on the female line and the prenatal period are necessary.

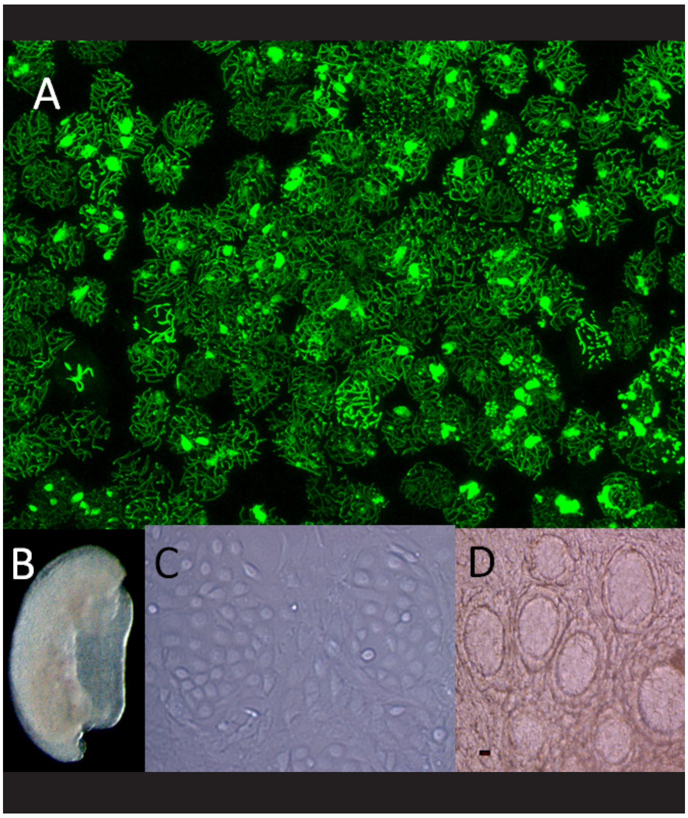
Characterization of the molecular and cellular mechanisms underlying the adverse effects of thousands of compounds on the reproductive systems can facilitate detection of biomarkers of reprotoxicity end points and analysis of the risk to the reproductive health at the individual and population level.

For years, the Molecular Biology of Gametogenesis group has been researching different aspects of the effects of endocrine disrupting compounds on germ cell differentiation into gametes (oocytes and sperm). Different lines of research have enabled us to evaluate how some endocrine disrupting compounds alter the normal expression of hundreds of genes important for the regulation of gametogenesis, with consequent effects on male and female fertility. These alterations in gene function affect the expression of genes encoding key proteins as well as other, more recently discovered elements involved in gene regulation (e.g. microRNAs, piRNAs). In recent years our research has demonstrated the following: defined patterns of gene dysregulation during spermatogenesis that differ depending on the endocrine disruptor to which experimental animals are exposed[3]; transgenerational effects, whereby these alterations in gene expression can be transmitted for up to 3 generations after initial exposure, affecting fertility in adulthood[4,5]; synergistic effects of mixtures of various disruptors, whereby combinations of compounds have more pronounced effects than the individual compounds, as evidenced by changes in both gene expression and hormone levels[6,7]. All of these findings have involved the use of cutting-edge methodologies for genetic, molecular, and cellular analyses that are continually advancing, from experimental approaches involving the creation and sequencing of differential gene expression cDNA libraries at the end of the 1990s, to current massive RNA sequencing technologies (NGS) and bioinformatics analyses.

More recently, we have used biotechnological approaches to study *in vitro* 3D gonadal cultures from both sexes, at the early stages of mouse embryonic development. In these studies we have analysed *in vitro* the process of germ cell generation and differentiation and performed molecular and cellular analyses to characterize the effects of disruptors (or other potential reprotoxic compounds). This approach provides an alternative method to animal experimentation (reducing the number of animals used for testing) and resolves the complex issue of studying the effects of these compounds on the female sex in mammals[8,9,10].

In parallel, we have made many important contributions to the analysis and characterization of the biological processes of gametogenesis in order to elucidate the complex regulatory mechanisms underlying the functional differentiation of these cells so crucial for perpetuation of species: gametes.

Gamete formation is a highly complex process involving many regulatory pathways and multiple genetic, cellular, metabolic, and environmental elements. Fertile gametes can only be produced through precise regulation of the entire process, from the embryonic stage to adulthood. Further studies in this area will broaden our knowledge of the processes involved and uncover mechanisms that can potentially be altered by compounds such as endocrine disruptors, ultimately allowing us to ensure the reproductive health of human and animal populations.



In vitro culture of mouse foetal ovaries. A) Confocal microscopy image of the meiotic process in oocytes developed *in vitro*. Immunofluorescence using antibodies that specifically recognize proteins of chromosomal structures during meiosis (protein of the synaptonemal complex). B) Mouse foetal ovary 12.5 days post-coitum, including accompanying mesonephros, before beginning culture. C) Phase contrast image showing clusters (cysts) of oocytes a few days after beginning culture. D) Phase contrast image of oocyte during culture. (Images taken by Silvia González-Sanz).

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Gabriella Morreale, from basic to applied endocrinology

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If we are to talk about biotechnology, we are obliged to dedicate this historical section to CSIC Research Professor Gabriella Morreale (Milan, 1930 - Madrid, 2017), a pioneer of modern endocrinology in Spain.

She joined the CIB in 1958, where she established her laboratory with her husband Francisco Escobar. In 1976 they moved to the UAM School of Medicine. Although their work in those years may not fall into the category of "biotechnology" as we know it today, Gabriella excelled in basic and applied research on thyroid hormones and was responsible for the implementation by the Spanish health system of the heel test for early detection of congenital hypothyroidism, as well as the recommendation of iodine supplementation in pregnant mothers to ensure correct brain development of the foetus and prevent cretinism.

Her exceptional scientific career and vast experience and knowledge of the consequences of iodine deficiency and the action of thyroid hormones in the brain made her receive multiple awards, including the National Award for Research in Medicine in 1977 (shared with her husband Francisco Escobar), the Reina Sofía Award for Research into Mental Retardation in 1982 (shared between her group and that of Magdalena Ugarte), the Gregorio Marañón National Award for Medical Research in 1997, and the Rey Jaime I Award for Medical Research in 1998.

Although Gabriella was born in Milan, she immediately moved to Vienna with her family, where her father Eugenio Morreale held the honorary position of Segretario del Fascio. In 1937 he was appointed consul in Baltimore, USA, where they remained until in 1941, when they left the USA and settled in Spain, where her father was appointed consul in Malaga. Gabriella received her secondary school education in Malaga, and was awarded the Extraordinary Prize in the State Bar Exam in

Gabriella Morreale in her CIB lab. Photo courtesy of María Jesús Obregón

1947. That same year she began her studies in chemical sciences at the University of Granada, where she met her future husband and scientific collaborator, Francisco Escobar. Together they embarked on a fruitful scientific career.

Gabriella Morreale's doctoral thesis, directed by Prof. Enrique Gutiérrez de los Ríos and presented at the University of Granada in 1955, focused on developing a method to assess levels of iodine in biological samples (serum and urine). This method allowed her to demonstrate that goitre, which was endemic in the Alpujarra region of Granada, was caused by a deficiency in this essential micronutrient, without which thyroid hormones cannot be synthesized.

After a 3-year postdoctoral stay at the University of

Leiden, where she was introduced to modern endocrinology and new techniques such as the labelling of thyroid hormones with radioactive isotopes and experimental approaches to study their metabolism, she joined the CIB in 1958. There she led the Thyroid Studies section of the Gregorio Marañón Institute between 1963 and 1975. Over this period she developed her own line of research, and made important contributions in the field of thyroid hormone metabolism, demonstrating the direct relationship between the biological activity of T4 hormone and its extrathyroid metabolism by deiodination, quantifying thyroid hormone concentrations in extrahepatic tissues, and demonstrating that thyroid hormones, and especially T3 and not T4, are essential for the synthesis of growth hormone.

Gabriella Morreale's contributions to clinical practice have proven crucial. Her studies led to the creation of the first protocol to study endemic goitre in Spain, subsequent characterization and classification of this disease, development of a method to measure iodine in urine, and the recommendation to quantify TSH as a means of diagnosing congenital hypothyroidism. Moreover, she even proposed a standardized means of collecting information for these purposes.

The period between 1976 and 1989 was a crucial stage in Gabriella's scientific career, with important social repercussions for Spain. During this time field studies on iodine deficiency were carried out in Las Hurdes (Cáceres), in the province of Madrid, and in other Spanish provinces, and a method was developed to screen or detect congenital hypothyroidism in newborn children. The aim of this trial was to detect as early as possible children born without a thyroid or with thyroid defects, and to treat them with thyroxin immediately and thereby avoid mental retardation resulting from the lack of thyroid hormones necessary for early brain development. Personnel responsible for carrying out this screening process were trained accordingly, and training sessions organized with gynaecologists, paediatricians, nurses, and midwives. Gabriella herself noted that when the program was implemented in the Community of Madrid in 1985, more than 250,000 newborns had been screened and 90 cases of congenital hypothyroidism had been detected.

This intense research work made Gabriella Morreale a world authority in the study of the effects of thyroid hormones on brain development and the consequences of nutritional iodine deficiencies, and an essential figure in basic and applied endocrinology research in the mid and late 20th century in Spain.

**Thanks to Prof. Flora de Pablo for providing the images included in this article*

Gabriella Morreale in "Nosotras biocientíficas españolas (We, Spanish female bioscientists)." L'Oréal For Women in Science, 2002.

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Biotechnology: from the laboratory to the classroom

The CIB Margarita Salas has recently created a project titled "Research in biotechnology and plant health at the CIB Margarita Salas: Combating climate change", funded by the Spanish Foundation for Science and Technology (FECYT) and the Fundación General CSIC, through the *Cuenta la Ciencia* call for proposals.

This project, aimed at ESO and Baccalaureate students, seeks to highlight key research carried out at the CIB in the field of global change and sustainability: bioplastic development, environmental microorganisms, pest control, crop resistance to stress (drought, cold, etc.), the use of enzymes to obtain high-value products, etc.

More than 500 students from schools throughout Spain participated in the organized activities, workshops, debates, and experimental projects, which students carried out under the supervision of researchers from our center.

Fundación General CSIC

FECYT FUNDACIÓN ESPAÑOLA PARA LA CIENCIA Y LA TECNOLOGÍA

The topics covered in the debates included space agriculture, antibiotic resistance, and the future of transgenic crops, related controversy, and misconceptions. Online workshops covered the use of PCR in the context of plant health and biotechnology and the use of microbial enzymes to generate products of interest from plant residues. The students also had the opportunity to walk in the researchers' shoes and to study how many bacteria were present in their schools, investigate diseases and pests that affect plants, and discuss the dilemmas of genetically modified organisms and public perceptions thereof. These projects were presented at an online congress held in June.

Do you have a question that you want our scientists to answer? Do not hesitate to write to us:
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