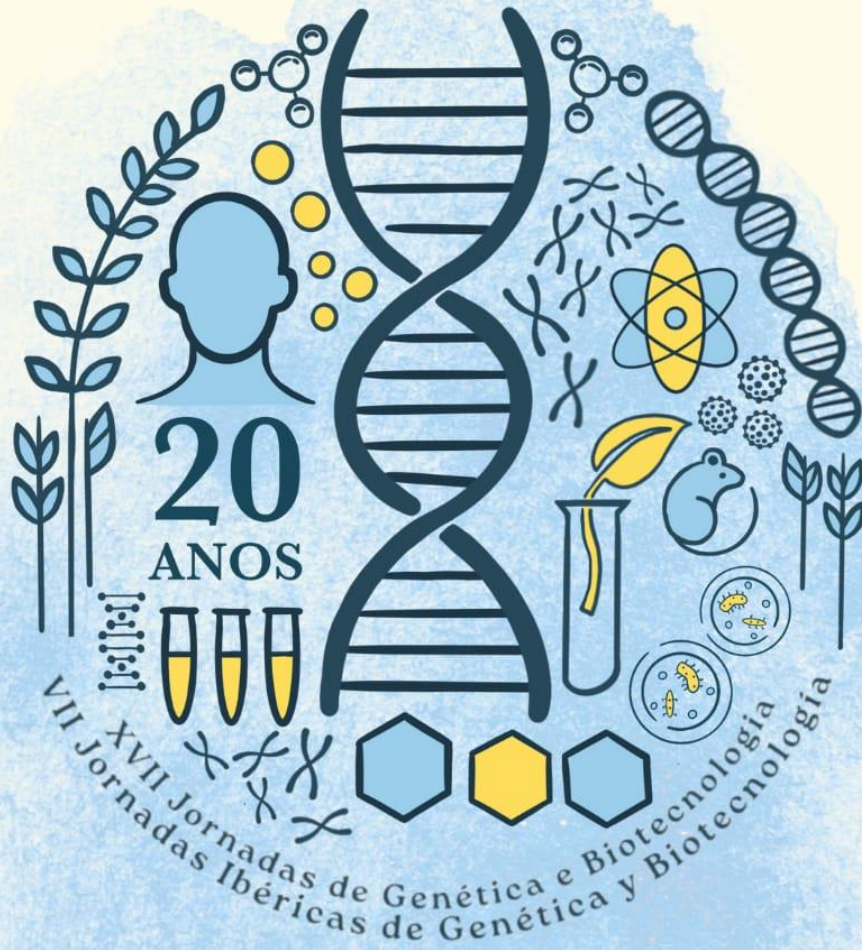


11th-15th March 2025



# BOOK OF ABSTRACTS

XVII Jornadas de Genética e Biotecnología | VII Jornadas Ibéricas de Genética y Biotecnología



**Título:** XVII Jornadas de Genética e Biotecnologia / VII Jornadas Ibéricas de Genética y Biotecnología - Book of Abstracts

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## XVII Genetics and Biotechnology Conference | VII Genetics and Biotechnology Iberian Conference

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The “Jornadas de Genética e Biotecnologia” (JGB) of the University of Trás-os-Montes and Alto Douro (UTAD) is an annual scientific event. It is organized jointly by the Nucleus of Students of Genetics and Biotechnology (ADNGB) of UTAD and the Direction of the Course of Genetics and Biotechnology in collaboration with the Department of Genetics and Biotechnology (DGB) teaching staff. As a result of the scientific-pedagogical partnership established between professors of DGB-UTAD and the Faculty of Biological and Environmental Sciences of the University of León (UL), Spain, it was considered essential to share the organization of this event between professors and students of the UTAD and UL designating it as “Jornadas de Genética e Biotecnologia | Jornadas Ibéricas de Genética y Biotecnología” (JGB | JIGB).

This year, the XVII JGB | VII JIGB will be held from March 11<sup>th</sup> to 13<sup>th</sup> at the School of Life and Environmental Sciences (ECVA), UTAD, Portugal, and from March 14<sup>th</sup> to 15<sup>th</sup> at the Faculty of Biological and Environmental Sciences of the University of León (UL), Spain.

The main objective of the XVII JGB | VI JIGB is to update knowledge in Genetics and Biotechnology. So, this event focuses on conferences given by renowned national and international scientists and thematic workshops that will constitute more practical sessions.

The XVII JGB | VII JIGB will also focus on interaction, exchange of experiences, and scientific debates among Portuguese and Spanish students and professors.

The best oral and poster presentations will be awarded, providing an excellent opportunity to showcase your research and gain recognition.

The target audience is Portuguese and Spanish students, researchers, university professors from the scientific areas of Biological Sciences and Biotechnology, and High School Biology teachers.

Various topics in genetics and biotechnology will be addressed, including neurogenetics, plant biotechnology, forensic genetics, bioinformatics, evolutionary genetics, microbiology, cancer research, and biotechnological applications.



# COMMITTEES





## HONOUR COMMITTEE

---

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Fernando Gonçalves

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(UL, Spain)

**Professor Asier Largo Gosens**

(UL, Spain)

**Professor Álvaro Mourenza Flórez**

(UL, Spain)

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**Professor Ana Isabel González Cordero**  
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**Professor Michal Letek Polberg**  
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**Professor Ana Margarida Ferreira**  
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**Dr. Ana Isabel Carvalho, Ph.D.**  
(UTAD, Portugal)

**Students and members of the Association of Biotechnologists of León (ABLE):**

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(President)

**Dña. Esther García Iglesias**  
(Vice President)

**D. David Dueñas Porqueras**  
(Undersecretary)

**Genetics and Biotechnology Students Association (ADNGB-UTAD):**

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(President of the Scientific Department)

**Maria João Magalhães**  
(Vowel of the Scientific Department)

**Maria Arroja**  
(Collaborator of the Scientific Department)



# PROGRAM





**Tuesday, 11<sup>th</sup> of March (Portugal – at UTAD)**

---

**WORKSHOPS (In person at UTAD)**

09:00 – 10:00 **Prof. Verónica Bermudez and collaborators**

*Learning from plants*

09:00 – 11:00 **Prof. Filomena Adegas and Prof. Filipe Pereira**

*The power of bioinformatics in cancer precision medicine*

10:00 – 11:00 **Prof. João Carrola and Prof. Francisco Peixoto**

*Desafios éticos emergentes na escrita científica, fotografia e produção/edição de imagens*

10:00 – 11:00 **Dr. Helena Henriques**

*Relevance of biosensors for the One Health approach*

11:00 – 12:00 **Prof. Maria Pires, Prof. Luana Travassos and Dr. Luana Martins**

*Descomplica a Histopatologia: a ciência por detrás do diagnóstico*

11:00 – 12:00 **Prof. Ana Novo Barros**

*Innovating Upcycled ingredients for sustainable beauty*

13:30 – 14:30 **Prof. Berta Gonçalves and collaborators**

*Physiological responses of fruit trees to climate change: challenges and innovative mitigation strategies*

13:30 – 14:30 **Prof. Isabel Pires, Prof. Justina Prada and Dr. Gabriela Maia**

*The use of Forensic Science in solving crimes against animals*

**Tuesday, 11<sup>th</sup> of March (Portugal – at UTAD)**

---

**WORKSHOPS** *(In person at UTAD)*

14:30 – 15:30 **Prof. Paula Lopes and Dr. Sara Barrias (Ph.D.)**

*Forensic Genetics applied to the food sector*

14:30 – 15:30 **Prof. Isaura Castro and Prof. Márcia Carvalho**

*SSRs for genotyping plant genetic resources*

15:30 – 16:30 **Prof. Patrícia Poeta, Prof. Telma de Sousa and Prof. Vanessa Silva**

*Antimicrobial Resistance in a One Health context: isolation and characterization of pathogenic bacteria*

16:00 – 17:00 **Prof. Ana Coelho, Prof. Fernanda Leal, Prof. Maria Manuela Matos and Prof. Marlene dos Santos**

*Eco-friendly fungus: harnessing fungi to combat fungi and parasites*

17:00 – 18:30 **ROUND TABLE WITH FORMER STUDENTS**

*Exclusive event for former and current students of the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> cycles in the field of Genetics and Biotechnology*

18:30 **MUSICAL MOMENT**

## Wednesday, 12<sup>nd</sup> of March (*morning session*) (Portugal – at UTAD)

---

UTAD (ECVA Auditorium): Zoom videoconference in streaming

### PROGRAM

PT	ES	
9:00	10:00	<b>OPENING SESSION</b>
9:30	10:30	Conference <b>Professor César Mendes (Nova Medical School)</b> <i>Neurogenetics of locomotion: from sensory-driven movement to freezing behaviour</i>
11:00	12:00	<b>Coffee break and Posters session</b>
11:15	12:15	Conference <b>Professor Fernanda Fidalgo (University of Porto)</b> <i>Resilient plants in a changing climate – research from the Plant Stress lab</i>

### ORAL COMMUNICATIONS – SESSION 1

12:15	13:15	<a href="#">Patrícia Afonso</a> - <i>Assessing salt resilience in European cowpea accessions during germination</i>
12:25	13:25	<a href="#">Marlene Santos</a> - <i>dPCR vs qPCR: How could be related and contribute to sweet cherry breeding programs?</i>
12:35	13:35	<a href="#">Elizabete Nascimento-Gonçalves</a> - <i>Effect of vermicompost from vine and wine industry by-products on growth and photosynthetic parameters of garden cress</i>
12:45	13:45	Discussion of all oral communications (Session 1)
13:00	14:00	<b>LUNCH</b>

**Wednesday, 12<sup>nd</sup> of March (afternoon session) (Portugal – at UTAD)**

---

*UTAD (ECVA Auditorium): Zoom videoconference in streaming*

**PROGRAM**

<b>PT</b>	<b>ES</b>	
14:00	15:00	Conference <b>Professor Hélder Maiato (University of Porto)</b> <i>Mechanisms of cellular adaptation to chromosome evolution</i>
15:00	16:00	Conference <b>Professor Alfonso Valencia (Barcelona Supercomputing Center)</b> <i>The limits and limitations of computation in Biology</i>
16:00	17:00	<b>Coffee break and Posters session</b>
16:30	17:30	Conference <b>Professor Octávio Paulo</b> <i>Forecasting species response to climate change using genomic prediction</i>
17:30	18:30	Conference <b>Dr. Luísa Fraga (University of Coimbra)</b> <i>Bridging Biotech and Genetics with One Health: exploring Molecular Ecology in soil biodiversity</i>
18:30	19:30	<b>MUSICAL MOMENT</b>

## Thursday, 13<sup>th</sup> of March (*morning session*) (Portugal – at UTAD)

---

UTAD (ECVA Auditorium): Zoom videoconference in streaming

### PROGRAM

#### ORAL COMMUNICATIONS – SESSION 2

PT	ES	
9:00	10:00	<b>Carolina Sabença</b> - <i>Evaluation of antibiotic resistance in environmental isolates of Klebsiella spp. and Raoultella spp.</i>
9:10	10:10	<b>Catarina Silva</b> - <i>Determination of antimicrobial resistance and the impact of Imipenem+Cilastatin synergy with Tetracycline in Pseudomonas aeruginosa isolates from septicemias</i>
9:20	10:20	<b>Telma de Sousa</b> - <i>Pseudomonas aeruginosa: One Health approach to deciphering hidden relationships in Northern Portugal</i>
9:30	10:30	<b>Jéssica Ribeiro</b> - <i>Genomic characterization of multidrug-resistant Escherichia coli in slaughterhouse broilers: a One Health perspective</i>
9:40	10:40	Discussion of all oral communications (Session 2)
10:00	11:00	Conference <b>Professor Laura Cainé</b> <b>(National Institute of Legal Medicine and Forensic Sciences)</b> <i>Potencialidades da Genética Forense</i>
11:00	12:00	<b>Talk Specanalitica</b> The foundation for your Next Generation Sequencing
11:15	12:15	<b>Coffee break and Posters session</b>
11:45	12:45	Conference <b>Professor Carlos Azevedo (CIIMAR)</b> <i>O mundo dos microparasitas observado ao microscópio</i>
12:45	13:45	<b>LUNCH</b>

## Thursday, 13<sup>th</sup> of March (*afternoon session*) (Portugal – at UTAD)

---

UTAD (ECVA Auditorium): Zoom videoconference in streaming

### PROGRAM

PT	ES	
14:00	15:00	Conference
		<b>Professor Paula Soares (University of Porto)</b>
		<i>Thyroid Cancer Genetics and recent advances in diagnosis and treatment</i>
15:00	16:00	Conference
		<b>Dr. Raquel Ruivo (CIIMAR)</b>
		<i>Gene loss, historical contingency and species resilience: a tale of the Anthropocene</i>
16:00	17:00	<b>Coffee break and Posters session</b>
		<b>ORAL COMMUNICATIONS – SESSION 3</b>
16:30	17:30	<b>Tatiana Afonso</b> - <i>Tracking zoonotic Microsporidia in seafood and assessing their repercussions for Food Safety and Public Health</i>
16:40	17:40	<b>Volodymyr Tkach</b> - <i>Potential Genotoxicant Bisphenol C2 and Sucralose Electrochemical Sensing. A theoretical insight</i>
16:50	17:50	<b>Carlos Vila-Verde</b> - <i>Decoding Human Satellite 1B in primates: insights from bioinformatic analysis</i>
17:00	18:00	Discussion of all oral communications (Session 3)
17:30	18:30	Conference
		<b>Professor António Almeida (University of Lisbon)</b>
		<i>Bionanotechnological approach to enhancing mucosal immunity: development of new vaccines</i>
18:30	19:30	Space reserved for sponsors
19:30	20:30	<b>MUSICAL MOMENT</b>



## Friday, 14<sup>th</sup> of March (Spain – at U. León)

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### WORKSHOPS (*In person at UL*)

- W1 - Introduction to R for Biology and Biotechnology data analysis
- W2 - Bioactive agents screening through bioassays: Exploring eco-sustainable alternatives to chemical pesticides
- W3 - Introduction to real-time qPCR
- W4 - Guided visit to the animal facility of the University of León

### LUNCH

#### PROGRAM

*University of León (“Aula Magna” - FCCBA): Zoom videoconference in streaming*

PT	ES	
14:00	15:00	Welcome by the Vice-Rector, Professor Michal Letek
14:30	15:30	Conference <b>Professor Elba Mauriz (University of León)</b> <i>Genosensors: Advanced technologies for precision Medicine</i>
<b>ORAL COMMUNICATIONS – SESSION 4</b>		
15:15	16:15	<a href="#">Sergio Fernández Martínez</a> - <i>Staphylococcus aureus in cancer: development of combined therapies for chronic intracellular infections</i>
15:30	16:30	<a href="#">Celia de la Puente-Ramos</a> - <i>Enhancing dark fermentation for biohydrogen and volatile fatty acids production through electrofermentation</i>
15:45	16:45	<a href="#">Tamara Joglar del Dago</a> - <i>Accelerating startup and efficiency in microbial electrolysis systems through advanced polymeric coatings</i>
16:00	17:00	<b>Coffee break and Posters session</b>
16:30	17:30	<b>ROUND TABLE</b> <b><i>Opportunities in the Biotech Companies</i></b> Prof. Raúl Mateos González (moderator); D. Mario Arcos Rodríguez (Wacker León SL); D. Luis E. Fontanetti Alves da Silva (mAbxience); Dña. Patricia De La Madrid Salmerón (53Biologics); Dra. Marta Rodríguez Saiz (Biotechnology Laboratory Manager) and Curia (Company)

## Saturday, 15<sup>th</sup> of March (Spain – at U. León)

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University of León (“Aula Magna” - FCCBA): Zoom videoconference in streaming

### PROGRAM

PT	ES	
8:30	9:30	Conference <b>Dr. Carla Calvo Peña (University of León)</b> <i>Exploring life inside olive roots: rhizosphere and endophytic Streptomyces</i>

### ORAL COMMUNICATIONS – SESSION 5

9:15	10:15	<b>Tania Martínez-García</b> - <i>Selection of traditional varieties of cucumber (Cucumis sativus L.) for drought tolerance</i>
9:30	10:30	<b>Daniel Fernández-García</b> - <i>Impact on cell wall composition of Arabidopsis thaliana pectin methylesterase inhibitor mutants susceptible to Pseudomonas syringae</i>
9:45	10:45	<b>Paula Gómez-de-Agüero-Campelo</b> - <i>Mutants in RGP genes show mucilage release defects in Arabidopsis thaliana seeds</i>
10:00	11:00	<b>Coffee break and Posters session</b>
10:30	11:30	<b>COMMUNICATIONS FLASH TALKS</b>

**García-Iglesias, E.:** Search for salt stress resistance genes in lentil (*Lens culinaris* M.)

**Nieto-Peña, E.:** Cut-dip-budding: simplifying plant genetic transformation without tissue culture

**Carrancio-Jato, P.:** Cell wall modifications in B73 maize seedlings in response to *Fusarium graminearum* infection

**López-López, L.:** Deepening the role of trichomes and lignin deposition as mechanisms of resistance of tomato plants to the parasitic plant *Cuscuta campestris*

**Mínguez-Hernández, A.:** Development and optimization of protocols for *Ascochyta* infection in lentils and analysis of the expression of candidate genes

**Galán Rodríguez, O.:** Novel bio-based strategies for sustainable phytopathogen control

**Llano-Verdeja, J.:** Penicillin-binding proteins in the control of phytopathogenic diseases

**Aguado-Rodríguez, I. and Collado Redondo, I.:** Anthropogenic activity affects the prevalence of antibiotic resistant bacteria in the soil. A study on antibiotic resistance and production

**Cid-González, A.:** Pathogens of the porcine respiratory disease complex in Spain: The role of molecular biology in their characterization

**González-Montero, M.C.:** Development of sheep and mouse liver organoids: Applications in drug screening and disease modelling

**Fraile-Roncero, N.:** History and evolution of Bioethics in animal experimentation

## Saturday, 15<sup>th</sup> of March (Spain – at U. León)

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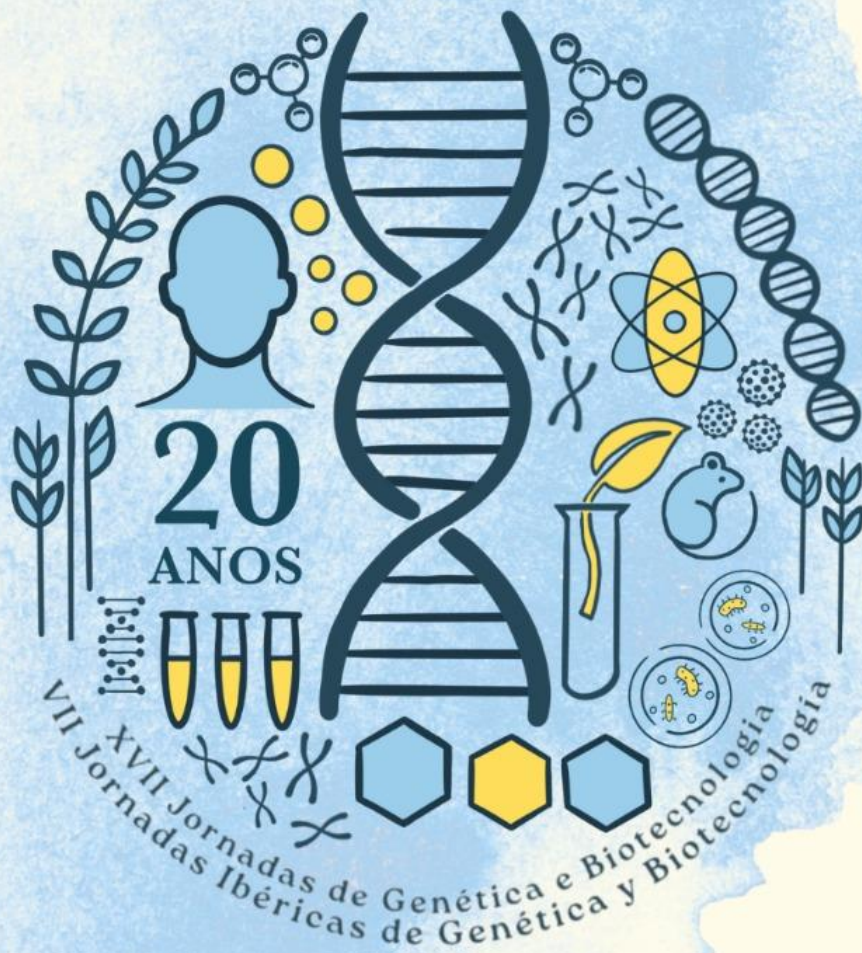
*University of León (“Aula Magna” - FCCBA): Zoom videoconference in streaming*

### PROGRAM

PT	ES	
11:30	12:30	<b>ELEVATOR PITCH “COMPANIES”</b> <ul style="list-style-type: none"><li>▪ <b>D. Antonio Llanos Canseco</b> - Legumbres Penelas SL</li><li>▪ <b>Dña. Pilar de la Torre Flórez and D. Adrián Fernández-Trapote</b> - Clinical Studies, Coordinators in the Hospital of León</li><li>▪ <b>D. Máximo Petrocchi-Rilo</b> - SYVA</li><li>▪ <b>D. Roberto Fernández González</b> - BIOGES Starters</li><li>▪ <b>D. Antonio Fernández Medarde</b> - Biomar</li></ul>
12:30	13:30	<b>AWARDS CEREMONY AND CLOSING SESSION</b>



# SPEAKERS







## Professor César Mendes

César Mendes is an invited assistant professor and a principal investigator at NOVA Medical School (NMS), Universidade Nova de Lisboa, where he leads a research group focused on the mechanisms controlling locomotion in health and disease. His academic journey began with a biochemistry degree at the University of Coimbra. He then joined the Gulbenkian PhD Program in Biomedicine (Portugal) and conducted his doctoral research at Rockefeller University, where he studied cell death mechanisms in the retina of the genetically tractable fruit fly, *Drosophila melanogaster*. Following his PhD, he moved to Columbia University Medical Center for his postdoctoral studies, focusing on the development and function of motor systems using mouse and *Drosophila* as model systems. During this time, he developed kinematic tools to study and quantitatively describe walking in both wild-type and mutant conditions. He returned to Portugal through an FCT Investigator grant and a Marie Skłodowska-Curie Reintegration grant, which laid the foundation for his establishment as a principal investigator at NMS. As a Principal Investigator, he leads a research group that investigates various aspects of motor control, ranging from the development of innate motor circuits to the role of sensory systems. His work also involves using kinematic data to identify optimal treatment options for patients with degenerative spine conditions. To address these research questions, his laboratory leverages the sophisticated genetic toolkit available for *Drosophila*, custom-made motor behavior setups, and advanced analysis tools to extract meaningful insights from large datasets. These datasets describe the multidimensional features of multi-jointed locomotion across species, from flies to humans.



### **Professor Fernanda Fidalgo**

Fernanda Fidalgo has a PhD in Biology (specialization in Plant Physiology) from the Faculty of Sciences, University of Porto (FCUP), where she is an Associate Professor with Habilitation in the Department of Biology and an integrated researcher at GreenUPorto (<https://www.fc.up.pt/GreenUPorto/pt/>). Her main research focuses on studying the physiological and biochemical responses of plants to abiotic stress (e.g., water deficit, high temperatures, salinity, and emerging contaminants), with an emphasis on redox metabolism. In addition, FFidalgo's team has been exploring stress mitigation strategies, such as organic soil amendments and the development of biological priming technologies. As the team leader of the Plant Stress lab, she has been fostering intra- and inter-institutional collaborations with researchers in Plant Physiology, Biotechnology, and Environmental Toxicology. To date, FFidalgo has coordinated or participated in 25 national and international multidisciplinary R&D projects, published more than 85 articles in high-impact ISI journals (approximately 90% in Q1), contributed to four book chapters, and presented over 280 communications at national and international congresses. In addition, she has been actively involved in supervision and tutoring, from the BSc to the PhD level, in evaluating applications for MSc and postdoc grants, as well as researcher contracts. She serves as a reviewer and editor for several international scientific journals in Plant Sciences and Environmental Sciences. These activities have been strongly complemented by initiatives for science outreach and dissemination, including organizing international scientific events and fostering collaborations with companies and local secondary schools.





### **Professor Hélder Maiato**

Hélder Maiato graduated in Biochemistry and holds a PhD in Biomedical Sciences from the University of Porto. He was a visiting PhD student at the University of Edinburgh (UK) and a Post-doctoral Research Affiliate at the New York State Department of Health (USA). At present, he is Coordinating Investigator at the Institute for Research and Innovation in Health (i3S) where he heads the Chromosome Instability & Dynamics Lab, and Invited Full Professor at the Faculty of Medicine of the University of Porto. He authored 120 scientific publications and has been invited to give seminars in more than 20 different countries, including major conferences in the field. He is recognized by his work on the role of CLASP proteins in the regulation of microtubule dynamics at kinetochores and the demonstration that kinetochore-driven microtubule organization is a key step in mitotic spindle assembly. More recently, his team uncovered a navigation system for chromosomes and a mitotic error code defined by tubulin detyrosination, as well as the mechanism behind spatial control of nuclear envelope reassembly during mitotic exit by an Aurora B activity gradient. Over the last 25 years, his main research interest has been the spatial, temporal and adaptive mechanisms underlying chromosome segregation fidelity in the context of normal physiology, disease and evolution. In this context, he is/has been the coordinator of three grants (Starting+Consolidator+Advanced) from the European Research Council. Between 2012- 2015 he served as National Counsellor for Science and Technology to the Portuguese Prime Minister. In 2015 he was honoured with the Young Investigator Award from the Louis-Jeantet Foundation (among ERC awardees), was elected EMBO Member in 2016 and was recognized with the Excellence in Scientific Research Award by the University of Porto in 2019. Together with his wife (a child psychologist), they founded Yscience ([www.yscience.pt](http://www.yscience.pt)), a pilot non-profit initiative that brings together scientists to promote science education and the scientific method with young children.



### **Professor Alfonso Valencia**

Professor Alfonso Valencia is ICREA research Professor, Director of the Life Sciences Department of the Barcelona Supercomputing Center, Director of the Spanish National Bioinformatics Institute INB/ELIXIR-ES and coordinator of the data pillar of the Spanish Personalised Medicine initiative, IMPaCT. His research interest is the development of Computational Biology methods and their application to biomedical problems. Some of the computational methods he developed are considered pioneering work in areas such as biological text mining, protein coevolution, disease networks and more recently modelling cellular systems (digital twins). He participates in some of the key cancer related international consortia. In terms of community services, he is one of the initial promoters of the ELIXIR infrastructure, founder of the Spanish and International Bioinformatics networks and former president of ISCB, the international professional association of Bioinformaticians. He is Executive Editor of the main journal in the field (Bioinformatics OUP).



### **Professor Octávio S. Paulo**

Associate Professor at the Universidade de Lisboa, Portugal, Department of Animal Biology. First degree in Biology 1988 and Ph.D. in 2001 at the Queen Mary and Westfield College, University of London. Currently is the Group Leader of the Computational Biology and Populations Genomics Group (CoBiG2 - <https://ce3c.ciencias.ulisboa.pt/team/CoBiG2>) of the cE3c – Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa encompassing 4 PhD members, 6 PhD students, 3 master students and one technician.

Main research areas are Evolutionary Biology, Genomics and Bioinformatics. The main research aim is to understand the genetic and genomic bases of the processes of evolutionary differentiation and local adaptation in natural populations of non-model organisms. This includes the research of the patterns and processes that originated and maintained the current genetic differentiations, structure and demographic history of the populations. The study of these processes at the genomic scale implies the development of Bioinformatics skills necessary to deal with large amounts of genomic data and carry out, complex and time-consuming analysis on them.

This research is under the framework of the emergent area of the response of organisms and populations to current environmental changes, i.e. understanding the ecological and evolutionary factors that allow the tracking of the process of climatic change by natural populations and its consequences for the population viability and conservation.



**Dr. Luísa Fraga, M.Sc.**

Graduated in Biology from the Faculty of Sciences of the University of Lisbon and holding a Master's degree in Applied Ecology from the University of Coimbra (UC), I have been focusing on the use of molecular methods for biodiversity mapping, particularly in soil fauna. Currently, I work at EnGeL (Environmental Genetics Laboratory - UC), where our mission is to integrate various fields, such as evolutionary ecology, phylogenetics, and conservation genetics/genomics of invertebrates, aiming at biodiversity conservation applications.

My main focus is on the use of next-generation sequencing (NGS) methods, such as metabarcoding, for studying soil macrofauna. I have a strong interest in both laboratory work and field sampling, along with curiosity in bioinformatics and data analysis. I am currently proposing a PhD that integrates DNA-based techniques, taxonomy, bioinformatics, anthropology, and archaeology to explore how historical human activities have shaped biodiversity in underexplored neotropical ecosystems in Amazonia.



### **Professor Laura Cainé**

Laura Cainé holds a PhD in Health Sciences from the Faculty of Medicine of the University of Coimbra, a Master's degree in Medicine from the Faculty of Medicine of the University of Porto (FMUP), and a Master's degree in Cell Biology from the Faculty of Science and Technology of the University of Coimbra.

Currently, she serves as a Board Member and Director of the Central Delegation of the National Institute of Legal Medicine and Forensic Sciences, I.P. (INMLCF). She is also a Member of the Medical-Legal Council. From 2003 to 2020, she worked as a Senior Specialist in Legal Medicine at the Forensic Biology and Genetics Service of the INMLCF.

She holds the position of Assistant Professor (Adjunct) at the Department of Public and Forensic Health Sciences and Medical Education at FMUP and at the Law School of the University of Minho, where she is responsible for various curricular units.

Dr. Cainé has published several articles in indexed international journals and book chapters in her field of expertise. She has delivered numerous invited lectures and presentations at scientific conferences in the field of Forensic Sciences.

She supervises undergraduate and postgraduate dissertations in Forensic Sciences and has participated as a jury member in various academic examinations at both Master's and PhD levels.

Additionally, she is a Researcher at LAQV-REQUIMTE (Associated Laboratory for Green Chemistry of the Network of Chemistry and Technology).



### **Professor Carlos Azevedo**

Carlos Azevedo - Full Professor of Cell Biology, Retired from the Institute of Biomedical Sciences Abel Salazar of the University of Porto (ICBAS/UP - Laboratory of Cell Biology) since 2004, where he was Director from 1975 to 2004; and Researcher Member of the Interdisciplinary Center for Marine and Environmental Research of the University of Porto (CIIMAR/UP).

He received the Master of Science (“Certificat de Zoologie”) (1972 -1974) in the University of Lausanne (Switzerland) and achieved his Ph.D. in Cell Biology (1974-1976), Specialty in “Physiology and Cellular Ultrastructure”, at the Faculty of Sciences of University of Porto.

As a researcher, developed several projects related to “Gametogenesis - Oogenesis and Spermatogenesis” (1975 -1984), and “Microparasitology” (1984 - 2004).

He formed a group of researchers who developed the theme “Microparasitology” of aquatic fauna, having published numerous articles.



**Professor Paula Soares**

Paula Soares, BSc, MSc, PhD, is Associated Professor of Biopathology at the Medical Faculty of the University of Porto and coordinates the Group of Cancer Signaling and Metabolism at the I3S/IPATIMUP. Paula Soares research interests include oncobiology of endocrine and neuroendocrine tumors, with special emphasis on genetic changes in cell signaling molecules involved in MAPK and PI3K / mTOR pathways. The metabolic deregulation in cancer is also an interest in her research. Paula Soares team has provided important insights into the genetics of thyroid tumors concerning the establishment of BRAF mutations and of TERT promoter mutations in thyroid cancer and become one of the most cited group in thyroid carcinogenesis. The group also made relevant contributions in establishing the prognostic value of TERT in thyroid and other cancers including glioblastoma, melanoma, squamous cell carcinoma and bladder cancer. Particular interest is given to translation studies concerning the clinical and pathological significance of cancer genetic alterations, and the development of useful biomarkers for monitoring and improving the diagnosis, follow-up and treatment of cancer patients. Paula Soares has over 300 papers (the majority of them addressing thyroid cancer) in peer-reviewed journals, with over 10000 citations and an h-index of 57. She is also a member of numerous scientific committees and evaluation boards and serves on grant review committees and in journal editorial and review boards.



**Dr. Raquel Ruivo, Ph.D.**

Raquel Ruivo is an Auxiliary Researcher at CIIMAR. Raquel Ruivo holds a Biology degree from the University of Aveiro (2005) and a PhD in Life Sciences, Biology and Health (2009) from the Paris-Sud University. She debuted her research career at the Cell Biology Center of the University of Aveiro (now part of IbiMed) addressing the molecular mechanisms underlying Alzheimer Disease pathology; and, at the Centre National de la Recherche Scientifique (CNRS) in Paris, focusing on the molecular mechanisms of pathogenesis of hereditary Lysosomal storage disorders. Raquel Ruivo joined CIIMAR as a Postdoctoral Fellow in 2011, working on the evolution, physiology and environmental disruption of nuclear receptors: a superfamily of metazoan transcription factors. Her current research interests include the genomic mechanisms underlying mammalian diversification, notably on the field of evolution by gene loss. In this scope, Raquel Ruivo contributed to unravel the role of gene loss in cetacean skin remodelling, cetacean sleeping behaviour, and sperm energetics, with consequences in reproductive strategies; as well as, the gene loss fingerprint of vestigial structures, such as the pineal gland in Cetacea, anteaters or pangolins. Currently, she addresses the trade-offs between ancestral adaptations and Anthropogenic environmental contamination scenarios. Notably, the case of marine mammals due to their status as iconic species, their susceptibility to contamination and their unique evolutionary history; namely, how the historical contingencies of their adaptations to the marine environment compromise their defence mechanisms in the current Anthropocene. In parallel to her research path, Raquel Ruivo completed a postgraduate course in Viticulture and Oenology from the Universidade do Porto (2015) and is currently attending the 3rd year of a Bachelor in History, with a minor in Anthropology, from Universidade de Coimbra.





### **Professor António Almeida**

António J. Almeida is a Full Professor of Pharmaceutical Technology and the President of the Scientific Council at the Faculty of Pharmacy, University of Lisbon (FFUL). He holds a degree in Pharmaceutical Sciences (Industrial Pharmacy) from FFUL (1987), a PhD in Pharmaceutical Technology from Aston University in Birmingham, United Kingdom (1993), and Habilitation in Pharmaceutical Sciences from FFUL (2005). He serves as a quality expert for the Medicines Evaluation Committee of INFARMED and the European Medicines Agency (EMA) and is a member of the Steering Committee of the National Medicines Laboratory. He was a Visiting Researcher at Free University Berlin, Germany (1995/1996), and at the Centre for Research in Molecular Medicine and Chronic Diseases (CIMUS) at the University of Santiago de Compostela (2012). Additionally, he is a Visiting Professor in the doctoral program in Pharmacy at the University of Seville. He leads the research group on Advanced Technologies for Drug Delivery at the Research Institute for Medicines (iMed.Ulisboa), focusing on the study of drug and vaccine delivery using polymeric and lipid micro- and nanoparticulate systems. He has supervised or co-supervised 17 doctoral theses and 40 master's theses and is the co-author of 6 patents and over 150 publications. He is also a member of the editorial boards of the journals J Biomed Nanotechnol, J Microencapsulation, J Drug Deliv Sci Technol, AAPS PharmSciTech, and Pharmaceutics.



### **Professor Elba Mauriz**

Elba Mauriz is a Senior Lecturer at the Faculty of Health Sciences at the University of León. She holds degrees in Nursing and Environmental Sciences. She completed her PhD at the Institute of Micro and Nanotechnology of the CSIC and her thesis was awarded the Extraordinary Doctorate Prize of the University of León. She is an expert in the development of bioanalytical applications for biosensor devices. Her research focuses on the design of real-time diagnostic strategies for the determination of chemical and biological agents in the fields of Medicine, the Environment and Food Safety. Throughout her scientific career she has collaborated in research activities and carried out short stays at prestigious national and international technological centres. She is the author of 41 scientific papers (77% as first author and 71% as corresponding author), including 4 book chapters, in high-impact journals in the fields of Chemistry, Biochemistry, Genetics and Molecular Biology, Medicine, Engineering, Computer Science, Physics and Astronomy, Environmental Sciences, Health Professions, Immunology and Microbiology, and Agricultural and Biological Sciences. Since 2022, she appears in the Stanford University ranking, among the 2% of the most cited scientists in the world.



**Dr. Carla Calvo Peña, Ph.D.**

I graduated in Biology from the University of Salamanca in 2015 and completed a Master's degree in Food Quality and Safety at the University of the Basque Country in 2016. Through the Galician Agency for Innovation, I worked for a year in the viticulture group at the MBG-CSIC in Pontevedra where I had my first approach to research. In 2018, I obtained a grant from the Junta de Castilla y León to work at the Instituto de Investigación de la Viña y el Vino (IIVV-University of León) applying microbiology and molecular biology techniques to research on grapevine trunk diseases. I later pursued my PhD there, under the supervision of Dr. Juan José Rubio Coque and Dr. Rebeca Cobos. My doctoral thesis focused on *Streptomyces* sp. as biocontrol agents against Verticillium wilt of olive caused by *Verticillium dahliae*. As a result, several indexed scientific articles were published, including a First Report of *Pleurostoma richardsiae* as an olive tree pathogen in Spain.

During my PhD, I completed a 3-month internship in the laboratory of Dr. Sebastjan Radišek at the Slovenian Institute of Hop Research and Brewing (Žalec, Slovenia) where we worked on the application of microorganisms against Verticillium wilt of hop caused by *Verticillium nonalfalae*. Additionally, I have participated in international conferences and I have taught university-level courses related to the fields of Biology and Environmental Sciences. Currently, I hold a position as a researcher in phytopathology at the IIVV-University of León. My main research interests include the search for new secondary metabolites with antifungal activity and the characterization of novel species of actinomycetes.



# CONFERENCES





## Neurogenetics of locomotion: from sensory-driven movement to freezing behaviour

Anna F. Hobbiss<sup>1,2</sup>, Alexandra M. Medeiros<sup>1</sup>, Mariana C. Franco<sup>2</sup>, Charlie Rosher<sup>2</sup>,  
Marta A. Moita<sup>2</sup> and César S. Mendes<sup>1\*</sup>

<sup>1</sup> NOVA Medical School | Faculdade de Ciências Médicas, Universidade Nova de Lisboa

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¶ Equal author contribution

**Keywords:** Locomotion; Neurogenetics; sensory systems; freezing behaviour; *Drosophila melanogaster*; optogenetics.

Locomotion is one of the most fundamental yet complex motor behaviors. It is essential for animals to navigate their environment, find food, escape predators, and locate mates. At the same time, it requires precise coordination between motor neurons, central pattern generators, sensory neurons, higher-order brain centers, and muscles.

While mechanosensation is known to play a critical role in coordinated walking, how specific mechanosensors engage locomotor circuits remains poorly understood. We investigated the role of mechanosensory structures in movement initiation by optogenetically stimulating specific classes of leg sensory neurons. We found that activating leg mechanosensory bristles (MsBs) is sufficient to initiate forward movement in immobile animals. This response is independent of the central brain, as decapitated flies still react strongly to bristle stimulation. Additionally, leg-MsB activation triggers robust avoidance behavior away from the stimulus source.

In a separate study, we explored the musculoskeletal system during freezing behavior, a conserved defensive response to threats where skeletal muscles maintain tension to sustain rigid postures for extended periods. Nevertheless, the neural and somatic mechanisms underlying freezing remain poorly understood. We found that freezing *Drosophila* display a striking novel pattern of leg muscle activation unique to immobility, a rhythmic pulsing in the distal tibia. This muscle, which we show to be a leg accessory heart, displays multiple activity modes and ramps up to movement onset, implying a preparation for movement. The frequency of pulsing is dynamically modulated as the fly integrates external threat or safety cues.

Together, these findings reveal the intricate interplay between neural circuits, sensory inputs, and physiological systems in adaptive behaviors. They provide a foundation for further research into the genetic and neural basis for movement and survival strategies across species.

## Resilient plants in a changing climate – research from the Plant Stress lab

Soares, C. and Fidalgo, F.\*

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**Keywords:** plant stress, redox metabolism, climate change, chestnut tree

As sessile organisms, plants are inevitably exposed to increasingly challenging conditions that compromise their growth and development, with significant consequences for ecosystems and agriculture. Thus, understanding plant responses to abiotic stress has long been a key focus in Plant Biology, not only for fundamental research but also for translation studies to the agronomic sector. Aiming to find new ways to boost crop stress resilience, *Plant Stress lab*, a research unit of GreenUPorto Research Centre, aims to uncover how environmental factors influence plant tolerance mechanisms. By integrating biochemical and molecular approaches, like transcriptomics, our research explores plant responses to abiotic stresses, such as high temperatures, salinity, drought, and emerging contaminants. A particular focus is also placed on the development of stress mitigation tools, like biostimulants and plant priming. Recently, within the scope of the CC&NUTS project (Fundação La Caixa/BPI & FCT), the team has been evaluating the role of stress priming in increasing the tolerance of chestnut trees (*Castanea sativa* Miller) to climate change. Preliminary results suggest that drought-mediated priming of young chestnut plants can activate plant memory, thereby enhancing tolerance to future severe drought episodes. Primed plants, compared to untreated ones, showed less decreases in biometric attributes, while also ensuring the maintenance of the redox homeostasis and photosynthetic potential.

Other current topics also include: i) characterization of pesticide-induced transgenerational effects on crops' physiology and epigenome; ii) use of plant- and macroalgae-based compounds as new biostimulants and biopesticides; iii) search for tolerance traits for domestic tomato using wild species as potential sources. From a global perspective, *Plant Stress lab* research aims to contribute valuable knowledge and solutions for improving plant adaptation and sustainability in an era of rapid environmental change.

**Acknowledgments:** Acknowledgments are due to the Promove Program of the “la Caixa” Foundation, in collaboration with BPI and the Foundation for Science and Technology (FCT), through the research project CC&NUTS (PD21-00007). This work was also partially supported by national funds through FCT (UID05748 - GreenUPorto).



## Mechanisms of cellular adaptation to chromosome evolution

Cardial-Dias, I.<sup>1,2</sup>, Okada, N.<sup>1,2</sup>, Soares-de-Oliveira, J.<sup>1,2</sup>, Pereira, A. J.<sup>1,2</sup> and Maiato H.<sup>1,2,3</sup> \*

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**Keywords:** mitosis, chromosome, kinetochore, evolution, muntjac, speciation, cancer

Chromosome number among eukaryotic species is highly divergent, ranging from  $n=1$  in haploid males of a primitive Australian ant, to  $n=ca\ 224-226$  in a diploid blue butterfly, or  $n=720$  in a polyploid fern. Likewise, large variations in chromosome size are also common among eukaryotes. Paradoxically, while karyotypic diversification underlies the emergence of new species, alterations in chromosome number and size within a given species are highly deleterious and have been implicated in cancer evolution, metastasis and drug resistance. Importantly, while significant progress has been made at the level of understanding how karyotypic evolution impacts organism physiology, disease and speciation, much less is known about the impact of karyotypic alterations on fundamental cellular processes. In particular, alterations in chromosome number and size pose significant challenges for cell division and we currently do not know how the cell division machinery adapts to cope with these challenges. To address this problem, we have been exploring natural karyotypic evolution in two related deer species with a very similar genome size and composition, but distinctively divergent chromosome number – 6/7 chromosomes in the female/male Indian muntjac and 46 chromosomes in the Chinese muntjac, as in humans. Here I will elaborate on how we have been using these unique systems for the mechanistic dissection of mitosis in mammals, while investigating how karyotypic alterations may be explored therapeutically in the treatment of human cancers.

**Acknowledgments:** ERC-2023-ADG 101140624

## The limits and limitations of computation in Biology

Alfonso Valencia

ICREA Professor

Barcelona Supercomputing Center

This talk will explore three major challenges in computational biology: computing capacity, data accessibility, and the ability to simulate biological processes at mechanistic level. First, I will discuss the limitations of computing capacity, focusing on the current developments in high-performance computing (HPC) in Europe, with a particular emphasis on the role of the Barcelona Supercomputing Center infrastructure (participated by Portugal) and its contributions to advancing large-scale biological simulations. Next, I will address data accessibility, describing the ongoing development of the European Health Data Space and how its implementation, particularly through federated systems, is set to transform access to and sharing of biological and health data. The core of the talk will focus on the challenges of simulating biological processes. Here, I will introduce the concept of “digital twins” for cellular systems—dynamic models designed to replicate and predict cell behavior. While these models represent a significant leap forward, they also highlight the immense complexity of biological systems and the computational barriers that remain. Finally, I will examine how artificial intelligence (AI) is revolutionizing computational biology. AI is accelerating data analysis, uncovering new insights, and improving predictive modeling, offering potential solutions to many of the current limitations at the cost of a significant lack of “scientific understanding at the mechanistic level”.

## Forecasting species response to climate change using genomic prediction

Paulo, O.S. <sup>1\*</sup>

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\* octavio.paulo@fc.ul.pt

**Keywords:** Genomic offset, Cork Oak, Holm Oak, GBS

The capacity to forecast species response to climate change has become a hot topic in applied genomics to biodiversity since Bay et al. paper in Science in 2018. The concept of “genomic vulnerability” and “genetic or genomic offset”, become central in framework of genomic prediction to climate changes. How good are the predictive models, their limitations and how validate the results are fundamental current concerns for this research area. Results from the KeePace project funding by FCT, named “Keep Pace: Selection of trees keeping pace with fast environmental changes, a science based approach for sustainable XXI century Oak forests LISBOA-01-0145-FEDER-029263 PTDC/ASP-SIL/29263/2017 show with oaks tress how these species can respond to future climate change and contribute towards the understanding of the potential and limitations of these models.

## Bridging Biotech and Genetics with One Health: exploring Molecular Ecology in soil biodiversity

Fraga Dornellas, L. <sup>1\*</sup>

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**Keywords:** One Health; Molecular Ecology; Taxonomy; Barcoding; Metabarcoding; Bioinformatics

The fields of biotechnology and genetics offer transformative tools that extend far beyond the laboratory, shaping our understanding of the natural world and its intricate connections. The concept of One Health, a holistic approach linking environmental sustainability, agriculture, and human well-being, provides an inspiring framework for applying molecular methods to real-world challenges. In this presentation, we explore how molecular methods can improve the study of biodiversity, with emphasis on soil macrofauna, which play an essential role in nutrient cycling and soil ecosystem stability.

By utilizing advanced molecular approaches such as next-generation sequencing (NGS) techniques, including metabarcoding, we can gain a profound insight into the rarely seen yet vital biodiversity beneath the ground. Metabarcoding, which uses high throughput sequencing of taxonomically informative genetic markers, enables the rapid and accurate identification of diverse soil organisms from whole-community DNA (wocDNA) and environmental DNA (eDNA) samples. This technique allows researchers to assess species composition, detect rare or cryptic taxa, and monitor ecosystem changes over time with unprecedented efficiency and resolution. By overcoming the limitations of traditional morphological taxonomy, metabarcoding enhances our ability to study a wide range of soil taxa, from microbial to macrofauna communities, leading to improved biodiversity assessments and conservation strategies.

This presentation aims to connect and inspire students in biotech and genetics by showcasing the vast applicability of their academic training! By integrating molecular methods into environmental sciences, we can develop innovative solutions for sustainable land management, agroecosystem resilience, and biodiversity conservation. I aim to highlight the power of interdisciplinary collaboration and encourage students to see the impact of their skills far beyond conventional biotech applications. I hope this will be a catalyst to inspire them to think boldly and creatively about how their expertise can lead to groundbreaking advancements across various fields.

**Acknowledgments:** Dr. Luis Cunha, the PI of our research group who allowed me to take over this presentation. António Nobre, from UTAD, who took the courage to get involved in our lab for a simple internship that ended up with a beautiful MSc Thesis collaboration.

## Potencialidades da Genética Forense

Laura Cainé

<sup>1</sup> National Institute of Legal Medicine and Forensic Sciences

*No abstract available*

## O mundo dos microparasitas observado ao microscópio

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**Palavras-Chave:** Ultraestrutura, Microparasitas, Ciclo de Vida, Procariotas, Protistas, Myxozoa.

Os microparasitas são microrganismos que ocorrem como infetantes num incalculável número de espécies pertencentes à generalidade dos grupos taxonómicos, desde bactérias a mamíferos.

Nesta apresentação, serão reportados aspetos morfológicos microscópicos de diferentes fases do ciclo de vida de microparasitas da fauna aquática que ocorrem em diferentes espécies de peixes, moluscos e crustáceos, na maior parte dos quais induzem ações patogénicas, como especial destaque do estudo dos esporos, usando a microscopia de luz (ML), microscopia de varrimento (“Scanning” e microscopia eletrónica de transmissão (TEM).

Serão reportados aspetos dos microparasitas procariotas (bactérias, rickettsias e micoplasmas), onde são focados aspetos invulgares ultraestruturais de associações pouco frequentes. Serão abordados detalhados aspetos da morfologia ultrastructural e histopatológica de espécies de microparasitas do grupo Eukaryota Protista, que incluem os “*phyla*” Apicomplexa, Perkinsozoa, Haplosporidia e Microsporidia, bem como, aspetos ultraestruturais de várias espécies do “*phylum*” Cnidaria (Myxozoa), com destaque da morfologia ultrastructural dos mixosporos e seus ciclos de vida.

Os resultados aqui apresentados correspondem a um estrato de algumas publicações do nosso grupo de Colaboradores de diferentes Universidades estrangeiras.

**Agradecimentos:** A todos os Colegas e Pessoal Técnico das várias Universidades, a colaboração na obtenção destes resultados que, na época, constituíram descrições de novas espécies.

## Thyroid Cancer Genetics and recent advances in diagnosis and treatment

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**Keywords:** Thyroid cancer, Prognosis, Genetics, Molecular diagnostics, Targeted therapy

Thyroid cancer is the most common endocrine malignancy, with a rising global incidence over the past decades. This increase is partly attributed to improved diagnostic techniques, but environmental and genetic factors may also contribute. Papillary thyroid carcinoma (PTC) is the most prevalent subtype, followed by follicular thyroid carcinoma (FTC), medullary thyroid carcinoma (MTC), and the rare but aggressive anaplastic thyroid carcinoma (ATC). While differentiated thyroid cancers (PTC and FTC) generally have an excellent prognosis, MTC and ATC remain more challenging due to their aggressive behavior and limited therapeutic options.

Genetic alterations play a crucial role in thyroid carcinogenesis. The most common mutations include BRAF and RAS mutations in PTC and FTC, RET mutations in MTC, and TP53 mutations in ATC. Recent discoveries, such as TERT promoter mutations, have provided valuable prognostic insights, particularly in aggressive thyroid tumors. Advances in molecular profiling have paved the way for targeted therapies, such as kinase inhibitors for advanced and refractory thyroid cancers, significantly improving patient outcomes.

In terms of diagnosis, ultrasonography and fine-needle aspiration biopsy remain the gold standard, but molecular testing has enhanced risk stratification, reducing unnecessary surgeries. Emerging biomarkers and liquid biopsy approaches hold promise for early detection and monitoring disease progression. Furthermore, novel treatment strategies, including immunotherapy and combination therapies, are being explored to improve responses in aggressive thyroid cancers.

Despite these advancements, challenges remain in managing high-risk and refractory cases. Future research focusing on precision medicine, novel therapeutic targets, and improved diagnostic techniques will be crucial in refining thyroid cancer management and improving patient survival.

**Acknowledgments:** To all the members of the Cancer signalling and metabolism group.

## Gene loss, historical contingency and species resilience: a tale of the Anthropocene

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**Keywords:** evolution, adaptation, gene loss, comparative genomics, mammals

Mammals display remarkable diversity, evolving unique adaptations that enabled them to colonize distinct habitats. Within mammals, secondary gene loss mechanisms were shown to accompany major habitat transitions and adaptations; for instance, paralleling abrupt habitat transitions (e.g. land-to-water) and life history trait adaptations in specific lineages (e.g. specialized diets). Cetaceans, for instance, provide a striking example. Evolved from fully terrestrial ancestors, they underwent significant morphological (e.g. skin), physiological (e.g. energy) and behavioural (e.g. sleep) modifications, with gene loss episodes recognized to parallel such adaptations to a fully aquatic environment. Here, we will discuss how gene loss events shaped Cetacea (in)ability to cope with contemporary environmental chemical build-up. We will address the contingency of present day responses to environmental changes with respect to past evolutionary events, their evolutionary trade-offs and consequences for species adaptation.

**Acknowledgments:** “Whales and polar bear in a petri dish: decoding marine mammal toxicology through in vitro and in silico approaches - Marma-detox” (Norges Forskningsrad (NFR) 334739 - FORSKER22).



## Bionanotechnological approach to enhancing mucosal immunity: development of new vaccines

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**Keywords:** particulate carriers, nanotechnology, vaccines, mucosal immunity

Vaccination represents one of the greatest advances in medicine, having played a decisive role in the eradication or significant reduction of certain infectious diseases and their associated mortality rates. The emergence of new infectious diseases and the increase in infections caused by antimicrobial-resistant strains have reinstated vaccination as a priority strategy in public health. Mucous membranes cover a large area that comes into contact with the environment and are frequently exposed to invading pathogens and antigens that elicit specific humoral immune responses. The uptake of microparticles and nanoparticles in mucous membranes closely correlates with local and systemic immunity, remaining an attractive area of research.

Antigens (proteins, mRNAs, bacterial lysates, or intact bacteria) can be attached to micro/nanoparticles suitable for immunization after mucosal absorption, facilitating both local and systemic immune responses relevant to diseases, such as tuberculosis. By adjusting particle physicochemical properties, including particle size, surface characteristics, and the inclusion of specific adjuvants and permeability enhancers, one can select the translocation route and manage the *in vivo* fate and effectiveness of vaccines.

This communication examines the potential of nanoparticles as adjuvants for mucosal antigen delivery and for rational vaccine design. An overview of the immune mechanisms involved in generating effective immune responses will be presented. Candidates currently under investigation in our laboratories will be highlighted, focusing particularly on the use of degradable biomaterials. Key factors for the effective design of nanoparticles for oral and intranasal vaccine delivery will be discussed.

**Acknowledgments** Fundação para a Ciência e a Tecnologia, I.P. (FCT) - UID 01438 - Research Institute for Medicines (iMed.Ulisboa).

## Genosensors: Advanced technologies for precision Medicine

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DNA biosensors are new-generation biomedical detection devices where the recognition element is DNA. Their physicochemical properties and versatility offer significant advantages over traditional analysis methods, such as the ability to detect a wider range of biomarkers, greater durability and lower production costs. These features make them ideal for applications in clinical diagnostics and precision medicine.

A key feature of DNA biosensors is the use of innovative structural configurations, which improve signal conduction on the sensor surface. This capability increases sensitivity and allows accurate real-time monitoring of molecular interactions, revolutionizing detection technology. Various strategies have been developed to improve the sensitivity and sequence specificity of DNA biosensors, allowing DNA to be detected down to the attomolar level and to discriminate differences in a single nucleotide.

The most recent advances in nanotechnology allow genosensors to be classified into: functional biosensors based on DNA chains, which bind to specific target molecules; DNA hybridization-based biosensors, which take advantage of complementary DNA pairing for detection; and DNA origami-based biosensors, which facilitate the assembly of functional components.

Addressing challenges such as stability, efficacy, and scalability will enable future strategic lines of research and development. Furthermore, overcoming these obstacles will fully ensure the transformative potential of DNA biosensors in a wide variety of applications.

## Exploring life inside olive roots: rhizosphere and endophytic Streptomyces

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**Keywords:** olive, *Streptomyces*, Verticillium Wilt, biocontrol, antifungal compounds, rapamycin

Olive (*Olea europaea* L.) is one of the most valuable tree species in the Mediterranean basin, with significant historical, social and economic importance. However, olive cultivation is threatened by Verticillium wilt, a vascular disease caused by the soil-borne fungus *Verticillium dahliae* Kleb. This pathology is considered the most serious biotic problem for olive trees with no successful control measures currently available.

Biological control represents an interesting, sustainable, and environmentally friendly approach within the integrated management strategies of Verticillium wilt of olive. In this context, actinomycetes of the genus *Streptomyces* are promising biocontrol agents (BCA) due to their production of multiple bioactive compounds.

The identification of the chemical compounds responsible for the antifungal properties of a particular strain is of great interest for developing formulations to suppress diseases under field conditions. Yet studies suggest that the genus is capable of making some 150,000 more bioactive compounds than all of those reported to date.

Our research resulted in the identification and characterization of several rhizosphere and endophytic *Streptomyces* species from the root system of olive, with a strong antifungal activity against *V. dahliae*, some of which were identified as putative new species.

One particular rhizosphere strain, *Streptomyces* sp. OR6, exerts a strong antifungal activity through the production of albocycline. Surprisingly, two of the endophytic isolates, identified as *S. iranensis* OE54 and the new species *Streptomyces* sp. OE57, produce rapamycin. This finding is particularly interesting since production of this compound is very rare among microorganisms. Rapamycin is highly effective against *V. dahliae* and it has important applications in biomedicine as an immunosuppressant, anticancer and anti-aging agent.

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# ORAL COMMUNICATIONS





## Assessing salt resilience in European cowpea accessions during germination

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**Keywords:** Salinity, Climate changes, *Vigna unguiculata* L. Walp, Gene expression, Lipid peroxidation

One of the most significant impacts of climate change is the soil salinity, negatively affecting plant growth and development. Seed germination and seedling emergence are among the most critical stages susceptible to salt stress, making it important to explore them to identify the most resilient accessions for improving crop yield. Cowpea (*Vigna unguiculata* L. Walp.) is a promising short-cycle, warm-season and multifunctional crop with a high ability to fix nitrogen, contributing to improved soil health. This study main objectives were to identify salt resilient cowpea accessions from a European collection and to evaluate their responses to salt stress during germination.

A total of 23 cowpea accessions from five European countries were subjected to two conditions: control (water) and salt stress (150 mM NaCl solution). The seedlings grew for ten days at 25±1°C. To evaluate the effects of salt stress, the germination and growth parameters and lipid peroxidation were determined. The results revealed significant differences in all the parameters (except for % germination) among accessions and treatments. The interaction accession × treatment only presented significant differences in root length. In this set was observed a high variation in salt responses among accessions, allowing the selection of three cowpea accessions (Co\_2, Co\_3 and Co\_14) as resilient to salt stress at germination stage. A total of five salt-related genes were selected and evaluated allowing the selection of two differentially expressed genes (*DREB2* and *VuEXO*) that were analysed through qPCR. In general was observed an increase of gene expression in both genes under salt stress treatment. These findings provide valuable insights for the early selection of salt resilient cowpea accessions, which may be considered for future breeding programs.

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## dPCR vs qPCR: How could be related and contribute to sweet cherry breeding programs?

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**Keywords:** dPCR, cracking, gene expression, qPCR, sweet cherry

Fruit cracking is a developmental disorder influenced by both genetic and environmental factors, negatively affecting fruit quality and yield. Sweet cherry (*Prunus avium* L.) is one of the fruits most affected by cracking. Identifying genetic markers could aid in breeding programs and post-harvest treatments. In this study, we compared qPCR and dPCR methods using 16 genes that exhibited differential expression between the cherry varieties 'Sweetheart' (low cracking susceptibility) and 'Burlat' (high cracking susceptibility). The expression of genes involved in wax biosynthesis and cell wall metabolism was analysed in healthy fruits from both cultivars. Overall, qPCR and dPCR showed a strong negative correlation of -0.90, highlighting the relationship between both methods in gene expression studies. In addition, the combined expression of *PaCER1*, *PaXTH*, *PaEXP1*, *PaEXP2*, *PaKCS6*, *PaWINA*, *PaWINB*, and *PaCER3* genes provided an expression pattern that allowed to distinguish cultivars with high and low cracking susceptibility, grouping it in two different clusters. Our findings emphasize the crucial role of wax biosynthesis and cell wall metabolism in cracking susceptibility, as its importance for future sweet cherry breeding programs.

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## Effect of vermicompost from vine and wine industry by-products on growth and photosynthetic parameters of garden cress

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**Keywords:** jiffy-7, photosynthetic pigments, sustainable agriculture

Vermicomposting provides a viable alternative for the management of viticulture and wine cellar activities by-products. To test various mixtures, a hybrid *in vitro* system has proved to be useful by combining plant multiplication in a culture medium with a testing system in standard peat. If the explants are seeds, the assays can start directly in standard peat. This study evaluated the effect of vermicompost derived from grape marc, grape stems and pruning wood on garden cress (*Lepidium sativum* L.) growth. For this, an aqueous extract of vermicompost (VC) was prepared at two different concentrations (10% and 20%, w/v) and incorporated into peat (Jiffy-7) in glass jars, where two seeds were cultivated under aseptic conditions and maintained under controlled temperature, light and moisture. Each treatment comprised 5 jars and was replicated three times. After five weeks, growth parameters (aerial height, root length, fresh weight of the aerial part, number of leaves, stem diameter) were measured. Leaf photosynthetic pigments (chlorophyll *a*, *b*, total chlorophyll, and total carotenoids) and total phenolic content were determined in lyophilized material. Growth parameters showed no significant differences between treatments. In contrast, both extracts significantly increased chlorophyll *a*, total chlorophyll and total carotenoids, suggesting that the vermicompost extracts can enhance the photosynthetic capacity of plants by improving nutrient supply and physical conditions of the substrate, even if no significant impact was observed on the growth of garden cress.

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## Evaluation of antibiotic resistance in environmental isolates of *Klebsiella* spp. and *Raoultella* spp.

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**Keywords:** *Klebsiella* spp.; *Raoultella* spp.; Antibiotic Resistance; ESBL

Antibiotic resistance is a growing global health concern, particularly in pathogenic bacteria such as *Klebsiella* spp. and *Raoultella* spp.. Studying the prevalence of antibiotic resistance in environmental isolates of these bacteria is critical to understand the spread of resistance and its potential impact on public health. A total of 18 water samples (1 sample per location) and 48 soil samples (3 samples per location) were collected between July 2022 and April 2023. Cellulose nitrate membranes (after water filtration) and 1g of soil from each plot, were placed in BHI broth for bacterial enrichment. For bacterial isolation, each sample was streaked on selective media after 24h at 37°C. Antibigrams were performed for 16 antibiotics according to EUCAST guidelines, and extended-spectrum  $\beta$ -lactamases (ESBL) production was tested by the double-disc synergy test. Whole-Genome Sequencing was used to identify the resistance genes. In water samples, 9 *Klebsiella* spp. isolates were recovered (50% of samples), and 4 *Raoultella* spp. isolates (22.2%). In soil samples, 4 *Klebsiella* spp. isolates were recovered (8.3%), and 3 *Raoultella* spp. isolates (6.3%). One *Klebsiella* spp. isolate from soil was resistant to six antibiotics, identified as an ESBL-producer, and carried multiple ESBL genes (*bla*<sub>CTX-M-15</sub>, *bla*<sub>TEM-1</sub>, *bla*<sub>SHV-28</sub>, *bla*<sub>OXA-1</sub>). Additionally, it harbored resistance genes for quinolones, sulfonamides, tetracycline, aminoglycosides, and chloramphenicol. Antibiotic resistance is not widespread in these environmental isolates, which is a positive epidemiological outcome. However, the presence of a single ESBL-producing *Klebsiella* spp. isolate highlights a potential hotspot for resistance emergence.

## Determination of antimicrobial resistance and the impact of Imipenem+Cilastatin synergy with Tetracycline in *Pseudomonas aeruginosa* isolates from septicemias

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*Pseudomonas aeruginosa* is a Gram-negative, aerobic, rod-shaped bacterium with a single flagellum. This pathogenic agent is one of the most ubiquitous in the natural world, exhibiting remarkable metabolic and physiological versatility, making it both widespread and highly adaptable. *P. aeruginosa* is one of the six "ESKAPE" pathogens included in the World Health Organization's list of "priority pathogens" due to antibiotic resistance.

Thus, the objectives of this study were to evaluate the effect of various antibiotics against this bacterium and its biofilm-forming ability. To achieve this, several methods were employed using *P. aeruginosa* isolates obtained from septicemia cases.

Based on the results of the antibiotic susceptibility test, the isolates demonstrated sensitivity to six of the tested antibiotics, with four of them showing nearly or fully 100% sensitivity. However, resistance was observed for most antibiotics, albeit at a minimal level.

Regarding biofilm formation, this bacterial species exhibited the ability to produce biofilms, which were predominantly of moderate intensity.

When determining the minimum inhibitory concentrations (MICs) for tetracycline and imipenem+cilastatin, a 100% resistance phenotype was observed for tetracycline. In contrast, imipenem+cilastatin displayed a predominantly intermediate phenotype (85.72%), with some degree of resistance (14.28%). The MIC-based microdilution sensitivity test for imipenem+cilastatin and tetracycline revealed several combinations that exhibited efficacy against distinct isolates. Following the staining of microtiter plates used to assess potential synergy between the two antibiotics, it was confirmed that specific concentrations of imipenem+cilastatin and tetracycline were capable of inhibiting biofilm formation.

This study highlights the resistance of *P. aeruginosa* to several antibiotics, particularly tetracycline, and its ability to form moderate biofilms. However, the synergy between imipenem+cilastatin and tetracycline showed potential in inhibiting biofilm formation, suggesting a promising therapeutic approach.

## ***Pseudomonas aeruginosa*: One Health approach to deciphering hidden relationships in Northern Portugal**

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**Keywords:** Antimicrobial resistance, One health, Portugal, Molecular Epidemiology, *Pseudomonas aeruginosa*, Virulence

Antimicrobial resistance in *Pseudomonas aeruginosa* poses a significant threat to both public and veterinary health, particularly within the One Health framework. This study aimed to characterize antimicrobial resistance, the presence of virulence genes, and the genetic diversity of *P. aeruginosa* isolates from diverse sources. A total of 737 isolates were collected from humans, domestic animals, and aquatic environments in northern Portugal. Antimicrobial susceptibility testing, genomic analysis for resistance and virulence genes, and multilocus sequence typing were performed to assess their profiles. The results revealed a high prevalence of multidrug-resistant isolates, with high-risk clones such as ST244 and ST446 predominantly found in hospital environments and wastewater treatment plants. Key resistance and virulence determinants, including efflux pumps and secretion systems, were identified. These findings emphasize the complex ecological dynamics of multidrug-resistant *P. aeruginosa* and the crucial role of genomic surveillance in understanding resistance mechanisms. Furthermore, this study underscores the necessity of integrated approaches to mitigate antimicrobial resistance, suggesting potential biocontrol strategies such as phage therapy, antimicrobial peptides, and targeted interventions in healthcare and agriculture.

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## Genomic characterization of multidrug-resistant *Escherichia coli* in slaughterhouse broilers: a One Health perspective

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**Keywords:** Antimicrobial resistance; Genomic characterization; Resistance genes; Virulence factors; Integrons; Phylogenetic analysis; Zoonotic potential

The widespread use of antibiotics in livestock has played a major role in the emergence and spread of antimicrobial-resistant bacteria, raising concerns about their potential transmission to humans. The detection of multidrug-resistant (*E. coli*) in poultry emphasizes the urgency of adopting a One Health approach to track and mitigate antimicrobial resistance. This study investigates the prevalence of *E. coli* in cloacal samples from broilers at a Portuguese slaughterhouse, characterizes their antimicrobial resistance patterns, and examines key genetic determinants, including resistance genes, virulence factors, integrases, and phylogenetic groups.

Genomic DNA was extracted from five *E. coli* isolates obtained from cloacal samples (n = 150) using the boiling method. PCR analysis revealed the presence of the *ampC* and *blaCTX-M* resistance genes in all isolates. Additionally, one isolate carried *blaVIM*, while another harbored *aadA5*, *tetB*, *sul2*, and *strB*. The virulence factors *aer* and *fimA* were detected in four and four isolates, respectively, with one isolate carrying both. The *int1* integrase gene was identified in one isolate. Phylogenetic analysis classified one isolate within group B1.

These findings highlight the prevalence of antibiotic-resistant *E. coli*, particularly cefotaxime-resistant *E. coli* in broilers in Portugal, reinforcing concerns about the dissemination of extended-spectrum  $\beta$ -lactamase (ESBL)-producing strains in poultry production and underscoring the importance of regional surveillance. The co-occurrence of resistance and virulence determinants in typically commensal phylogroups suggests bacterial adaptation to selective pressures in poultry environments. Strengthened antimicrobial stewardship—through the reduction of critically important antibiotics, the promotion of alternative strategies such as probiotics and vaccination, and improved biosecurity measures—is essential to mitigate the risk of zoonotic transmission. Future research should focus on tracking resistance gene mobility and transmission pathways to inform targeted public health interventions within a One Health framework.

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## Tracking zoonotic Microsporidia in seafood and assessing their repercussions for Food Safety and Public Health

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**Keywords:** Microsporidia; Seafood, Zoonotic Parasites, qPCR; Pouting.

Microsporidia are emerging as significant parasites in seafood, presenting considerable risks to public health and food security. These parasites can cause gastrointestinal and systemic infections in humans, making them a concern for consumers of marine products. Traditional detection methods often lack the sensitivity and speed required for effective monitoring resulting in underreporting and delayed response to outbreaks. Recent advancements in molecular techniques, such as real-time polymerase chain reaction (qPCR) offer rapid, sensitive, and specific detection of microsporidia in seafood. Given the potential presence of microsporidia in coastal waters and edible marine fish, it poses a risk for fish consumers.

This study aims to assess the likely presence of the main microsporidia zoonotic species, namely *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi*, in the edible muscle of pouting (*Trisopterus luscus*) collected from Portuguese fisheries throughout all four seasons of the year. The research focuses on targeting the internal transcribed spacer (ITS) sequence for *E. bieneusi* and the small subunit ribosomal ribonucleic acid (SSU rRNA) gene sequence for *Encephalitozoon spp.* Samples from pouting will be analyzed to determine the seasonal prevalence and distribution of these microsporidia species in Portuguese fisheries, using molecular detection methods, like qPCR. High-quality DNA was successfully extracted from pouting muscle tissue, ensuring reliable molecular analysis. Furthermore, qPCR assays were optimized for the specific detection of *E. bieneusi* and *Encephalitozoon spp.*, establishing a robust framework for ongoing surveillance of microsporidia in seafood. Integrating molecular tools into routine seafood safety represents a crucial step toward proactive identification and managing microsporidia-related risks and improving public confidence in seafood products.

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## Potential genotoxicant Bisphenol C2 and Sucralose Electrochemical Sensing. A theoretical insight

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**Keywords:** Chloroorganic genotoxicants, sucralose, bisphenol C2, food contact substances, electrochemical determination, electrochemical oscillations, stable steady-state

Sucralose is a widely used sweetener. Nevertheless, it is an environmentally unfriendly and potentially genotoxic chloroorganic compound, which tends to be accumulated in the environment. A recent investigation has shown that the sucralose, if consumed by pregnant women, passes by a placental barrier, enters the breast milk, and thereby causes changes in baby gut microbiota, leading to the metabolic disorders in babies and the possibility of type 2 diabetes development. Another negative effect of sucralose and its industrial precursor (6-acetylsucralose) is genotoxicity, reason why the development of an efficient sucralose and lactic acid sensor for diabetic and obese pregnant women is actual, and, taking into account that both of the substances are electrochemically active, the electrochemical sensing may be an interesting approach for this task.

On the other hand, bisphenol C2 is one of 16 xenoestrogenic compounds, which are used as monomers in polycarbonates and polyterephthalates in food packages and may penetrate the food. Although bisphenol A is the most used of them, bisphenol C2 is potentially the most genotoxic, reason why the detection of its presence in the presence of sucralose is really up to date. Bisphenol C2 is synthesized from phenol and dichloroacetone by aromatic electrophilic substitution

In this work, the possibility for electrochemical determination of sucralose and bisphenol C2, potentially genotoxic compounds, has been evaluated. The electroanalytical process may be realized either cathodically or anodically. Nevertheless, due to the electroanalytical properties of both of the compounds the anodic process is preferred. As for the electrochemical removal from the environment, cathodic process is more preferable.

## Decoding Human Satellite 1B in primates: insights from bioinformatic analysis

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**Keywords:** Satellite DNA, T2T assemblies, primates, NGS, bioinformatics

A significant amount of the human genome is made by satellite DNAs - tandem repetitive sequences that are present in the chromosomes pericentromeric constitutive heterochromatin regions. Their highly repetitive and complex nature has hampered their accurate representation on the human genome assemblies. Recent advances were made by the Telomere-to-Telomere (T2T) consortium that have made these regions more accessible for study, by including them in the most recent assembly of the human and non-human primate (NHP) genomes. Human satellite 1B (HSat1B), is a 2.5 bp satellite repeat located on the short arms of acrocentric chromosomes in human and other primates, including some primates Y chromosome. HSat1B is a constituent of pericentromeric regions, yet its role in genomic function is not fully understood. This work aimed to address the most recent sequencing data from several species of primates and explore HSat1B genome location (at chromosome level), and amount in these species' genome by using bioinformatic tools.

Our analysis reveals HSat1B's genomic organization, abundance and its distribution across different primate species, shedding light on its sequence variation and evolutionary conservation across primate species. In some species genome HSat1B is intercalated with other satellite repeats, such as HSat1A and HSat3 (both in gorilla and human Y chromosomes), which suggest potential functional interactions or even co-evolution. These findings will increase our understanding of HSat1B's contribution to genome biology and our understanding of the complexities of satellite DNA and their implications for genome architecture and evolution.

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## ***Staphylococcus aureus* in cancer: development of combined therapies for chronic intracellular infections**

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**Keywords:** *Staphylococcus aureus*, intracellular, infection, cancer, antibiotic resistance, drug repositioning, compounds combinations

*Staphylococcus aureus* is an opportunistic pathogen capable of infecting a wide range of tissues. A key aspect of its pathogenicity lies in its ability to invade and persist within host cells, adopting a facultative intracellular lifestyle that poses significant challenges for treatment and immune clearance. Intracellular infections by *S. aureus* have recently been implicated in the onset and progression of different cancers, contributing to cellular transformation and tumor development.

This study aims to develop novel antimicrobial therapies targeting intracellular *S. aureus* by combining repurposed drugs with other antimicrobial agents. Using a cellular infection model, we are testing a range of anticancer drugs identified from a previous high-throughput screening of 6,995 repurposing candidates. Initially, these compounds are assessed for their antimicrobial activity against *S. aureus* and will subsequently be tested in vitro in combination with host- and pathogen-targeted anti-infective agents.

Preliminary findings from cellular models reveal that certain anticancer drugs demonstrate notable antimicrobial activity against intracellular *S. aureus*. When combined with other therapeutic agents, these drugs show synergistic effects, significantly enhancing bacterial eradication. These results suggest that combinatorial therapies targeting both the host cell and the pathogen offer a promising approach to treating *S. aureus* infections in melanoma. Future work will focus on optimizing these strategies and validating their clinical potential, paving the way for innovative treatments that address the dual challenge of intracellular infections and cancer progression.

**Acknowledgments:** We express our gratitude for the funding provided by MCIN/AEI, the European Union (NextGenerationEU/PRTR), Junta de Castilla y León (LE044P20), and FGULEM (TCUE plans 2018–2023). We also acknowledge the fellowships supporting our team, including predoctoral and postdoctoral grants from JCyL, AECC, Margarita Salas, Beatriz Galindo, and FPU programs.

## Enhancing Dark Fermentation for biohydrogen and volatile fatty acids production through Electrofermentation

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**Keywords:** dark fermentation (DF), electrofermentation (EF), bioelectrochemical system (BES), cheese whey (CW), biohydrogen, volatile fatty acids (VFAs), waste valorization

Dark fermentation (DF) is a biological process in which anaerobic bacteria consume organic substrates under anaerobic conditions and in the absence of light, generating mainly biohydrogen (H<sub>2</sub>) and volatile fatty acids (VFAs). This process offers a promising approach for the valorization of organic waste. However, its efficiency may be limited by the accumulation of metabolic by-products and the low conversion of substrates.

Integrating a bioelectrochemical system (BES) into the fermentation reactor could help overcome these limitations. By modifying the fermentation broth's redox potential through the application of electrical potentials, the microbial metabolism can be directed toward target compounds. This electrofermentation (EF) approach aims to enhance the conversion of organic substrates, thereby improving the efficiency of DF.

This study analyzed the implementation of a BES in the DF of cheese whey, using digested sludge from a local wastewater treatment plant as inoculum. The impact of electrodes on H<sub>2</sub> production and fatty acids profile was evaluated. Compared to conventional DF, EF enhanced hydrogen yield while modulating VFAs composition, demonstrating its potential to improve fermentation efficiency. These results highlight the potential of BES technology for sustainable valorization of organic waste.

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## Accelerating startup and efficiency in microbial electrolysis systems through advanced polymeric coatings

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**Keywords:** Bioelectrochemical systems; Hydrogels; Biocathode; Volatile fatty acids; Start-up

This research explores innovative strategies to improve microbial electrolysis systems to produce valuable biochemicals, such as methane and volatile fatty acids, through the implementation of polymeric coatings on carbon-based bioelectrodes. The intrinsic hydrophobicity of carbon materials impedes microbial colonization, which is crucial for effective bioelectrochemical transformation. Hydrophilic hydrogel coatings provide a promising solution by enhancing water retention and creating a biocompatible interface that fosters microbial adhesion and activity.

The study will systematically investigate the optimization of these coatings, considering factors such as polymer composition, application techniques, and cross-linking mechanisms to develop a durable, uniform, and functionally efficient layer. Key performance indicators, including electron transfer capability, current density output, and system stability, will be thoroughly assessed.

This approach has the potential to significantly enhance the operational efficiency and sustainability of microbial electrolysis technologies. Additionally, it promotes the generation of high-value compounds, positioning this method as a sustainable alternative for renewable resource utilization and the advancement of eco-friendly energy solutions.

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## Selection of traditional varieties of cucumber (*Cucumis sativus* L.) for drought tolerance

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**Keyword:** cucumber, traditional accessions, water deficit, morphological traits, selection

Climate change is modifying environmental conditions in agricultural areas, especially in the Mediterranean area. One of the most important changes is drought due to lack of water, mainly rainfall. Lack of water can affect growth and plant production. Knowing accessions that can adapt to water-deficient soil conditions is one of the strategies that can mitigate that problem. Cucumber (*Cucumis sativus* L.) is a species belonging to the *Cucurbitaceae* family that is widely distributed in tropical and subtropical regions. The objective of the present work is to evaluate and identify plant and fruit traits of traditional cucumber accessions under two irrigated conditions of cucumber: under irrigated (100 %) and deficit irrigation (50 %) conditions. In addition, a selection of accessions will be proposed for their adaptation to water deficit conditions. This work was carried out within the framework of the project AGROALNEXT/2022/025 in the spring-summer season 2024 in the *Cucurbitaceae* laboratory of the COMAV at the Universitat Politècnica de València. 35 cucumber accessions were evaluated, mainly from the COMAV Germplasm Bank, although some accessions were requested from the Murcia and Zaragoza Germplasm Banks too. The plot was divided into two zones, one for each treatment. Two randomized blocks were included for each zone. The evaluated traits (5 for plant and 10 for fruit) were analyzed with a Linear Mixed Model in which the water regime, the accessions and its interaction were included as a fixed effect and the block effect was included as a random effect. Deficit irrigation reduced the number of days from transplanting to harvest of the first commercial fruit, the number of fruits and yield, among others. Differences were also observed between the 31 cucumber accessions of various cucumber types evaluated (short, long, oriental, ...) as well as in the interaction between water regime and accessions. Eleven accessions out of 35 are proposed for selection.

## Impact on cell wall composition of *Arabidopsis thaliana* pectin methylesterase inhibitor mutants susceptible to *Pseudomonas syringae*

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**Keywords:** Cell wall, pectins, PMEIs, methylesterification, *Arabidopsis thaliana*, *Pseudomonas syringae*, Pathogen resistance

The cell wall is a metabolically active structure that serves as a defense barrier against external agents. Among its main components, pectins stand out, with homogalacturonan (HG) being the predominant pectic polysaccharide. This polysaccharide is deposited in the cell wall with a high degree of methylesterification and is subsequently demethylated *in muro* by the action of pectin methylesterases (PMEs). PME activity is tightly regulated by protein inhibitors (PMEIs), and this control is essential for processes such as the defensive response against pathogens.

Recently, we discovered that mutants in PMEIs of *A. thaliana* exhibited a loss of resistance to a non-natural pathogen, *Pseudomonas syringae* pv. *phaseolicola* (Pph). This study aims to analyze changes in the susceptibility of *pmei* mutants to infection by a non-pathogenic strain (Pph) and a pathogenic strain of *A. thaliana* (*P. syringae* pv. *tomato DC3000*) and to assess modifications in cell wall composition that could explain this susceptibility to Pph.

All analyzed *pmei* mutants displayed increased susceptibility to both strains of *P. syringae*. Analysis of cell wall composition revealed that infection with *Pseudomonas* alters the extractability of polysaccharides such as pectins, suggesting changes in polysaccharide interactions due to the presence of the pathogen.

In conclusion, the results suggest that PMEIs of *A. thaliana* play a key role in *Arabidopsis* resistance to *Pseudomonas* infection.

## Mutants in *RGP* genes show mucilage release defects in *Arabidopsis thaliana* seeds

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**Keywords:** *Arabidopsis thaliana*, arabinose, mucilage, pectins, plant cell wall, reversibly glycosylated polypeptides

The cell wall surrounds the cells of plants and is primarily composed of cellulose, hemicelluloses, and pectins. The latter contain arabinofuranose in their side chains, and although the deficiency of this monosaccharide causes severe developmental defects, its function in the cell wall is still unknown. Therefore, mutants in *RGP* genes (*RGP1-5*), which encode for an enzymatic complex responsible for performing the last step in the synthesis of UDP-arabinofuranose, an activated precursor necessary for the synthesis of polysaccharides which contain arabinofuranose. In an attempt to understand the role of arabinose in the cell wall, we have studied the mucilage of *Arabidopsis thaliana* seeds, a structure primarily composed of pectins that facilitates its study. Through the use of molecular, biochemical, and cytological techniques, it has been determined that, among the genes expressed in the seed coat, *RGP4* and *RGP5* show an anomalous phenotype in mucilage release, particularly the *rgp5-2* insertional mutants. However, it is necessary to continue studying the *RGP* genes to understand better the role of arabinose in the plant cell wall.

# POSTERS







## Evaluation of a commercial disinfectant's efficacy against bacterial isolates from shelters and households

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**Keywords:** Disinfectants, Escherichia coli, Staphylococcus spp., animal Shelter; households

The presence of animals in shelters and households can contribute to the transmission of zoonotic pathogens, emphasizing the importance of proper hygiene practices in these environments. Disinfectants are essential in reducing microbial contamination and preventing infections. This study evaluated the bactericidal activity of the commercial disinfectant Cillit Bang® against 10 bacterial isolates: five Escherichia coli (E. coli) and five Staphylococcus spp.

The isolates originated from two different environments: 2/5 E. coli samples were collected from an animal shelter, while the remaining 3 were from households. Similarly, 3/5 Staphylococcus spp. isolates came from the shelter, and two were from household settings. The methodology followed the EN 1040 standard, which employs a suspension test to assess disinfectant efficacy. The bacterial isolates were exposed to the disinfectant for 5, 15, and 30 minutes, and the experiment was conducted in triplicate. After 24–48 hours, colony-forming units were counted, and results were compared to control samples. According to EN 1040 standards, a disinfectant is considered effective if it achieves at least a 5-log (99.999%) reduction in bacterial growth within 5 minutes of exposure. In 80% of E. coli samples (n=4) the disinfectant was effective with a 100% reduction in bacterial growth. In 20% of samples (n=1) the disinfectant was ineffective at both 5 and 15 minutes of contact time, only having a 99.731% reduction rate of bacterial growth, equivalent to a 3-log reduction. However, at 30 minutes a 100% reduction in bacterial growth was observed. The disinfectant was considered effective, in all contact times, in 100% (n=5) of the Staphylococcus spp. samples tested. The findings of this study reinforce the importance of testing disinfectant efficacy, particularly in environments where bacterial contamination is a concern, such as animal shelters and households.

## *Aspergillus* spp. and hygiene: evaluating disinfectant efficacy through a phenotypic lens

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**Keywords:** *Aspergillus felis*, *Aspergillus fumigatus*, antifungal activity, disinfection

The persistence of *Aspergillus* spp. in environments highlights the need for effective antifungal disinfection strategies. Inadequate cleaning protocols may contribute to fungal proliferation, increasing the risk of infection for both animals and humans. Therefore, evaluating the efficacy of disinfectants against these fungi is essential for improving hygiene practices and minimizing contamination. The aim of this study was to evaluate the antifungal potential of the commercial disinfectant Biocidal active substance (PT2/AL): 0.5g/100g didecyldimethylammonium chloride (CAS: 7173-51-5) (Sanytol®s) against *Aspergillus felis* and *Aspergillus fumigatus* using the mycelial growth method. Ten *Aspergillus* isolates (4 *A. felis* and 6 *A. fumigatus*) originated from two different environments animal shelter, and households were analyzed. A mycelial 4 mm-disk of *A. felis* and *A. fumigatus* colonies grown on Potato Dextrose Agar (PDA) for 3-5 days was placed in the center of a new PDA Petri dish with 0.005% of the disinfectant. The radial growth of colonies was measured, and growth zones were assessed on the third, fifth and seventh day, to determine antifungal activity. Colony growth was compared to the control, converting the difference in percentage of inhibition. The highest inhibition percentage for *A. felis* was observed in the dishwasher isolate (household) (85.1%), while the lowest was recorded in the cat paw isolate (81.5%). For *A. fumigatus*, the highest inhibition percentage was also found in the dishwasher isolate (household) (84.8%), whereas the lowest was in the air sample from the shelter (72.8%). No statistically significant differences were observed between the inhibition means of *A. felis* and *A. fumigatus* ( $t = 1.046$ ;  $p = 0.326$ ), suggesting comparable susceptibility to the disinfectant. The commercial disinfectant chloride showed notable antifungal activity against both fungal species. However, variations in efficacy across different isolates highlight the need for further research into optimizing disinfection protocols.

## Optimization of DNA extraction and PCR conditions to identify microbial communities in vermicompost

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**Keywords:** Vermicompost, DNA extraction, soil microbiology, PCR amplification

Complex organic materials like soil, organic residues, and composts host diverse microbial communities. Traditional microorganism culture methods often overestimate microbial diversity due to difficulties in growing many species. However, DNA-based techniques, which directly extract genetic material from environmental samples, provide a more accurate assessment of microbial communities. This study aimed to use DNA-based methods to investigate the evolution of denitrifying bacterial communities during vermicomposting, a process where organic residues are decomposed through the combined actions of earthworms and microorganisms. Thus, this study represents the first attempt to optimize DNA extraction and amplification techniques specifically for vermicompost samples. Vermicompost samples were derived from composted mixtures of vineyard pruning wood, sewage sludge, and solid cattle manure slurry. Two formulations were tested: one with 30% pruning wood, 50% sludge, and 20% cattle manure, and another with 50% pruning wood, 30% sludge, and 20% cattle manure. Samples were collected at three time points: the start of vermicomposting, at 55 days, and at 98 days. DNA was extracted using the E.Z.N.A.<sup>®</sup> Soil DNA Kit with modified protocol steps to enhance yield and purity. Spectrophotometric analysis confirmed DNA integrity, with 260/280 absorbance ratios indicating no or minimal contamination. Specific primers were selected for PCR amplification targeting bacteria, fungi, archaea, and the associated with denitrification *nirK* gene. Despite some technical issues, agarose gel electrophoresis confirmed successful amplification in all samples. Although no significant changes in the amplified DNA were observed for the PCR conditions used, this study shows that the used protocols are effective for DNA extraction and amplification of vermicompost samples. Future improvements, as optimization of PCR conditions are expected to allow detection of microbial shifts. These advancements will contribute to better understanding how microbial communities evolve during vermicomposting.

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## Earthworm avoidance test for ecotoxicological evaluation of a developing herbicide

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**Keywords:** *Eisenia fetida*, Avoidance behavior; Herbicide; Soil ecotoxicology

The excessive use of herbicides disrupts soil ecosystems by affecting habitats, food webs, and soil organisms. To ensure safety, herbicides require thorough testing before commercialization. Earthworms, such as *Eisenia fetida*, serve as key bioindicators for assessing soil ecotoxicological risks and establishing pollutant safety thresholds. The “earthworm avoidance test” provides a sensitive, fast, and straightforward method for screening soil contamination. Since soil organic matter content can influence herbicide bioavailability and toxicity, this study evaluated the avoidance behavior of *E. fetida* in response to a non-glyphosate herbicide (composition under patent) in two natural soils with distinct organic matter content.

The avoidance tests were conducted in accordance with guideline ISO 17512-1 (2007) for a dual test chamber. Briefly, two sections were created within the test container: one with the herbicide and one without. Earthworms (n=10/replicate) were placed in the central area and the avoidance response was measured by counting the number of earthworms that migrate away from the herbicide after a 48h-period. Eight herbicide concentrations were used based on the manufacturer’s recommended field application concentration (40 g/L/ha). These included two concentrations above, the recommended dose and the remaining below it (1.8 dilution factor). Boric acid (750 mg/kg soil dry weight) was used as a positive control to assess the sensitivity and reliability of the test.

In both soil types, only the concentration 5.8x lower than the recommended dose induced an avoidance response in the earthworms (>20%). No differences were observed related to soil organic matter content, except for the positive control, in which the soil with higher organic matter content elicited a higher avoidance response (double). These findings indicate that earthworms avoid this herbicide at a low dose but not at the recommended field dose. Further studies are needed to assess its effects on earthworm physiology, e.g., the OECD reproduction test.

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## Fungal biodiversity in hedgehogs (*Erinaceus* spp.) from a rescue center

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**Keywords:** Hedgehogs (*Erinaceus* spp.), Fungal biodiversity, Dermatophytes, Zoonotic fungi, Wildlife health

Fungal infections are a growing concern in both wild and captive animal populations, with potential implications for animal health and zoonotic transmission. Hedgehogs (*Erinaceus* spp.) can harbor a variety of fungal species, some of which may be opportunistic pathogens. This study aimed to assess fungal biodiversity in hedgehogs from a rescue and interpretation centre. A convenience sample of 100 hedgehogs from a rescue and interpretation centre was examined for the presence of fungus using the toothbrush technique. About 34 hedgehogs presented clinical signs suggestive of fungal disease. A total of 100 samples were inoculated in Potato Dextrose Agar medium (PDA) and Dermatophyte Test Medium (DTM). Based on the observation of microstructures and colony morphology, the fungal isolates were identified at genus level. Fungal growth was observed in 65% of the PDA cultures, whereas only 29% of the DTM cultures tested positive. Among the 34 hedgehogs exhibiting clinical signs suggestive of fungal disease, 70.6% (24/34) of the PDA cultures and 41.17% (14/34) of the DTM cultures were positive. In asymptomatic hedgehogs (n=66), fungal growth was detected in 62.1% (41/66) of the PDA cultures and 22.7% (15/66) of the DTM cultures. A total of 19 fungal genera were isolated from the collected biological samples. The overall occurrence of dermatophytes in this study was 19.0% (95% CI: 11.8-28.1%). Various filamentous fungi were identified, including *Aspergillus* spp., *Penicillium* spp., *Scopulariopsis* spp., *Chaetomium* spp., *Fusarium* spp., *Chrysosporium* spp., *Rhizopus* spp., *Talaromyces* spp., *Trichoderma* spp., *Alternaria* spp., *Phoma* spp., and *Onychocola* sp. or *Arthrographis* spp., listed in order of frequency. The identification of dermatophytes and opportunistic fungi reinforces the need for routine fungal screening in rescued hedgehogs. Further research is necessary to understand the clinical relevance of these fungal isolates and their potential impact on hedgehog health, zoonotic risks, and conservation efforts.

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## Prevalence of *Aspergillus* spp. in Portuguese Hedgehogs (*Erinaceus* spp.)

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**Keywords:** *Aspergillus* spp., Hedgehogs (*Erinaceus* spp.), Fungal infections, One Health

The significance of aspergillosis has grown in recent decades, affecting both humans and a wide range of animal species. *Aspergillus* spp. are globally distributed fungi that can colonize various hosts, including domestic and wild animals, as well as humans. These fungi can cause diverse health conditions, ranging from localized infections to severe systemic diseases, in addition to allergic reactions triggered by inhaled spores. In wildlife, *Aspergillus* infections can pose serious health threats, particularly in immunocompromised or stressed individuals. Understanding the occurrence and impact of *Aspergillus* spp. in wild species is essential for improving disease surveillance, wildlife conservation, and public health within a One Health framework. Fur and spines were collected from 100 hedgehogs (*Erinaceus* spp.) from a hedgehog rescue and interpretation centre using the toothbrush technique. Samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25°C and 37°C, for 3-7 days. Occurrence of *Aspergillus* was as follow: *Aspergillus* spp. (12.0%; 95% CI: 6.4-20.0%), *A. niger* (1%; 95% CI: 0.0-5.0%), *A. felis* (1%; 95% CI: 0.0-5.0%), *A. fumigatus* (1%; 95% CI: 0.0-5.0%), *A. versicolor* (1%; 95% CI: 0.0-5.0%). *Aspergillus* spp. are widespread saprophytic fungi found in air, soil, and decomposing organic matter. They can cause a variety of health issues, from allergic reactions to severe invasive infections in both humans and animals. In this study, the presence of *Aspergillus* spp. was notably high. Given its role as an opportunistic pathogen, further research is needed to better understand the implications of its presence on the skin of hedgehogs and its potential impact on both animal and public health.

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## Pathogen reduction in vermicomposting of vine pruning residues with sewage sludge

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**Keywords:** microbial safety, sanitation, waste, public health

Vermicomposting (VC) is a sustainable process that enhances organic residue decomposition while potentially reducing the presence of pathogenic microorganisms, such as *Escherichia coli* and *Salmonella* sp. This study assessed their presence during the VC of vine prunings with sewage sludge, in samples collected at the beginning (T0), after 100 (T100), and after 140 days (T140). For *E. coli* detection, samples were cultured on CHROMagar™CC medium across three dilutions and incubated aerobically at 37°C for 24 hours; microbial quantities were measured as colony-forming units (CFU) per gram. For *Salmonella* sp., a 5 g sample was inoculated in Buffered Peptone Water for pre-enrichment, followed by incubation in Modified Semi-Solid Rappaport-Vassiliadis medium at 42°C with novobiocin. Selective solid media were used to verify bacterial growth. Results showed a decrease in *E. coli* concentration over time across all dilutions. At T0, *E. coli* was present at  $1.63 \times 10^2$ ,  $1.50 \times 10^2$ , and  $1.67 \times 10^2$  CFU/g for the 1:10, 1:100, and 1:1000 dilutions, respectively. At T100 and T140, *E. coli* was only detected in the 1:10 dilution,  $2.8 \times 10^1$  CFU/g and  $1.17 \times 10^1$  CFU/g, respectively. While the 1:100 and 1:1000 dilutions showed no growth in both timepoints. *Salmonella* sp. was detected at T0 but was absent at T140. Current findings suggest that vermicomposting may be a viable strategy for this pathogen reduction, improving its microbiological safety for agricultural and environmental applications.

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## *Aspergillus* spp. isolated from household items

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**Keywords:** *Aspergillus*, filamentous fungi, ISSR-PCR, microbiology, molds, RFLP-PCR

The genus *Aspergillus* is ubiquitous, being present in several types of environments, including our homes. Several *Aspergillus* species are known to be human pathogens, but the most common one is *Aspergillus fumigatus*, the main cause behind aspergillosis in humans and other animals. Further species that are the main causes for this disease are *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans*. In this study, samples were taken from 37 households. Objects sampled were pillows, bedsheets/blankets, kitchen sponges/rags, rugs and bath towels, as well as a pet if existing. These samples were collected by dipping a swab in distilled water and swiping it 3 times on the object's surface, followed by placing the swab in a sealed tube with sterilized distilled water until inoculation. Additionally, in each house, a Petri dish of Potato Dextrose Agar (PDA) was exposed to the air in the room for 10 min at 10 cm from the floor. In total, 253 samples were obtained. Samples were inoculated in PDA and left to grow for 7 to 14 days at 28 °C. Identification was performed with a modified tape method using cotton blue lactophenol dye and microscopic observation at 400x ampliation. Using PCR, 8 samples were confirmed as belonging to the *Aspergillus* genus. Through RFLP-PCR, we identified *A. fumigatus* and *A. niger* in 2 samples each and *A. nidulans* in 1 sample. These are three of the most clinically significant species in this genus. Through ISSR-PCR, we determined there was a high degree of polymorphism, which indicates high genetic variability in the samples studied. These results highlight the presence of clinically significant *Aspergillus* spp. in household objects that we contact with daily, emphasising the need for awareness relating to potential exposure to these fungi in domestic environments.



## Development of High-Resolution Melting assays targeting the identification of fungal species among the Botryosphaeriaceae family

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**Keywords:** Grapevine Trunk Diseases, Botryosphaeriaceae, Botryosphaeria dieback, High Resolution Melting, Real-Time PCR

Viticulture represents a substantial role in Portuguese economy. A major threat to vineyards is grapevine trunk diseases (GTDs), which are caused by several fungal pathogens that reduce the life-span of vineyards and yield. In this study we focus on the Botryosphaeriaceae family, which englobes fungal species responsible for Botryosphaeria dieback, such as *Botryosphaeria dothidea*, *Neofusicoccum parvum*, *N. luteum*, *N. ribis*, *Lasiodiplodia theobromae*, *Dothiorella viticola*, *Diplodia seriata* and *Diplodia mutila*. Accurately identifying these diseases in the grapevine is challenging due to similar symptomatology with other GTDs. Also, the lack of distinct morphological markers makes challenging distinguishing between closely related species. This study aimed to develop an assay capable of differentiating between Botryosphaeriaceae species, through unique melting curves obtained by High Resolution Melting (HRM). An optimized DNA extraction protocol followed by PCR with specific primers (HRM2bot and Bt2b), and HRM analyses were conducted with fungal pathogens samples belonging to Botryosphaeriaceae family and negative controls from other fungal families. PCR amplification was successful, retrieving a specific band of approximately 130 bp in Botryosphaeriaceae samples. HRM analysis effectively distinguished species based on melting curves profiles which range from 82.4-92.4°C, proving it to be a valuable tool for rapid and precise pathogen screening. Since there are no known curative measures, the best employed strategies are based on prevention and mitigation. Thus, pathogen detection in early stages of infection improves the vineyard management strategies by applying cost-effective measures and avoiding increased costs for the wine producer.

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## Assessment of surface contamination in a shelter before and after disinfection or washing process

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**Keywords:** Bacterial contamination, disinfection efficacy, animal shelter hygiene

The presence of contaminants on environmental surfaces and equipment is a major concern, particularly in animal shelters. This study evaluates surface and equipment contamination before and after cleaning and disinfection procedures. Samples were collected from various locations, objects, and surfaces within a Portuguese shelter before and after the disinfection and/or washing process. In total, 20 samples were taken from different sources using sterilised test tubes and cotton-tipped swabs moistened with normal saline. Aseptic techniques were maintained throughout. For bacterial isolation, HiCrome™ Coliform Agar and MacConkey Agar were used for *Escherichia coli* (*E. coli*), incubated at 37°C for 24 hours. Colonies were identified based on morphology and confirmed via classical biochemical tests (Gram staining, catalase, oxidase, indole, Methyl-Red-Voges-Proskauer, citrate) and the API 20E system (BioMérieux, France). Mannitol Salt Agar (MSA) was used for *Staphylococcus aureus* (*S. aureus*), incubated at 37°C for 24 hours, and identified through Gram staining and the catalase test. *E. coli* was detected in 2 samples (10.0%; 95% CI: 1.2-31.7%), one from a washing machine containing animal clothes before disinfection and another from unwashed shelter dogs' clothes. *Staphylococcus* spp. was found in 65% (95% CI: 40.8-84.6%), while *S. aureus* was present in 40.0% (95% CI: 19.1-64.0%). The contaminated sites included air, floors, furniture, clothing, and animal paws. These findings emphasise the need for improved hygiene protocols and continuous monitoring to mitigate bacterial contamination risks in shelters, ensuring safer conditions for both animals and staff.

## Between homes and animal shelters – what fungi live within four walls? A phenotypic study

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**Keywords:** Shelters, households, fungi, occurrence, phenotype

Fungi threaten human and animal health, causing various health conditions ranging from mild, treatable allergic illnesses to deadly infections. This study identified filamentous fungi on objects and surfaces in 13 private homes and one animal shelter for dogs and cats. Five different samples were collected in each of the 13 houses, and in the animal shelter were 10, with a total of 75 samples collected. After identification, the hemolytic activity of *A. felis*, *A. fumigatus*, *A. niger*, *Penicillium* spp., *Talaromyces* spp. and *T. marneffeii* was evaluated.

The most common filamentous fungi in homes were the *Aspergillus* spp. and *Penicillium* spp. with equal occurrence (16.9%; 95% CI: 8.8-28.3%). In the animal shelter, the fungi that occurred most frequently belonged to the genus *Aspergillus* spp. (30.0%; 95% CI: 6.7-65.3%), *Chrysosporium* spp. (20.0%; 95% CI: 2.5-55.6%) and fungi compatible with the order Mucorales (20.0%; 95% CI: 2.5-55.6%). The hemolytic activity was evaluated in 29 isolates obtained from homes and animal shelters, of which 4 (13.8%) were *A. felis*, 6 (20.7%) were *A. fumigatus*, 2 (6.9%), were *A. niger*, 15 (51.7%) were of the genus *Penicillium* spp., 1 (3.4%) was *Talaromyces* spp. and another was *Talaromyces marneffeii* (3.4%). Hemolytic activity was observed in 58.6% (n=17) of these isolates. The occurrence of hemolytic activity in this study was 58.6% (95% CI: 38.9-76.5%). Identification of fungi present in homes and animal shelters is essential to understand the contamination risk and how to prevent their proliferation. Identifying fungi, such as *Aspergillus* spp., *Penicillium* spp. and *Talaromyces* spp., in this work provides information on possible health risks and allows the adoption of effective control and prevention measures.

## Prevalence of *Malassezia* spp. in dogs and cats

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**Keywords:** *Malassezia* spp., otitis externa, fungal infections

*Malassezia* spp. are commensal yeasts that colonize the skin and mucosa of homeothermic animals, including humans, depending on lipids for growth. In dogs and cats, they are commonly associated with external otitis, dermatitis, allergies, superficial pyoderma and chronic infections. In humans, *Malassezia* spp. can cause various skin conditions and systemic infections, including fungemia. Due to their zoonotic potential, *Malassezia* spp. are of significant concern in public health, as multiple species can infect both animals and humans. This study investigated the presence of *Malassezia* spp. in samples collected from the ears of dogs and cats. A total of 47 samples were obtained from 18 dogs and 29 cats, including 45 healthy animals and two dogs diagnosed with external otitis. Sampling was performed using a cotton-tipped swab moistened with sterile saline solution (0.9%). The samples were inoculated onto Potato Dextrose Agar (PDA) supplemented with olive oil and incubated at 30-35°C for up to five days. Identification of *Malassezia* spp. was conducted through macroscopic and microscopic morphological evaluation using methylene blue staining. *Malassezia* spp. was isolated in 13 animals (27.7%; 95% CI: 15.6-42.6%). The occurrence was higher in dogs (50.0%; 95% CI: 26.0-73.9%) compared to cats (13.8%; 95% CI: 3.9-31.7%), with a statistically significant difference ( $p=0.017$ ). Regarding sex, occurrence in females was 26.9% (95% CI: 11.6-47.8%) and 28.6% in males (95% CI: 11.3-52.7%). Both animals diagnosed with external otitis tested positive for *Malassezia* spp.. These findings highlight the importance of *Malassezia* spp. in veterinary and public health, emphasizing the need for further research and preventive measures.

## Genetic characterization of *Aspergillus* species using ISSR-PCR

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**Keywords:** anemophilous fungus; genetic variability; ISSR-PCR; dendrogram

The genus *Aspergillus* belongs to the Trichocomaceae family and is characterized as a filamentous fungus. This genus includes more than 200 species, 20 of which are considered harmful to humans, primarily affecting the respiratory tract. Certain species are opportunistic pathogens that can develop within the bodies of animals, leading to a disease known as aspergillosis. The spores produced by *Aspergillus* disperse easily and can remain airborne for extended periods, continuously exposing humans to inhalation risks. Using the ISSR-PCR technique, we characterized *Aspergillus* species from seven different samples isolated from various environments. The primers used in this study were UBC807, UBC835, UBC865, and UBC880. The results obtained from the gel analysis indicate that the primer with the highest percentage of polymorphism was UBC864, with a value of 55.6% and length variation between 450-1200 bp. In contrast, the primer with the lowest percentage of polymorphism was UBC880, with a value of 37.3% and length variation between 420-2050 bp. The dendrogram analysis showed that sample 35B, isolated from a sheet, exhibited the greatest genetic difference, with a correlation factor of 0.48. The most genetically similar samples were 32A and 37C, isolated from a pillow and a sheet, respectively, with a correlation factor of 0.76. The identification of polymorphic markers offers significant insights into the genetic relationships among various *Aspergillus* strains, which may be beneficial for further research on fungal pathogenicity and environmental adaptation.

## Evaluation of the anti-cancer potential of RNA fractions isolated from edible mushrooms

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**Keywords:** *Cantharellus cibarius*, small RNA, anti-cancer activity, MTT assay

Edible mushrooms are widely recognized for their nutraceutical properties, including potential anti-cancer activity. Recent studies have highlighted small RNAs (sRNAs) isolated from *Cantharellus cibarius* as bioactive molecules capable of inhibiting tumor cell proliferation. This study's aim is to evaluate the anti-cancer potential of sRNA-enriched fractions isolated from *Cantharellus cibarius*.

RNA fractions were obtained through anion exchange chromatography, and their RNA nature was confirmed by RNase digestion and migration in denaturing gel electrophoresis. The anti-proliferative activity of these fractions was assessed in *Caco-2* (tumor) and *HDFn* (normal) cell lines using MTT assays.

Using anion exchange chromatography, it was possible to isolate a fraction enriched in nucleic acids. This fraction showed to be RNA-enriched by the absence of nucleic acid after RNase digestion and a size above 100 base pairs. Results demonstrated that the RNA-enriched fraction significantly reduced the viability of *Caco-2* cells while having minimal effects on *HDFn* cells, suggesting selective anti-cancer potential. After purification with RNA-specific columns, the anti-cancer effect was highly reduced, suggesting that this sRNA-enriched fraction also contains a molecule that facilitates cancer cell penetration.

These findings support the potential of *C. cibarius* as a source of bioactive RNAs with therapeutic relevance. Further studies should focus on identifying the specific RNA sequence responsible for this effect, as well as its target, and exploring their mechanisms of action in cancer therapy.

## Renal physiology in cats: understanding the key mechanisms behind kidney function and homeostatic regulation

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**Keywords:** feline, renal function, glomerular filtration rate, juxtaglomerular apparatus, renin-angiotensin-aldosterone system

Cats, as companion animals, are important subjects of study due to their close interaction with humans and their role in One Health. By understanding feline physiology, particularly their renal function, we can improve veterinary care and gain invaluable insight into health issues that affect both animals and humans. This knowledge is essential to improve our ability to address shared health challenges, such as infectious diseases, environmental threats, and metabolic disorders. As vital organs, the kidneys play a fundamental role in maintaining homeostasis, with renal failure leading to severe physiological imbalances or death within days. One of their key functions is glomerular filtration, which removes metabolic waste while retaining essential molecules. This process occurs through a specialized filtration membrane that selectively allows substances to pass, depending on their size, charge and capillary pressure. The glomerular filtration rate (GFR) is tightly regulated by intrinsic and extrinsic mechanisms, including renal autoregulation, hydrostatic pressures, and neurohormonal influences. Renal arteriolar vascular tone is critical for the stabilization of GFR, in particular through the juxtaglomerular apparatus (JGA) and the renin-angiotensin-aldosterone system (RAAS). The JGA modulates blood flow via myogenic reflexes and tubuloglomerular feedback, adjusting arteriolar constriction in response to sodium levels. Meanwhile, the RAAS ensures renal perfusion by inducing vasoconstriction and promoting water and sodium retention. Together, these coordinated processes highlight the complexity of feline renal physiology, underscoring its fundamental role in maintaining overall homeostasis and ensuring the health and well-being of felines.

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## Molecular survey on the occurrence of zoonotic bacteria in wild birds from Portugal

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**Keywords:** *Anaplasma*, *Bartonella*, *Francisella*, *Mycoplasma*, PCR, *Rickettsia*

Wild birds are a considerably diverse group of species that occupy a variety of ecological niches. Concerns have arisen about the ability of birds to host and transmit zoonotic pathogens, mainly due to the fact that they fly long distances, as well as their tendency to gather and travel in large flocks. The importance of wild birds in the spread of human pathogens is widely described, either through the transport of infected ticks, through contamination of the environment or other types of interaction, including proximity to humans. The occurrence of some avian zoonotic bacteria has already been reported in various species from different European countries. This study aimed to assess the occurrence of five potentially zoonotic pathogens (*Anaplasma* spp., *Bartonella* spp., *Francisella* spp., *Mycoplasma* spp. and *Rickettsia* spp.) in wild birds of Portugal. Blood and tissue samples were taken from 103 birds admitted at wildlife rehabilitation centres. All samples were screened for detection of bacterial DNA by polymerase chain reaction (PCR) assay. PCR amplifications were performed using specific primers for each bacterium. Using conventional PCR our findings indicate no evidence of circulation of these pathogens among the studied bird populations. Following a One Health approach, it is of utmost importance to know how far avian populations are playing a role in the epidemiology of these infectious agents, in order to better take preventive measures and mitigate the risks of spreading from wildlife to domestic animals and humans. Besides, understanding the impacts and distribution of bacterial zoonoses in wildlife populations can help in planning for future conservation efforts, particularly in endangered species, and wildlife disease monitoring.



## A forensic approach to reveal grapevine genotypes

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**Keywords:** *Vitis vinifera L.*, SSRs, Multiplex PCR, Grapevine identification, forensic, sample identification.

*Vitis vinifera L.* is a valuable economic species, widely grown all over the world and employed primarily for the production of wine. Identification of the various varieties of this species is important since it plays a deciding role in the quality of wine. The demand for rapid and efficient methods of varietal identification is growing. For this purpose, techniques based on the analysis of molecular markers, and more specifically microsatellites, have been utilized extensively. The objective of this work was to identify 25 unknown grapevine samples through the amplification of nine microsatellites by multiplex PCR and subsequent capillary electrophoresis. The samples were used for DNA extraction using the DNeasy Plant Mini Kit and the CTAB protocol. After evaluating the quality and concentration of the obtained DNA, two multiplex PCR reactions were performed, for amplification of the selected markers and subsequent analysis by capillary electrophoresis. The alleles of each marker were determined for each sample, which resulted in 17 identifications according to correspondences with the profiles in the VIVC database. Microsatellite profiles were also obtained for four samples that may represent new genotypes that have not yet been identified. It will be necessary to review and optimize the methodology for four samples extracted by the kit, where it was not possible to obtain amplification of some *loci*. This study demonstrates the efficacy of this methodology in identifying grapevine varieties, and highlights its applicability in different forensic analysis relying in unknown sample identification.

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## Fungal diversity in zoo-housed tortoises - a survey of skin mycobiota

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**Keywords:** Tortoises, *Penicillium*, *Aspergillus*, Fungal diversity

Tortoises can harbour a diverse range of fungal species on their skin, some of which may have implications for both animal and human health. This study aimed to investigate the fungal diversity in tortoises housed in a Portuguese zoo, identifying the most prevalent genera and assessing potential zoonotic risks. A convenience sample of 19 tortoises from 8 different species (belonging to a Portuguese zoo) was examined for the presence of fungus in the skin. Samples were inoculated in Potato Dextrose Agar medium and Sabouraud Dextrose Agar medium and incubated at 25°C and 37°C, for 3-7 days. Based on the observation of microstructures and colony morphology, the fungal isolates were identified at genus level. In this study, 8 genera were identified. The most occurrent genera were *Penicillium* spp. (31.6%; CI 95%: 12.6-56.6%), *Aspergillus* spp. (26.3%; CI 95%: 9.51-51.2%), *Trichoderma* spp. (15.8%; CI 95%: 3.4-39.6%), *Paecilomyces* spp. (15.8%; CI 95%: 3.4-39.6%), *Scedosporium* spp. (10.3%; CI 95%: 1.4-33.1%). The other genera isolated were *Mucor*, *Acremonium* and *Alternaria* with 5.3% each (CI 95%: 0.0-26.0%). This study provides new insights in biodiversity of mycobiota of the integument of zoo tortoises, showing many saprophytic genera. Our findings also raise public health concerns as veterinarians and other professionals, especially those who are immunosuppressed, have a high risk of infection when handling samples from these species.

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## Occurrence of fungi in the enrichment areas of zoo animals

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**Keywords:** Phenotype, zoo habitats, fungal diversity, mycobiota, occurrence

Fungi are essential components of ecosystems, contributing to decomposition and nutrient cycling. However, their presence in zoo environments may pose potential risks to both animal health and environmental quality. Zoo habitats and enrichment areas can serve as reservoirs for fungal species, including opportunistic pathogens that may affect captive animals and pose zoonotic risks to humans. This study aimed to investigate the fungal diversity present in the habitats and enrichment areas of birds, reptiles, and mammals housed in a zoo. Fungal isolates were identified at the genus level, providing insight into their distribution across different animal environments. Samples were collected from 12 different habitats (enrichment areas) and inoculated in Potato Dextrose Agar and Sabouraud Dextrose Agar media. Cultures were incubated at 25°C and 37°C for 3 to 7 days, and fungal identification was based on colony morphology and microscopic characteristics. A total of eight fungal genera were identified in seven habitats. *Acremonium* and *Alternaria* were found in the Alpine marmot (*Marmota monax*) habitat, while *Aspergillus* was present in the common marmoset (*Callithrix jacchus*) and golden tegu (*Tupinambis teguixin*) habitats. *Cunninghamella* was isolated from the reticulated python (*Malayopython reticulatus*) habitat, and *Fusarium* was detected in the grey parrot (*Psittacus erithacus*) habitat. *Mucor* was identified in both the common tegu (*Salvator merianae*) and Cuban boa (*Chilabothrus angulifer*) habitats. Additionally, *Scedosporium* was isolated from the grey parrot habitat, while *Scopulariopsis* was found in the common marmoset habitat. This study provides valuable insights into fungal biodiversity in zoo environments, highlighting the ability of various fungal genera to colonize different habitats. The findings underscore the importance of monitoring fungal communities in controlled environments, particularly to assess potential health risks for both animals and zoo personnel.

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## Investigating fungal in zoo mammals - animal and public health implications through a One Health perspective

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**Keywords:** Zoo mammals, biodiversity, fungal, One Health

Fungal communities play an essential role in animal health and environmental ecology. This study aimed to investigate the fungal biodiversity on the fur of various mammal species in a zoo, as well as the prevalence of different fungal genera. Samples were collected from the fur of mammals using a toothbrush following the Mackenzie technique. The samples were inoculated on Potato Dextrose Agar and Sabouraud Dextrose Agar media and incubated at 25°C and 37°C for 3 to 7 days. Fungal growth was analyzed and identified at the genus level by evaluating the colony morphology and microscopic features. Fungal growth was observed in samples collected from different mammal species. In the Barking Deer (*Muntiacus muntjak*) (n=3), samples yielded *Fusarium* spp., and *Penicillium* spp.. The Common Marmoset (*Callithrix jacchus*) (n=2) samples presented *Aspergillus* spp., *Scopulariopsis* spp., *Penicillium* spp., and *Talaromyces* spp.. No fungal growth was observed in the Green Monkey (*Chlorocebus sabaeus*) (n=1) and Suricate (*Suricata suricatta*) samples. The Marmot (*Marmota monax*) (n=1) sample revealed *Acremonium* spp., and *Alternaria* spp.. In the Bennett's Wallaby (*Macropus rufogriseus*) (n=1) sample, *Aspergillus* spp., *Curvularia* spp., and *Fusarium* spp. were isolated. The Tiger (*Panthera tigris*) (n=1) sample showed *Aspergillus* spp., while the Lion (*Panthera leo*) (n=1) sample only revealed unidentified fungus. The findings stress the importance of monitoring fungal biodiversity in zoo mammals, particularly regarding the potential health risks to both animals and personnel. Further research on fungal colonization in animals is essential for improving zoo biosecurity and animal welfare practices.

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## Assessment of solid lipid nanocarrier toxicity: integrating *in vitro* and *in vivo* approaches

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**Keywords:** Solid lipid nanoparticles; Toxicity; Cell viability; *Drosophila melanogaster*

Nanotechnology has revolutionized drug delivery systems by enhancing drug protection, controlled release, and targeted delivery. Among nanocarriers, solid lipid nanoparticles (SLN) stand out for their biocompatibility and potential safety in pharmaceutical applications. However, the assessment of their possible toxicity remains underexplored in the literature. This study investigates the toxicity of SLN and their components through *in vitro* and *in vivo* approaches. *In vitro* studies were conducted using a normal human fibroblast cell line. Positively charged SLN (SLN<sup>+</sup>) exhibited higher cytotoxicity than negatively charged SLN (SLN<sup>-</sup>), particularly at concentrations of 10, 20, and 100 µg/mL, likely due to stronger interactions with cell membranes. Additionally, the main SLN components (Precirol® ATO 5, Tween® 80, and benzalkonium chloride) were tested at their highest formulation concentrations. Among them, benzalkonium chloride was the only component displaying significant cytotoxicity. For *in vivo* evaluation, *Drosophila melanogaster* was exposed to SLN<sup>+</sup> (100 µg/mL) and its components under chronic and periodic conditions. Toxicity was assessed based on egg count, hatch rate, and sex distribution. No significant differences were observed in chronically exposed flies for these parameters. However, when F<sub>1</sub> flies (chronically exposed) were crossed with untreated flies, gender-related effects were noted across different tested components. The discrepancy between *in vitro* and *in vivo* findings suggests that while SLN exhibit cytotoxicity in cell cultures, their toxicity is less evident in whole organisms. This may result from biological processes such as metabolism and detoxification, emphasizing the importance of comprehensive *in vivo* studies for a more accurate toxicity assessment of nanocarriers.

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## Plant defences and biological control: strategies for managing pathogens and contaminants

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**Keywords:** biocontrol, contaminants, in vitro, pathogens, antifungal activity

Plant health is constantly challenged by various pathogens, including fungi, bacteria, and nematodes, which impact crop productivity and sustainability. Traditional synthetic fungicides and pesticides, although effective, pose environmental and health risks, as well as contribute to the emergence of resistant strains. This review explores alternative strategies for the control of phytopathogens, focusing on plant-induced secondary metabolites, biocontrol agents, and contamination management in plant tissue cultures.

Plants produce bioactive secondary metabolites, such as tannins, alkaloids, and flavonoids, which exhibit antifungal properties. These metabolites represent a sustainable alternative to chemical fungicides. Additionally, the biocontrol potential of *Metarhizium anisopliae*, an entomopathogenic fungus isolated from suppressive soils, has been demonstrated against the cereal cyst nematode *Heterodera avenae*, with high pathogenicity at optimal temperatures of 27–35°C.

On the other hand, contamination by these pathogens is a major challenge in plant tissue cultures, significantly impacting the success of *in vitro* propagation. Bacteria, fungi, and yeasts contribute to culture losses. Effective sterilisation, contamination detection, and aseptic handling are essential to maintain culture integrity and prevent the spread of latent pathogens.

By integrating plant defence mechanisms, biocontrol agents, and contamination prevention strategies, sustainable and effective plant disease management can be achieved, reducing reliance on synthetic agrochemicals and promoting agricultural resilience.

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## Antifungal properties of strawberries and blueberries against two *Aspergillus* species

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**Keywords:** red fruits, fungal infections, dose-dependent inhibition, antimicrobial properties

The growing incidence of fungal infections caused by *Aspergillus* species presents a significant challenge in veterinary care, as these fungi are responsible for a range of diseases in animals, including respiratory and skin infections, leading to high morbidity and mortality. Effective methods to control these infections are vital for animal health and for preventing zoonotic transmission to humans. Strawberries and blueberries are known for their nutritional benefits, but their potential antimicrobial properties have also attracted attention. The antioxidant effects and other bioactive compounds, in these fruits, have been widely studied for their health-promoting qualities in humans.

This study aimed to assess the ability of strawberry and blueberry leaves to inhibit the growth of *A. niger* and *A. fumigatus*, as part of efforts to develop new approaches for preventing fungal infections in veterinary medicine.

In the experimental approach, macerated leaves of both strawberries and blueberries were prepared at various concentrations (5, 10, 20 and 30 mg/mL) in Potato Dextrose Agar supplemented with water. Fungal inoculum was applied to the plates, and fungal growth was measured over a week.

The results showed that blueberry leaves had no effect on the growth of either fungus, whereas strawberry leaves successfully inhibited *A. fumigatus*, with complete inhibition at the highest concentrations. Additionally, a dose-dependent response was observed, where higher concentrations of strawberry leaf extract led to greater inhibition.

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## Fungal contamination in a shelter dog of the Centre of Portugal: assessing the presence of *Aspergillus* spp. and other opportunistic fungi

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**Keywords:** *Aspergillus* spp., shelter dogs, fungal identification

Filamentous fungi such as *Aspergillus* spp. are widely distributed in the environment and can cause opportunistic infections in both animals and immunocompromised humans. Contact with animals may increase the risk of transmission of these pathogenic agents to humans. Canine aspergillosis is predominantly caused by *Aspergillus fumigatus*. In this study, hair samples were collected from 50 dogs at the Official Collection Center (CRO) of Castelo Branco. The hair samples were placed in individual paper envelopes and kept at room temperature until inoculation. The samples were inoculated on Potato Dextrose Agar (PDA) and Dermatophyte Test Medium (DTM) and incubated for 5 to 21 days at 28°C in a dark and humid environment. Fungal identification was performed using the lactophenol cotton blue staining technique, and microscopic observations were conducted at different magnifications. The three most frequently occurring fungal genera were *Aspergillus* spp. with 19.6% (95% CI: 11.3–31.8%), followed by *Alternaria* spp. with 10.7% (95% CI: 5.0–21.5%), and *Chrysosporium* spp. with 8.9% (95% CI: 3.9–19.3%). The results of this study demonstrate a significant presence of *Aspergillus* spp., *Alternaria* spp., and *Chrysosporium* spp. in hair samples from dogs at the CRO of Castelo Branco. These findings emphasize the need for fungal surveillance in animal shelter environments and encourage further research on preventive measures and the clinical impact of these infections in both animals and humans.



## Detection and genetic analysis of *Aspergillus fumigatus* and *A. nidulans* in hair canine samples

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**Keywords:** *Aspergillus* spp., *Aspergillus fumigatus*, *Aspergillus nidulans*, PCR–ISSR, PCR–RFLP

Fungi of the *Aspergillus* genus are saprophytic and ubiquitous. While most species within this genus are harmless, certain species, such as *Aspergillus fumigatus* and *Aspergillus nidulans*, can cause aspergillosis in both humans and animals. In dogs, aspergillosis is predominantly caused by *A. fumigatus*. In this study, 50 samples of *Aspergillus* spp., previously isolated in dogs, were analyzed using PCR to amplify the  $\beta$ -tubulin gene with the primers BT2a and BT2b in the extracted DNA samples. These samples were further analyzed using PCR–RFLP, which confirmed the *Aspergillus* genus of the samples and the presence of *A. fumigatus* and *A. nidulans* species.

Additionally, PCR–ISSR analysis with eight primers revealed a polymorphism percentage of 91.11%, with three primers (UBC 835, UBC 864, and UBC 889) exhibiting 100% of polymorphism. A presence/absence matrix was constructed based on gel banding patterns, and a dendrogram was subsequently generated. This study highlights the significant presence of *Aspergillus* spp. in canine hair samples, with molecular identification confirming the occurrence of *A. fumigatus* and *A. nidulans*. The high polymorphism percentage observed in ISSR analysis suggests substantial genetic variability within the fungal isolates from the same genus and some of them from the same species. These findings reinforce the importance of fungal surveillance in shelter environments and emphasize the potential zoonotic risk posed by *Aspergillus* spp. to both animals and humans.

## Case report of a multiple myeloma patient with a complex karyotype

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**Keywords:** Cytogenetics, clinical report, diagnosis, hemato-oncology

Hemato-oncological diseases affect the production, quality and function of blood components through abnormal and accelerated cell growth. In this way, the study of hemato-oncological diseases has contributed to increasing patient survival through the timely determination of diagnosis, prognosis and therapies to be used. Multiple Myeloma (MM) is a malignant hemato-oncological disease characterized by the presence of a high number of abnormal plasma cells in the bone marrow involving the formation of monoclonal proteins. Currently, this is the second most common hemato-oncological cancer and is responsible for approximately 10% of all hemato-oncological diseases and approximately 1% of all cancers. MM mainly affects male individuals between the ages of 60 and 70 who present bone deterioration, hematopoietic dysfunction and organ failure. The appearance of primary chromosomal alterations is common at the onset of this disease, while secondary chromosomal alterations appear more frequently at a later stage. Conventional and molecular cytogenetic studies were requested on a bone marrow sample from a 63-year-old male individual with suspected MM. The methods used to prepare the karyotype and the Fluorescent *In Situ* Hybridization (FISH) technique were performed in accordance with the protocols established by the Genetics Laboratory of ULSTMAD. Conventional cytogenetic analysis revealed the presence of a complex karyotype involving human chromosomes 1,2,3,7,8,10,15,19 and X. The result of the FISH technique was negative for the probes panel used for the chromosomal alterations del(13q), del(17p), t(4;14) and t(11;14). Several studies in the literature have shown that the presence of a complex karyotype in individuals with MM, as determined by conventional cytogenetic analysis, is associated with poor prognosis. Thus, conventional cytogenetics was extremely important for detecting these complex alterations that were not detected by the FISH technique.

## ***In vitro* germination of a variety of *Vaccinium corymbosum*: 'Legacy'**

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**Keywords:** *Vaccinium corymbosum*, *in vitro* germination, gibberellic acid, light conditions

*Vaccinium corymbosum* is one of the major species of the Ericaceae family. Originally from North America and Oriental Asia, blueberries, as it's commonly known, are highly heterozygous tetraploid crops domesticated in the 20th century. Introduced in Portugal in the 1990s, particularly in Sever do Vouga, its production has significantly expanded, positioning the country on the top 10 global producers. Most of it is due to its beneficial health properties, including anticancer, anti-inflammatory, antibacterial, antifungal and antidiabetic effects. Blueberries are rich in antioxidants, particularly anthocyanins, which contribute to the prevention of chronic and degenerative diseases. Traditionally propagated via staking, blueberries benefit greatly from micropropagation techniques, which enhance growth and morphology. *In vitro* germination provides a controlled and efficient method to overcome the challenges previously mentioned, ensuring optimal seedling development before field transplantation.

Gibberellic acid (GA3) is a key factor in overcoming dormancy, and its effects varying depending on concentration. The main goal of this work was to evaluate the seed's response under different light conditions (24 hours dark, 24 hours light, and 16 hours of photoperiod) and different times of exposure to GA3 (0h, 3h, 6h, 12h, 24h), allowing a better understanding of the interaction between light and hormonal regulation in seed germination. Overall, Woody Plant Medium under 24h light with an exposure of 6h to GA3 granted the best results to this work. Ultimately, this study contributes to a better understanding of how light and gibberellic acid influence blueberry seed germination, offering practical insights for improving propagation techniques and enhancing production efficiency.

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## Laser Capture Microdissection (LCM) application to microdissection chromosomes for cytogenetics applications

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**Keywords:** Laser capture microdissection, WGA, WCP

Laser Capture Microdissection (LCM) is an automated technique that enables the separation of cell subgroups, specific cells, or chromosomes from a heterogeneous population. Using an inverted microscope, a laser, and a sample collection system, LCM allows for cellular microscopic observation, precise cutting of regions of interest, and subsequent sample collection in a single operation. This technique is widely used in various scientific fields, particularly in cytogenetics, to isolate specific chromosomes or chromosomal regions. However, amplification is necessary due to the low quantity of DNA obtained. Among the tested amplification methods, the commercial kit GenomePlex WGA4 Single Cell was used. In this work it was possible to isolate chromosomes using LCM, amplify and labelling them using the whole genome amplification (WGA) methodology, to use them as Whole Chromosome Paints (WCP) in molecular cytogenetic analyses.

## A Bioinformatic's pipeline for earthworm diversity: assessing biofertilized soils health

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**Keywords:** Fertilizers, Earthworms, diversity, Bioinformatics

As the Earth's population increases, the need for sustainable food production has become a priority in agricultural research. However, the growing demand for food has led an increase of intensive agricultural practices, often leading to soil degradation. One major consequence is soil biodiversity loss, which significantly impacts soil ecosystem functioning.

Earthworms are fundamental for organic matter decomposition, soil aeration, nutrient cycling and aggregate formation contributing to soil fertility. They are also well-established bioindicators of soil quality, since they are sensitive to environmental changes, easy to collect, visible in the field, and cost-effective. The presence and diversity of different species can provide information on the conservation status of different land-use systems.

By integrating high-throughput sequencing, computational analysis, and data visualization techniques, bioinformatics can offer a more comprehensive and efficient assessment of earthworm diversity in soils treated with different fertilizers.

Here we present a bioinformatics pipeline for studying earthworm diversity, utilizing OTU analysis for species identification, alpha and beta diversity assessments to evaluate community structure across soil conditions, and advanced visualization techniques to interpret the impact of biofertilizers on soil health.

## Precision Medicine in NSCL: a dual approach with DoctorVida and NGS

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**Keywords:** Lung Cancer, Next-Generation Sequencing (NGS), DoctorVida device, Liquid Biopsy, Personalized Medicine

Lung cancer is the most commonly reported malignant cancer worldwide, with one of the lowest survival rates. Over the years, interest in LC and the factors that increase the risk of developing the disease have grown significantly. This type of cancer is often associated with a poor prognosis, so when it is detected, precision and speed are two essential factors in increasing the patient's survival rate.

In the last decade, several genes have been associated with different subtypes of lung cancer. This work focuses on non-small cell lung cancer (NSCLC) subtype, where deletions and single nucleotide polymorphisms (SNP's) are often linked to the disease. The detection of such mutations provides key information for both diagnosis and treatment, therefore, early screening of these biomarkers is of vital importance. The need for fast, reliable, and early detection methods for NSCLC has driven the development of a highly sensitive portable device capable of detecting cancer-associated mutation, known as DoctorVida. This device is a promising alternative for initial detection, where the in-frame deletion in exon 19 and L858R substitution in exon 21 can be rapidly identified, enabling early diagnosis and treatment selection, being a near-to-patient approach. As a second approach, the sample undergoes Next-Generation Sequencing (NGS) which allows the detection of all occurring mutation and enables a more precise treatment strategy.

In this study, we report the development of a streamlined procedure designed to improve patient care. By utilizing liquid biopsy, patients can receive personalized therapy based of the results of both DoctorVida (first test) and NGS (second test) ultimately increasing their survival rate.

## Upscaling and production of theanine-enriched wheat flour: chemical and immunological characterization

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**Keywords:** Theanine, Transglutaminase; Wheat; Functional food

L-theanine, an amino acid naturally present in tea, is known for its diverse health benefits, including anti-anxiety and relaxing effects, cognitive performance and mood enhancement, neuroprotection, anti-inflammatory properties, blood pressure reduction, and even anti-cancer activity. These beneficial effects make L-theanine an ideal candidate for functional foods.

A recent investigation described a microbial transglutaminase-catalyzed transamidation process in which gluten proteins were modified using ethylamine as an amine nucleophile, effectively converting a substantial portion of glutamine residues into theanine residues.

In this study, we scaled up the process to the kilogram level using a commercial wheat flour with a protein content of 9.6% and performed a thorough chemical and immunological characterisation of the resulting flour. High-performance liquid chromatography (HPLC) analysis confirmed a theanine content of approximately 0.2%. Furthermore, reactivity with the anti-gliadin R5 antibody, associated with celiac disease, was reduced by nearly 90% compared to native gluten, highlighting not only the functional potential of the product but also its possible use for celiac disease management.

## Evaluating root mitosis of *in vitro*-grown grapevine plants upon exposure to a new herbicide

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**Keywords:** Cell cycle; Cytogenetics, cytotoxicity; herbicide, *in vitro* culture, *Vitis vinifera* L.

Herbicide persistence in soil and water could affect non-target plant species. A Portuguese company recently developed a new synthetic herbicide for vineyards. Before its release to the market, under an ongoing R&D project, additional and complementary analyses were required of our team. This work aimed to determine the lowest and highest cytotoxic concentrations of herbicide in grapevine plants cultured *in vitro* based on the cytological evaluation of newly regenerated roots. Single node stems of “Malvasia Fina” (MF) were used as explants. Filtered herbicide solutions with different concentrations (C2- 0.00096 mg/L; C3- 0.0135 mg/L and C6- 0.75 mg/L) were incorporated in the culture medium. A medium without herbicide (C0 - 0 mg/L) was used as a control. Herbicide exposure lasts for 21 days. Newly regenerated roots were used for mitotic preparations and cell cycle analysis. Briefly, the average of normal and irregular dividing cells (in different mitotic phases) significantly differed ( $p < 0.05$ ) among treatments. The lowest frequency of dividing cells with anomalies (DCA) was detected in the control medium. The average DCA increased with the herbicide concentration. As expected, the lowest and highest cytotoxic concentrations were C2 and C6, respectively. Higher vegetative growth was seen in C2. This work also revealed that Cytogenetics and *in vitro* culture are suitable tools for cytotoxicity studies in grapevine.

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## Molecular instability assayed by ISSRs in grapevine *in vitro* plants under herbicide exposure

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**Keywords:** DNA amplification, Herbicide, *in vitro* culture, molecular instability, *Vitis vinifera* L.

Given the significance of wine production, the use of herbicides has steadily increased over time. As a result, it is essential to evaluate the potential adverse effects of these compounds on the molecular stability of non-target crops, such as grapevines. In this study, we aim to assess the impact of a new herbicide (with confidential chemical composition) on the DNA of *Vitis vinifera* L. (variety 'Malvasia Fina') grown *in vitro*, exposed to varying herbicide concentrations: 0.00096 mg/L (C2), 0.0135 mg/L (C3), and 0.75 mg/L (C6). These concentrations were incorporated into Murashige and Skoog (MS) medium, with MS without herbicide as the control (0 mg/L). After 21 days of herbicide exposure, the leaves were collected and immediately frozen. Genomic DNA was extracted from the leaves to identify potential polymorphisms (loss or gain of bands) between the pesticide-exposed explants and the control, using Inter-Simple Sequence Repeat (ISSR) molecular markers for amplification. Polymorphisms were detected across the treatments with the primers used. In future studies, we plan to investigate the genetic polymorphisms in plants exposed to varying concentrations of glyphosate (widely used in viticulture) to compare its genotoxic effects on non-target species.

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## Effect of *Ascophyllum nodosum* application on the physiology and expression of *PvLEA3* gene involved in resistance to water stress in green beans

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**Keywords:** Abiotic stress, Biostimulants, Gene expression, Plant physiology, *Phaseolus vulgaris*

The excessive use of agrochemicals harms ecosystems, prompting the EU to restrict toxic pesticides. With sub-Saharan Africa's population projected to reach 3.1 billion by 2100, sustainable alternatives like biostimulants are gaining attention. Beans, an important nutritional source, suffer significant losses due to abiotic stresses, especially drought, which affects growth, transpiration, photosynthesis, and protein accumulation. Gene expression plays a key role in drought tolerance, with the *PvLEA3* gene being a relevant marker. This study evaluates the effect of *Ascophyllum nodosum* on drought tolerance by analysing plant physiology and *PvLEA3* gene expression. Four treatments were tested: (T1) irrigation at 100% field capacity (FC), (T2) irrigation at 50% FC, (T3) irrigation at 100% FC with foliar biostimulant application, and (T4) irrigation at 50% FC with foliar biostimulant application. A total of 36 plants were assessed for leaf gas exchange, relative water content, membrane permeability, biochemical composition, *PvLEA3* gene expression related to water stress, and total production. The results generally showed a low *PvLEA3* expression, suggesting minimal water stress. No significant differences were found between plants irrigated at 50% and 100%, with or without biostimulant, in terms of *PvLEA3* gene expression. However, biostimulant application slightly improved pod production in plants irrigated at 50% (10 % more healthy pods and 10% fewer reject pods). Plants treated with biostimulant showed less membrane damage, which was observed from the irrigation at 100% (44.68%) to 50% (33.33%) and from the irrigation at 50% without biostimulant (40.53%) to 50% with biostimulant (30.33%). These findings highlight *Ascophyllum nodosum*'s biostimulant potential, especially under moderate drought. Further research is needed to understand the growth factors in algae and their mechanisms of action on plant development to optimize their agricultural application.

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## Impact of a new fungicide for downy mildew control on the microbiome of *Vitis vinifera* cv. 'Tinta Roriz' leaves

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**Keywords:** Grapevine, leaf microbiome, *Plasmopara viticola*, ITS, 16S, crop protection

Downy mildew, caused by *Plasmopara viticola*, is the most significant grapevine disease, leading to severe yield and quality losses. Management of the disease relies on fungicide applications, cultural practices and resistant varieties. However, with the reduction of approved fungicides on the market, environment and health concerns, the search for new molecules to maintain productivity, competitiveness and simultaneously the sustainability of the wine sector is crucial. In this study, a new fungicide for downy mildew prevention/control was tested and its impact on the microbial community of grapevine leaves was accessed through a metagenomic approach. A field trial was installed at UTAD with the cultivar 'Tinta Roriz', and sprayings were carried out in 2024 from leaves unfold until veraison in a total of nine foliar applications. The conventional sprayings for powdery mildew were also done. Six different treatments for downy mildew control were tested: M1 - control, without any spray; M2 and M3 - two different concentrations of the new fungicide; M4 - the new fungicide combined with elicitor (fungicide +Prevatect®, chitosan based); M5 - the new fungicide combined with elicitor Equiset®, *Equisetum arvense* L. based and M6 - conventional fungicide. Leaf samples were collected before the first spraying and after the last one. Metagenomic libraries for ITS and 16S were prepared and sequenced using Illumina platform. In general, the most abundant bacterial phylum was Actinomycetota, with *Candidatus Protofrankia californiensis* as the dominant species. Among fungi, Ascomycota was the most prevalent phylum, with *Pyrenophora avenicola* dominating before sprayings and *Erysiphe necator* after treatments. The treatments M2 and M3 revealed an increased diversity and abundance of bacteria after sprayings. Notably, an overall increase in fungal species diversity was observed in all treatments. Thus, the new fungicide increased bacterial abundance and altered fungal diversity.

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## Breaking to build the Human Y Chromosome - Recent approaches sequencing and exploring its role

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**Keywords:** Human Y chromosome; Assemble sequences; 1000 Genomes Project; T2TY; MSY

The journey to understand the human Y chromosome began some decades ago and despite multiple efforts in assembling it, researchers were unable to complete the sequence, due to the chromosome's complex structure and abundance of repetitive sequences. These challenges made it difficult to fully reconstruct the Y chromosome, even when high-quality segments were obtained.

To overcome these barriers and as part of the 1000 Genomes Project, advanced sequencing techniques were employed to analyze 43 samples from individuals across five continents. This allowed for a comprehensive and precise representation of the Y chromosome's diversity. The reconstruction process utilized several technologies, including PacBio HiFi, Oxford Nanopore Technologies (ONT) and the Verkko tool, which combined both methods to create highly contiguous reconstructions. Only three of the 43 Y chromosomes were fully assembled without gaps.

The reconstructed chromosomes showed significant variability in size and structure, ranging from 45.2 to 84.9 Mb. They included structural variants, insertions, deletions, and single nucleotide variants. The heterochromatic regions exhibited more structural variation, while euchromatic regions showed less variability in size and copy number. MSY analysis highlighted the high conservation of single-copy genes, which are essential for male sex determination and other biological functions, while multi-copy genes were more variable.

Most chromosomes recombine during division which helps repair damaged genes, however the Y is mostly isolated leading to buildup and gene loss, as well as inversions. This phenomenon could be explained by the DNA repair mechanism of double-strand breaks via intrachromatid recombination. Lineage research revealed differences up to 182.900 years between the oldest and newest variants of the Y chromosome, providing valuable insights into its evolution and genomic stability.

Overall, the sequencing marks an important advancement in understanding the Y chromosome's evolution by itself or in comparison with other chromosomes and its role in human health, traits and fertility.

## Senolytic potential of *Rheum ribes*: a phytochemical approach for healthy aging

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**Keywords:** *Rheum ribes*; senolytics; aging; quercetin; fisetin; cellular senescence; phytochemicals

The accumulation of senescent cells plays a central role in age-related tissue dysfunction and chronic disease development. Senolytics, agents that selectively clear these dysfunctional cells, have emerged as a promising strategy to extend healthspan and mitigate aging-related disorders. *Rheum ribes* (Syrian rhubarb), long utilized in traditional herbal medicine, exhibits a complex phytochemical profile, rich in bioactive flavonoids such as quercetin, fisetin, and their glycosides. These compounds are recognized for their antioxidant and anti-inflammatory properties, and mounting evidence suggests that they may also exhibit senolytic activity.

Recent preclinical studies have demonstrated that natural senolytic agents can reduce senescence-associated secretory phenotype (SASP) factors, modulate inflammatory signaling via pathways such as NF- $\kappa$ B, and enhance cellular stress responses through activation of the Nrf2/ARE pathway. A 2019 study conducted by Wen Li and colleagues demonstrated that natural compounds with senolytic properties are effective in delaying aging and extending lifespan. Additionally, Zhang et al. (2023) provided mechanistic insights into how flavonoid treatment improved tissue homeostasis, alleviated age-related pathologies, and extended both median and maximum lifespan. This study further supports the idea that natural compounds like fisetin have been shown to extend the replicative lifespan of various species in vivo.

This work proposes an in-depth investigation into the senolytic potential of *Rheum ribes*. By correlating its traditional use with modern phytochemical analyses and preclinical data, we aim to assess whether extracts of *Rheum ribes* can effectively eliminate senescent cells and improve healthspan. The findings highlight *Rheum ribes* as a potential natural senolytic agent, supporting the development of plant-based therapies to extend healthspan and counteract aging-related disorders.

## Natural Bioactive Compounds and Genomic Stability: The Antigenotoxic Potential of Lemon Essential Oil and Olive Oil

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**Keywords:** Antigenotoxicity, Applied genomics, Comet assay, DNA damage, Genomic stability, Lemon essential oil, Natural bioactive compounds

Applied genomics explores natural compounds for their role in protecting genetic material from damage. This study evaluates the antigenotoxic effects of lemon essential oil and olive oil in human peripheral blood mononuclear cells, focusing on their potential to mitigate oxidative stress-induced DNA damage. Human peripheral blood mononuclear cells were treated with different concentrations of lemon essential oil (0.2%, 0.5%, 1%, 2%, 3% w/v) and olive oil alone and combined with streptonigrin, a known DNA-damaging agent. DNA integrity was assessed using the comet assay, measuring the DNA percentage in the tail to indicate damage. Lemon essential oil exhibited a concentration-dependent protective effect, with 1% w/v reducing DNA damage to 3.13%. Olive oil also demonstrated protective properties, lowering the tail DNA percentage from 7.50% (control) to 6.00%. In streptonigrin-treated cells exhibiting severe DNA damage (47.06% DNA in the tail), lemon essential oil at 3% w/v significantly reduced damage to 11.81%, suggesting strong antigenotoxic potential. Olive oil also mitigated damage, reducing it to 36.88%. These findings reinforce the relevance of natural bioactive compounds in genomic stability and their potential applications in precision medicine and biotechnology. The protective effects of lemon essential oil and olive oil against oxidative stress-induced DNA damage highlight their promise in functional nutrition and therapeutic interventions. Further studies are needed to elucidate the molecular mechanisms and explore their translational applications.

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## Assessment of 'crioulo' common bean diversity through microsatellite markers

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**Keywords:** Plant genetic resources, Agrobiodiversity, SSRs, Conservation genetics, Bean germplasm

The study of the genetic diversity of bean seeds in active germplasm banks (BAG) is an important strategy for maintaining the genetic variability explored by farmers and crop researchers. The common bean (*Phaseolus vulgaris* L.) is one of the oldest cultivated plants in the world. Studies indicate that the crop was domesticated in the regions of Central and South America where the native peoples have been cultivating it for over 9,000 years. Beans are of great economic and social importance in many parts of the world. This crop has a protein content of 20 to 25%, which is rich in amino acids such as lysine and threonine, normally deficient in cereals. There is great genetic variability in bean germplasm cultivated in Brazil and around the world. This variability is of fundamental importance for agriculture, as the selection of materials adapted to its agroecological and socioeconomic conditions are different from those found in more technologically advanced crops. The preservation of the genetic variability of beans, evaluated, organized and made available to the community, provides significant genetic gains for the crop and for agriculture. A collection of 45 common bean accessions, including improved and unimproved varieties from a cultivar germplasm bank from the Federal Technological University of Paraná (Brazil), was analysed for genetic diversity using three microsatellite markers (BM211, GATS91 and PV-AT007) previously described as highly polymorphic. The three loci together allowed the discrimination of all the 45 genotypes. The number of alleles per locus ranged from 16 (BM211) up to 21 (PV-AT007) with an average of 18.3 alleles per locus. The locus with higher heterozygous genotypes was GATS91 (51%) and the lowest BM211 (29%). Clustering of the germplasm set allowed to infer the genetic similitude between accessions revealing groups encompassing cultivars 'crioulas', improved, and both, highlighting their common genetic background. Microsatellite markers revealed a high fingerprinting capacity unveiling the genetic diversity within the set of Brazilian bean germplasm under study.

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## High-throughput screening of a phenolic compound library for trypanothione reductase inhibitors as potential anti-leishmanial agents

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**Keywords:** Trypanothione reductase, high-throughput screening, *Leishmania*, enzyme inhibition, anti-leishmanial agents, oxidative stress, neglected tropical disease

Trypanothione reductase (TryR) is a crucial enzyme in the oxidative stress defence system of *Leishmania* parasites, making it a promising target for leishmaniasis treatment. In this study, we employed a high-throughput screening (HTS) platform to assess a library of 1,216 phenolic compounds for their ability to inhibit TryR activity.

The HTS approach enabled the rapid evaluation of a large compound library using miniaturized enzymatic assays. Each compound was examined for its capacity to reduce TryR activity. Our screening identified a subset of phenolic compounds that effectively impair TryR function, with 8 of the tested compounds exhibiting strong inhibition, reducing TryR activity in a range between 60% and 100%.

These findings highlight the potential of phenolic compounds as a valuable source of TryR inhibitors. Further investigations will focus on characterizing the most potent inhibitors in terms of structure, mechanism of action, and *in vitro* efficacy. This research contributes to the ongoing efforts to discover novel therapeutic strategies against leishmaniasis, a neglected tropical disease with significant global health implications.



## ***In vitro* evaluation of the impact of biofilm formation on the antimicrobial resistance of *Streptococcus suis***

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**Keywords:** *Streptococcus suis*, biofilm, antibiotics, crystal violet assay, MTT assay

*Streptococcus suis* is a major pathogen in the swine industry, causing systemic infections such as meningitis, septicemia, arthritis, and pneumonia. Its serotype diversity influences both virulence and prevalence, with serotypes 1, 2, 7, and 9 being the most clinically relevant. A key challenge in treating *S. suis* infections is its ability to form biofilms, which enhance antimicrobial tolerance and hinder eradication. In this state, bacteria exhibit reduced metabolic activity, decreased antibiotic penetration, and increased horizontal gene transfer of resistance determinants. These factors reduce treatment efficacy and contribute to pathogen persistence in both the host and the environment. Given that biofilm formation varies among serotypes, evaluating their differential susceptibility to antibiotics is essential for optimizing therapeutic strategies.

This study assesses the impact of biofilm formation on *S. suis* antimicrobial susceptibility and compares the effectiveness of different antibiotics in preventing and eradicating biofilms.

Sixty *Streptococcus suis* isolates belonging to serotypes 1, 2, 7, and 9 were selected to evaluate biofilm inhibition in response to antibiotics. The isolates were cultured in 96-well plates using supplemented THB medium, and different concentrations of ampicillin, ciprofloxacin, clindamycin, erythromycin, and tetracycline were tested through serial dilutions. After incubation, biofilm formation was quantified using crystal violet staining.

In a second assay, biofilm formation was allowed before exposure to antibiotics at various concentrations. Following incubation, cell viability was assessed using the MTT assay. These experiments enable the evaluation of both biofilm formation inhibition and the efficacy of antibiotics against preformed biofilms.

Preliminary expectations suggest that biofilm formation will confer increased antimicrobial resistance, with variations among serotypes. The study aims to identify antibiotics with higher efficacy in preventing biofilm formation and eliminating preformed biofilms, contributing to improved therapeutic strategies for *S. suis* infections.

## Cell wall modifications in B73 maize seedlings in response to *Fusarium graminearum* infection

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**Keywords:** Maize, *Fusarium graminearum*, cell wall, gene expression, stalk rot disease, seedlings

Stalk rot disease, caused by the fungus *Fusarium graminearum*, is a major factor limiting maize yield. While this disease has been extensively studied in adult plants, little is known about the infection process in seedlings. To address this gap, 10-day-old seedlings of the B73 inbred line—previously identified as susceptible—were either infected or not infected, and the expression of various defence genes was analysed in both the epicotyl and root at different time points. Additionally, changes in the composition and structure of the cell wall during seedling development and infection were examined. For this purpose, a qualitative FTIR analysis was performed, along with an assessment of lignin content and composition. The results highlighted the importance of modifications in pectins and lignification, particularly in the epicotyl, during both development and infection. Moreover, cellulose content increased in seedlings under both conditions. However, cell wall fractionation followed by sugar quantification revealed a decrease in hemicellulose extractability, especially during epicotyl development, suggesting cell wall reinforcement. Notably, this reinforcement was less pronounced under infection, aligning with the known susceptibility of B73 to *Fusarium graminearum*.

## Targeting Leishmania: evaluating compounds disrupting DNA repair and proteasome pathways

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**Keywords:** Leishmania, compounds, IRFP, viability, neglected tropical disease

Leishmaniasis is a neglected tropical disease caused by protozoa of the *Leishmania* genus, transmitted through the bite of infected sandflies. It affects millions of people worldwide, and due to the limited efficacy of current treatments and the growing resistance to drugs, the development of new therapeutic strategies is crucial.

In this study, we evaluate the effect of various compounds on genetically modified axenic *Leishmania* amastigotes expressing the fluorescent IRFP protein, allowing real-time monitoring of parasite viability. The compounds were tested at different concentrations to determine their impact on parasite survival. Among them, we analyzed an Ataxia-telangiectasia mutated (ATM) and ATM and RAD3-related (ATR) inhibitors, which are key proteins in DNA repair pathways, combined with a topoisomerase I inhibitor. Additionally, we tested a proteasome inhibitor, essential for cellular homeostasis, combined with a drug commonly used to treat leishmaniasis. Inhibiting these pathways may compromise *Leishmania* proliferation and viability, providing new targets for innovative therapy development.

The results of this study will contribute to identifying compounds with potential leishmanicidal activity, offering valuable insights into critical cellular mechanisms for parasite survival.

## Pathogens of the Porcine Respiratory Disease Complex in Spain: the role of Molecular Biology in their characterization

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**Keywords:** Porcine Respiratory Disease Complex, PCR, serotypes, virulence factors

The swine farming sector is a fundamental pillar of the agri-food economy in Spain, ranking as the fourth-largest global power in pork production and export and the second-largest within the European Union. However, diseases associated with the Porcine Respiratory Disease Complex (PRDC) pose a significant challenge, causing substantial economic losses to the industry.

Among the bacterial agents most affecting PRDC are *Streptococcus suis*, *Staphylococcus hyicus*, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Glaesserella parasuis*. These bacteria not only compromise animal health but also present significant epidemiological and sanitary risks, as some are involved in zoonotic diseases. As a consequence of the pathologies caused by these bacteria, the livestock industry suffers severe economic losses every year.

To study the prevalence of these bacteria in Spanish pig farming, samples were collected from various farms to perform isolations that would allow the identification and characterization of the disease-causing agents in the animals. Using PCR techniques, different genes were analyzed to identify specific serotypes of these organisms, as well as genes expressing virulence factors.

These results will provide a basis for the development of targeted vaccines and more effective treatments, adapted to the particular characteristics of the most prevalent strains and serotypes in specific locations.

**Acknowledgments:** We would like to express our acknowledgements to Laboratorios SYVA S.A. and to the veterinarians who provided the isolates used in this study.

## Anthropogenic activity affects the prevalence of antibiotic resistant bacteria in the soil. A study on antibiotic resistance and production

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**Keywords:** bacteria, soil, antibiotic resistance, antibiotic production, anthropogenic activity

The increasing number of antibiotic resistant bacteria is a global health problem that is escalating, since the WHO estimated that by 2050 the rate of annual deaths caused by this type of infections will rise to 10 million people.

The aim of this study is to determine whether the human activity influences the number of antibiotic resistant bacteria found in the soil, as well as to identify if these strains are capable of producing antimicrobial compounds. In order to achieve this, bacteria present in the soil from two areas with different human influence were isolated and used in different antibiograms and bioassays.

For the bioassays, the strains were selected by their resistance to a variety of antibiotics used nowadays. For the antibiograms, some soil strains were tested against other bacteria known as ESKAPE, a group of multi-resistant human pathogenic bacteria, to assess whether these strains could inhibit their growth. Those that produced an inhibition halo were selected. Strains with the ability to resist or produce antibiotics were identified by a Gram stain and the sequencing of 16S gene fragment in order to determine the species they belonged to.

This study led to the conclusion that more microorganisms from the highly human influenced area were found to be resistant compared to those from the other sample. Moreover, a bacterium from that same soil, identified as *Bacillus cereus* with a 94.49% identity, was capable of inhibiting the growth of all the human pathogenic bacteria tested (*Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*).

**Acknowledgments:** We would like to extend our heartfelt thanks for the grant CNS2022-135378 funded by MCIN/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR, and to the Junta de Castilla y León (JCyL), Spain, for funding our research through the grant LE044P20.

## Optimization of gene expression analysis associated with biofilm formation in different *Streptococcus suis* serotypes

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**Keywords:** *Streptococcus suis*, biofilm, gene expression, serotypes, RT-qPCR, virulence, RNA

*Streptococcus suis* is a pathogen of great importance in the swine industry, responsible for meningitis, septicemia, arthritis, and pneumonia in pigs. Multiple serotypes have been identified, differing in prevalence and virulence, with serotypes 1, 2, 7, and 9 standing out. Among them, serotype 2 is the most frequently associated with clinical cases.

One of the key factors in *S. suis* pathogenicity is its ability to form biofilms, which enhance bacterial resistance to antibiotics and the host immune response, promoting persistence in the environment and within the host. Biofilm formation plays a crucial role in bacterial survival, making infections more difficult to eradicate. Previous studies have shown that biofilm formation capacity varies among serotypes, suggesting a differential regulation of the genes involved in this process.

The objective of this study is to analyze the differences in gene expression associated with biofilm formation among the most prevalent *S. suis* serotypes.

Sixty *S. suis* isolates from serotypes 1, 2, 7, and 9 were selected. The strains were cultured in THB medium supplemented with fetal bovine serum, and biofilm formation was established in 96-well plates for 24 hours. The adhered biofilm was then recovered, and RNA was extracted through lysis and purification using affinity columns. The relative expression of *luxS*, *fbps*, *pdh*, and *otc* genes, all associated with biofilm formation, was analyzed by RT-qPCR, using *16S rRNA* as an internal reference and the  $\Delta\Delta C_t$  method to determine expression differences between serotypes.

Currently, bacterial culture, biofilm formation, and RNA extraction phases have been completed, and gene expression analysis is in progress. Based on previous studies, serotypes with greater biofilm formation, such as 7 and 9, are expected to show higher gene expression. Conversely, serotype 2, despite its high prevalence in clinical cases, has shown a lower biofilm formation capacity, suggesting lower expression levels of these genes.

## History and evolution of Bioethics in animal experimentation

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**Keywords:** Alternative, Animal experimentation, Bioethics, Legislation, Suffering

Animal experimentation has been essential for biomedical progress, although its development has raised various ethical dilemmas. Its origins date back to Ancient Greece, where it was practiced without restrictions, but in the 19th century, greater ethical awareness emerged, which led to the creation of the first legislation in the 20th century. In 1959, Russell and Burch proposed the principles of the 3Rs (reduction, refinement and replacement), to minimize the use and suffering of animals.

Currently, alternative methods such as “*in vitro*” models, computer simulations, organs on chips and organoids exist to replace animal testing in most of the commonly studied biomedical fields. However, ensuring the reliability and applicability of these methods remains a challenge, since the extrapolation of data from animal models to humans is limited by phylogenetic differences. Therefore, until now it has not been possible to completely dispense with experimental animals.

The increase in knowledge about animal cognition and capacity for suffering has provided a deeper understanding of the minds, behaviours and needs of animals. This knowledge has led to greater ethical concern about their treatment and welfare, which has led to stricter regulations, such as Directive 2010/63/EU, in force in Europe, or Royal Decree 53/2013, in force in Spain.

Despite these advances, the debate persists between those who consider animal experimentation to be essential for scientific progress and those who defend animal rights. In this context, my TFG consists of a review of animal experimentation to date, analysing its scientific contributions, the ethical and legislative challenges it raises and how the growing awareness of animal suffering has influenced regulations and the development of alternative methods.

## Search for salt stress resistance genes in lentil (*Lens culinaris* M.)

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**Keywords:** Salt stress, resistance, lentil (*Lens culinaris* Medik), phenotyping, genotyping, genetic markers, salinity

Salt stress is one of the main abiotic stresses that plants face. This increase in soil salt concentration also leads to a rise in osmotic pressure, making it difficult for plants to absorb water and nutrients. As a result, plant growth and productivity decrease, potentially threatening the plant's survival. However, certain lentil (*Lens culinaris* Medik) varieties have been observed to possess a higher natural resistance to this stress.

In this study, a method is developed to evaluate salt stress in lentils, optimizing tools to measure the response of this species to high salinity conditions. Previously genotyped lentil varieties will be analyzed, classifying them based on their resistance or susceptibility to this abiotic stress and identifying key phenotypic differences among the studied varieties.

The phenotyping data obtained will be integrated with genotypic information to explore genetic markers and candidate genes associated with salt stress resistance. These results will contribute to a better understanding of the genetic mechanisms underlying salt tolerance in lentils, laying the groundwork for the development of improved varieties with greater resistance to this stress.

**Acknowledgments:** I would like to thank my tutor, Beatriz San Miguel de Vega from Physiology Department, for the opportunity to do this TFG.



## Development of sheep and mouse liver organoids: applications in drug screening and disease modelling

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**Keywords:** liver organoids, 3D culture, sheep, mouse, biotechnology, disease modelling, drug screening

Liver organoids are an innovative platform for studying liver physiology and pathology, closely mimicking the organ's in vivo functions in a 3D culture. This study focuses on developing liver organoids derived from sheep and mouse, aiming to explore their relevance in biotechnology, disease modelling and translational research.

Protocols for isolating and culturing primary liver cells were optimized for each species. The resulting organoids were analysed for morphology and viability, evaluating their ability to maintain a stable 3D structure and general liver tissue functionality.

These models show great potential in drug screening, providing a more realistic platform for evaluating drug toxicity and efficacy in a more representative environment than conventional 2D cultures. Additionally, they offer opportunities to investigate molecular mechanisms of liver diseases and interspecies differences in physiological and metabolic processes.

## Epigenetic factors related to cancer

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**Keywords:** Epigenetics, cáncer, DNA methylation, histone modifications, non-coding RNA

Cancer is a disease of exceptional clinical importance, where countless factors are relevant to understanding cancer development, but in this work we highlight the epigenetic aspect. Epigenetics is a branch of biology that studies changes in gene expression inherited through cell division that do not involve changes in the DNA sequence. The main epigenetic mechanisms crucial in cancer development are: histone modifications, DNA methylation, and non-coding RNA. All these marks are interconnected and interact to generate changes in the phenotype through different stimuli, favouring or blocking the expression of certain genes. Epigenetic activity controls numerous biological processes, and its alteration can lead to the development of diseases. Since the formation of epigenetic marks is a reversible process, the manipulation of these epigenetic mechanisms has been proposed as an innovative therapeutic option compared to conventional treatments.

## Penicillin-Binding Proteins in the control of phytopathogenic diseases

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**Keywords:** Peptidoglycan, PBPs, Cell Wall, Antimicrobial, Sustainability, Phytopathogen

In prokaryotic organisms, the cell wall is essential for survival, ensuring cell integrity and resistance to different kinds of stress. It is primarily composed of peptidoglycan, a polymer of glycan chains crosslinked by short peptides and its synthesis relies on penicillin-binding proteins (PBPs). Targeting PBPs is a well-established strategy for combating bacterial pathogens, particularly through  $\beta$ -lactam antibiotics, such as penicillin, which have been in use since 1941. These antibiotics inhibit PBPs, thereby disrupting peptidoglycan synthesis and causing bacterial cell lysis. However, widespread antibiotic use has led to the emergence of resistance mechanisms, such as low-affinity PBPs, including PBP2a, which confers methicillin resistance in *Staphylococcus aureus*. Given the growing threat of antibiotic resistance, global initiatives have been implemented, including restrictions on routine antimicrobial and prophylactic antibiotic application in agriculture and farms, and a goal to reduce by 50% pesticide use in 2030.

For this reason, new alternative approaches must be explored to control phytopathogenic bacteria. One of most economically significant bacterial pathogen of tomatoes (*Solanum lycopersicum*) is *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), the causal agent of bacterial canker. Historically, *Cmm* infections have been managed using chemical pesticides and synthetic antimicrobials, affecting negatively to both environmental and human health. As a safer and more sustainable alternative, targeting essential bacterial proteins, such as PBPs, with biodegradable natural compounds presents a promising strategy. This work analyses potential strategies to disrupt bacterial cell wall synthesis, identifying hypothetical targets to control the disease while mitigating the risks associated with traditional chemical treatments.

**Acknowledgments:** The authors acknowledge the financial support provided by MCIN/AEI/10.13039/501100011033, the European Union NextGeneration EU/PRTR; Junta de Castilla y León (LE044P20) and the FGULEM (TCUE plans 2018-2020 and 2021-2023). They also acknowledge the fellowships that supported their team members including predoctoral and postdoctoral grants from JCyL, AECC, Margarita Salas, Beatriz Galindo and FPU program.

## Assessment of mutagenic compounds using the Ames test in *Salmonella* spp.

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**Keywords:** Ames Test, *Salmonella typhimurium*, Mutagenicity, Mutagens, Reverse mutation, Base-pair substitution mutations

Mutagenic compounds increase the rate of spontaneous mutation in the cell, and these genetic mutations can lead to carcinogenesis. The Ames test, a widely used bacterial reverse mutation assay, assesses the mutagenic potential of chemicals using *Salmonella typhimurium* strains with specific mutations. Since DNA structure and mutation mechanisms are conserved across species, substances that cause mutations in *Salmonella* often have similar effects on human cells.

In this study, we assessed the mutagenic activity of two compounds (sodium azide and instant coffee) using *Salmonella typhimurium* strains TA98 and TA100, which reverse their mutations by frameshift and base pair substitution mechanisms, respectively. The *Salmonella typhimurium* strains used in the test have specific mutations that make them highly sensitive to mutagens, allowing researchers to observe genetic changes caused by chemical compounds. The strains studied also possess mutations that make them auxotroph for histidine. When grown in minimal medium (without histidine), only those that, due to the mutation that occurred in each case, have acquired the necessary mechanism to produce histidine grow.

The results showed that sodium azide showed strong mutagenic effects on the TA100 strains, while instant coffee affected the TA98 strains. The observation of a high mutation rate in the TA100 strains exposed to sodium azide confirms that this substance causes mutations by base substitutions. Finding some mutations when using instant coffee in the TA98 strain suggests that, under the conditions tested, there could be components in coffee that can induce frameshift mutations. However, the effect of coffee seems to be less pronounced compared to the strong mutagenic effect observed with sodium azide.

These findings emphasize the importance of continued study and evaluation of environmental and industrial chemicals, as early detection of mutagenic properties is essential to prevent genetic damage and reduce potential carcinogenic risks.

## Deepening the role of thichomes and lignin deposition as mechanisms of resistance of tomato plants to the parasitic plant *Cuscuta campestris*

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**Keywords:** haustorium, trichomes, *Cuscuta campestris*, *Solanum lycopersicum*, *Vigna radiata*, lignin and resistance

*Cuscuta campestris* is a parasitic plant with a wide host range, including important crops such as soybean or alfalfa, resulting in significant losses when a crop is infested. However, it has been observed that some species develop variable levels of resistance to invasion, such as tomato (*Solanum lycopersicum*) plants. However, the mechanisms underlying this resistance response are still poorly understood. In this work, the evolution of the interaction between *C. campestris* and the tomato variety “Minibel” was characterised using a series of microscopic techniques, focusing on the variation of anatomical and histological structures. For this purpose, after germination of *Cuscuta* seeds, young green soybean (*Vigna radiata*) plants were used as intermediate hosts, before allowing neighbouring one-month-old tomato plants to be invaded. The set of results allow us to identify the interaction between *Cuscuta* and tomato plants in five successive phases (F/R). During F1 the *Cuscuta* adheres to the tomato stem, in F2 the formation of prehaustoria was observed, and in some cases the formation of haustoria took place (F3). After F2 or F3, the resistance response appeared (R1) and, after few days, haustoria appeared dried and dead (R2). During the resistance phases, tomato stems were characterised by the presence of a deposit of components such as lignin or suberin in cortex cells, as a physical barrier. An increase in the number of darkened type VI glandular trichomes was also observed near the interaction surface, suggesting that they play a relevant role in the response against this parasitic plant. This study provides new information on the defence mechanisms of the tomato plant against *C. campestris*, and highlights the potential role of lignin and suberin deposits and trichomes in the defence against the parasitic plant.

## Development and optimization of protocols for ascochyta infection in lentils and analysis of the expression of candidate genes

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**Keywords:** *Ascochyta lentis*, lentil, *Lens culinaris*, plant disease, qPCR, resistance

Ascochyta blight is a fungal disease that causes significant productivity losses in legumes. Lentil cultivation is one of the most affected by *Ascochyta lentis*, making the development of resistant cultivars a key strategy, as it represents the most cost-effective, efficient, and environmentally friendly method to combat the pathogen. Therefore, it is essential to create and optimize resistance identification protocols that are accessible, reliable, and high-throughput for disease screening.

In this study, the *MAT1* and *AI/Avr1* types present in the *Ascochyta* strains analyzed were confirmed. Three inoculation methods were evaluated using two strains with distinct pathotypes, a susceptible cultivar, and another initially classified as resistant but ultimately showing signs of infection, proving to be tolerant rather than resistant. The detached leaf drop inoculation protocol yielded the best results, while the spray inoculation protocol in whole plants was the least effective. It was concluded that the *AI/Avr1-2* pathotype was the most aggressive.

Finally, the expression of four candidate genes was measured using quantitative PCR, as they were expected to be affected by infection. An increase in Lc26438 expression was observed in all plants, while Lc24669 was overexpressed in all groups except for cultivar 5588 inoculated with AL-84. A general decrease in Lc27513 expression was observed, except in cultivar 5588 inoculated with AL-84. For the last gene, Lc27905, no changes in expression were detected.

## ***In vitro* analysis of the effect of hypoxia in a knockout model of HIF-1 $\alpha$ in human hepatocellular carcinoma**

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**Keywords:** Hepatocellular carcinoma, HIF-1 $\alpha$ , hypoxia, knockout, 3D models, survival

Hypoxia is a common feature of solid tumours such as hepatocellular carcinoma (HCC) and plays a key role in their development and progression. Hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) promotes tumour cell proliferation and resistance to systemic therapies. This study aimed to analyse the role of HIF-1 $\alpha$  in cell survival in three-dimensional (3D) *in vitro* models of human HCC.

To this end, a stable HIF-1 $\alpha$  silencing model was generated and subsequently 3D spheroid-based cultures were established that mimic physiological conditions of hypoxia in tumours. In these models, the effect of silencing on cell proliferation, viability and death was evaluated. The results showed that inhibition of HIF-1 $\alpha$  reduced cell growth and viability, while increasing cell death, suggesting that the adaptive response to hypoxia is key to HCC cell survival in 3D models. This suggests that therapeutic strategies aimed at blocking HIF-1 $\alpha$  may be a promising avenue to improve the treatment of advanced HCC and its prognosis.

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## Cut-dip-budding: simplifying plant genetic transformation without tissue culture

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**Keywords:** plant biotechnology, non-sterile gene delivery, *Agrobacterium rhizogenes*, plant genome engineering, agriculture biotechnology

Plant tissue culture has been a very useful tool in plant biotechnology during the past few decades, especially for obtaining genetically modified plants with unique characteristics. The most common biological method for this matter is the use of *Agrobacterium tumefaciens*, a molecular parasite capable of transferring its T-DNA into the plant cell, where it integrates into the plant genome.

However, it is often a tedious and costly process, requiring multiple protocols and equipment. Additionally, it is not applicable to all plant species, particularly woody plants, recalcitrant species, and plants with alternative propagation systems. Many tree species have a low regeneration capacity in vitro due to their high phenolic content and long-life cycles. These limitations represent several challenges for the agricultural industry and have restricted the genetic modification of a wide range of plants due to genotype dependency.

This review examines the recently developed technique called cut-dip-budding (CDB) delivery system, which uses *Agrobacterium rhizogene* to obtain genetically modified plants under non-sterile conditions and without the use of tissue culture.

This method allows for the introduction of transgenes into plant cells, leading to the formation of genetically modified roots that regenerate transgenic shoots through root suckering. Therefore, the transformed shoots can be directly planted into soil after selecting the positive root segment.

These findings suggest that CDB has the potential to revolutionize plant biotechnology by offering a simpler, more efficient, and widely applicable approach to plant genetic transformation.



## Advancing our understanding of the genetic basis of photoperiod sensitivity in *Vigna unguiculata*

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**Keywords:** Cowpea, Flowering, Genome-wide association studies (GWAS), Photoperiod sensitivity, Plant adaptation, *Vigna unguiculata*

Cowpea (*Vigna unguiculata*) is a short-day legume originated in Africa that is widely cultivated in many tropical and subtropical regions of the world. Cowpea is well-known for its good adaptation to drought and high-temperatures, as well as for its important role in food and nutritional security. Photoperiod sensitivity is one of the most important agronomic traits, determining geographical adaptation and crop production. The loss of photoperiod sensitivity in some individuals was a key for the expansion of cowpea to higher latitudes. The gene or genes involved in the response to photoperiod are still unknown.

This study aims to get a better understanding of the genetic basis of photoperiod sensitivity in cowpea by analysing a collection of 368 cultivated germplasm accessions, representative of the species' global diversity. We are employing two different approaches: (i) Genome-wide association studies (GWAS) of flowering data from different environments and (ii) study of orthologs of known photoperiod-related genes in closely related species.

The findings of this research will contribute to gain insights into the understanding of the genetic control of photoperiod-sensitivity, providing breeding programs with markers for developing varieties better adapted to new agroclimatic conditions, with direct implications for food security and agricultural sustainability.

## Novel plant protection compounds derived from macroalgal industrial leftovers

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**Keywords:** cell wall, plant defense, macroalgae, industrial leftovers, subcritical water extraction

Macroalgae are a diverse group of photosynthetic eukaryotic organisms characterised, among other things, by the presence of a carbohydrate-enriched extracellular matrix (cell wall). Apart from the scientific interest in understanding the structure and composition of the cell wall of macroalgae, these extracellular matrices are a source of valuable industrial products, generating leftovers with potential bioactive compounds. Despite their importance and decades of research, the detailed composition and structure of macroalgal cell walls are only partially understood.

The aim of this work was to better understand the cell wall composition of selected species of marine macroalgae, focusing on representatives of industrial interest and real industrial leftovers. These residues were fractionated using subcritical water extraction technology, an environmental-friendly methodology and analysed using a wide range of analytical techniques.

Once extracted, these fractions could be tested for their ability to trigger immune responses in plants in search of new compounds with agrobiological potential. In this way, macroalgae waste from industrial processes could be revalorized through efficient and environmentally friendly processes in the context of the circular economy.

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## Gene therapy focused on type I Diabetes

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**Keywords:** Diabetes, glucose levels, gene therapy, viral vectors, ectopic expression, antiapoptotic molecules

Type 1 diabetes (T1D) is a chronic autoimmune disease in which cytotoxic T cells destroy insulin-producing  $\beta$  cells in the pancreatic islets, leading to insulin deficiency and recurrent hyperglycemia. Affected individuals require daily insulin injections for life to prevent potentially fatal diabetic ketoacidosis (DKA).

Gene therapy for T1D can be approached using viral or non-viral vectors; here, we focus on viral vectors. A viral vector is a modified virus that delivers foreign genetic material into a cell. Among them, retroviral vectors can only transduce dividing cells and may integrate the transgene randomly, increasing the risk of insertional mutagenesis. Adenoviral vectors, capable of transducing both dividing and nondividing cells, trigger strong immune responses and result in short-term gene expression. In contrast, lentiviral vectors efficiently modify both dividing and nondividing cells, integrate permanently into the host DNA, and do not induce strong immune responses.

Ectopic gene expression involves expressing genes in cell types that do not typically express them. This can be achieved using viral vectors. In T1D, this strategy aims to recreate  $\beta$ -cell functions in alternative cell types such as hepatocytes, fibroblasts, muscle cells, keratinocytes, neuroendocrine cells, and other endocrine cells, thereby avoiding immune system attacks. To achieve this, a new functional copy of the *INS* gene can be introduced into these cells, so that they start producing proinsulin and process it into active insulin. Another option may involve using viral vectors to introduce different transcription factors, such as Pdx1, MafA, or Ngn3, into the cells to be modified in order to activate the endogenous *INS* gene of the target cells.

In early stages or before disease, genetic manipulation of islets can induce the expression of anti-apoptotic molecules, such as Bcl-2, or block ligands like TNF- $\alpha$  or FasL, protecting them from apoptosis induced by cytotoxic agents.

## Study of the implications of the renin-angiotensin system on baseline inflammation in obese patients candidates for bariatric surgery

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**Keywords:** inflammation, inflammasome, NLRP3, obesity, PBMC, renin-angiotensin

The World Health Organisation (WHO) has identified obesity as a critical factor in the global burden of disease, affecting 59% of the European population. It is estimated that the increase in prevalence cannot be halted in any country by 2025.

Evidence suggests that alterations in the classical and non-classical pathway of the renin-angiotensin system can have several effects on obesity-related inflammatory processes. Specifically, it has been estimated that in people with obesity, elevated levels of angiotensin-converting enzyme (ACE) 2 are associated with increased activity of the pyrin-domain NOD-like receptor family inflammasome 3 (NLRP3), creating an environment that favours chronic inflammation and cell damage.

This study aims to analyse the protein expression of various components of the renin-angiotensin system and its relationship with systemic inflammation in patients with obesity, specifically its relationship with NLRP3 inflammasome activation.

To achieve the described objective, the study included blood samples from 10 men and postmenopausal women, aged between 50 and 60 years, diagnosed with central obesity according to WHO criteria, provided by the CAULE. Peripheral blood mononuclear cells (PBMC) were isolated from healthy adults and obese patients and the expression of various components of the renin-angiotensin pathway, such as ACE, ACE2, angiotensin II type 1 receptor (AT1R), angiotensin II type 2 receptor (AT2R) and Mas receptor (MasR) was analysed. Protein expression analysis was performed by Western blot, followed by statistical analysis.

Preliminary results indicate that there is a trend towards increased ACE2 protein expression in obese individuals compared to the expression observed in non-obese control individuals.

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## Detection of novel microorganisms with BHET-degrading capacity as an intermediate in PET degradation

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**Keywords:** Plastics, Bioremediation, Polyethylene terephthalate (PET), Bacteria, Degradation, BHET (bis(2-hydroxyethyl) terephthalate), 16S rRNA sequencing

The environmental impact of plastics is a global concern. The stability of their chemical structures hinders natural degradation, resulting in massive accumulations in the ecosystem. Among the most widely used plastics, polyethylene terephthalate (PET) is commonly employed in packaging manufacturing. In recent years, advances in biotechnology have enabled the identification of various microorganisms capable of degrading plastics such as PET; however, their efficiency has not been sufficient for industrial applications. The use of these microorganisms in bioremediation is aimed at reducing environmental pollution. In this study, bis(2-hydroxyethyl) terephthalate (BHET), an intermediate in PET degradation, is proposed as a substrate for the isolation of bacteria with potential PET- degrading capabilities. To achieve this, bacteria from other bioremediation projects were cultivated in defined media with BHET as the sole carbon source, allowing for the selection of microorganisms capable of utilizing BHET as a carbon and energy source. Throughout the process, the growth of the selected bacterial strains was monitored by measuring optical density to identify the most efficient strains in BHET utilization. Simultaneously, visual characterization of morphology and other phenotypic traits of the bacterial strains was performed. Subsequently, the fastest-growing and most stable bacterial strains were subjected to 16S rRNA gene sequencing for taxonomic identification and to determine their phylogenetic relationships with previously described plastic-degrading microorganisms. Ultimately, physiological and biochemical characterization will be conducted to evaluate their metabolic properties and potential role in PET degradation. The results of this study will contribute to a deeper understanding of PET biodegradation and will support the development of more efficient and sustainable biotechnological strategies to address plastic pollution.

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## Targeting melanoma: targeted therapies based on specific genetic alterations

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**Keywords:** BRAF mutation, BRAFV600E, Immunotherapy, MEK inhibitors, Melanoma, Targeted therapy

Melanoma is an aggressive and increasingly prevalent form of skin cancer, primarily driven by genetic alterations in melanocytes. Among these, mutations in the BRAF gene—particularly the BRAFV600E variant—are found in approximately 50% of patients and lead to constitutive activation of the MAPK signaling pathway, driving uncontrolled tumor growth. Advances in targeted therapies have significantly improved patient outcomes, offering more effective treatment options.

BRAF inhibitors, such as vemurafenib and dabrafenib, have demonstrated high efficacy in BRAFV600E-mutant melanoma by blocking aberrant BRAF activity and halting tumor progression. However, resistance often develops through MAPK pathway reactivation, alternative signaling (e.g., PI3K-AKT), or tumor microenvironment adaptations. To counteract this, MEK inhibitors (e.g., trametinib) are combined with BRAF inhibitors, improving response durability and delaying disease progression. Despite these advances, resistance remains a challenge, necessitating novel treatment strategies.

In parallel, melanoma has shown a remarkable response to immunotherapy, particularly immune checkpoint inhibitors such as anti-CTLA-4 (e.g., ipilimumab) and anti-PD-1 antibodies (e.g., nivolumab), which enhance the immune system's ability to eliminate cancer cells. Recent clinical trials suggest that combining targeted therapy with immunotherapy provides synergistic benefits, potentially overcoming resistance and leading to more durable responses. Early-phase studies of triplet therapy (BRAF + MEK inhibitors + anti-PD-1 therapy) show promising results, improving progression-free and overall survival in patients with advanced melanoma.

This work explores the evolving landscape of BRAF-targeted therapies, their limitations due to resistance, and the potential of combination strategies, including triplet therapy, to revolutionize melanoma treatment and improve patient outcomes.

## Novel Bio-Based strategies for sustainable phytopathogen control

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**Keywords:** Antagonistic microorganisms, Basic substances, Bioassays, Bioactive agents, Phytopathogens

Horticultural crop phytopathogens present an ongoing challenge, traditionally managed with synthetic pesticides, with a well-known significant environmental impact. Besides, plasticulture, specially using mulching films, presents great agronomical advantages, although significantly contributes to the accumulation of micro- and nano-plastics in agricultural soils and waters. To mitigate both issues, biodegradable platforms based on functionalized bioplastics have emerged as environmentally responsible substitutes, where bioactive agents, such as basic substances or beneficial microorganisms, are gaining attention as a sustainable alternative to provide effective pests control with reduced ecological impact. The European BioBIVE project (no. 101130442) is focused on developing biodegradable delivery systems designed for the controlled release of bioactive agents targeting fungal pathogens in three relevant horticultural crops in Europe: tomatoes, strawberries, and carrots. This initiative incorporates three bio-platform technologies (bioplastic mulch, biochar, and sprayable mulch), each one functionalized with different bioactive compounds to enhance their efficiency. Initial results have demonstrated that several basic substances exhibit strong fungicidal properties against key phytopathogenic fungi, such as *Rhizoctonia solani*, *Botrytis cinerea*, and *Fusarium oxysporum*. These findings support their potential as viable eco-friendly alternatives to conventional chemical fungicides. Moreover, bioassays using antagonistic bacteria such as *Bacillus* spp. have shown an interesting ability to decrease pathogens growth, reinforcing their synergistic role in sustainable crop protection when they are combined with basic substances. As a main conclusion, the concept of the bioactive agents release through functionalized biodegradable systems is supported as a promising replacement of traditional pesticides. They minimize the environmental impact while maintaining effective crop protection in eco-sustainable agricultural practices.

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## Two strategies for 8-oxoguanine determination for DNA mutation monitoring. A theoretic comparison

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**Keywords:** mutagenesis, ROS, adenine, guanine, 8-oxoguanine, electrochemical sensing, stable steady-state

8-oxoguanine is one of the main products of reactive oxygen species (ROS) reaction with DNA, resulting in mutagenesis leading to genome alterations. Its formation results in a mismatched pairing with adenine resulting in G to T and C to A substitutions in the genome. In humans, it is primarily repaired by DNA glycosylase OGG1. Therefore, 8-oxoguanine, as purine oxidation product is important as an indicator for mutation monitoring and its determination is highly recommended to detect possible mutations.

Although 8-oxoguanine is guanine oxidized form, both cathodic and anodic determination may be applied to it. The electrode modifiers for both of the scenarios may be suggested from the reaction mechanism. By anodic way, 8-oxoguanine will be oxidized analogously to caffeine and other uric acid derivatives. Yet on cathode it will be reduced via 8-oxogroup or its carbamide moiety, resulting in the methanol formation.

Two different electroanalytical processes for 8-oxoguanine have been described theoretically. The anodic process may be conducted by cobalt (III) oxyhydroxide, conducting polymer, copper sulphide or another hydroxyl-donating oxidant or an electrode modifier, yielding it *in situ*. As for cathodic process, it will be conducted by trivalent vanadium or bivalent chromium compound. The use of conducting polymer may also be included.

By comparison of both of the models, it was possible to conclude that both cathodic and anodic scenarios are suitable for the determination of 8-oxoguanine as a DNA mutation indicator. Nevertheless, considering the reaction mechanisms and steady-state stability requirements, the cathodic route is more preferable.



# JUNIOR POSTERS





## JUNIOR POSTERS

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### *Agrupamento de Escolas D. Sancho II (Alijó, Portugal)*

- **Prémio Nobel da Medicina ou Fisiologia de 2007 “Targeted Gene Modification in mice using embryonic stem cells”**  
**Authors:** Ana Ribeiro, João Cunha and Lara Constantino
  
- **Nobel de Fisiologia e Medicina de 2009 “Descoberta: como os cromossomas são protegidos pelos telómeros e pela enzima telomerase”**  
**Authors:** Lucas Silva and Matilde Carvalho
  
- **Nobel da Medicina de Svante Pääbo “Descobertas sobre os genomas de homínídeos extintos e a evolução humana”**  
**Author:** Tomás Garcias Denis



# WORKSHOPS







## Learning from Plants

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The study of natural materials, such as plant leaves, has become a significant area of research due to their unique properties, including self-cleaning, anti-icing, and superhydrophobicity. By understanding the chemical composition and microstructure of waxes in the epicuticular layer of the plant leaves, researchers can develop materials that mimic these properties, leading to improved performance in various sectors. The first step to achieving this type of bio-inspired materials involves understanding the waxes' function on the leaves. This workshop aims to illustrate the specific process of leaf waxes extraction, and familiarize the participants with static contact angle measurements via the sessile drop method. Wax content will be determined by immersing leaves in chloroform, while Polarized Optical Microscopy (POM) will provide detailed insight into leaf surface morphology. Additionally, moulds to reproduce the leaves' morphology will be analysed by POM to further clarify the leaf microstructure.



## The power of bioinformatics in cancer precision medicine

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**Keywords:** Bioinformatics, Variant analysis, Case studies, Cancer

The advances in the Omics' technologies and bioinformatic tools of the last years are unveiling a huge number of genomic variants that can be associated to particular diseases. This information is of paramount importance in the diagnosis and prognosis, but also at the therapeutic decision level, increasingly enabling the application of precision medicine according to the mutational profile a patient presents. Old and new sequencing technologies, such as Sanger or Massive parallel (Nextgeneration) sequencing, are widely used for mutation detection in clinical settings, but only a solid and reliable in silico analysis using bioinformatic tools of base calling, read alignment, variant identification, and annotation allows to process the data generated. Moreover, the proficiency of this type of analysis is undeniably faster and cheaper than lab experimental procedures. Variant analysis encompasses the comparison of a patient DNA sequences to a reference one to identify whether there are any variants in the interest target. The annotation and interpretation steps are set to identify and classify each variant and predict its clinical significance. In the present workshop, the participants will be challenged to "solve" clinical cases, identifying the presence/absence of genic mutations through the analysis of sequence data from putative cancer patients and their families (hereditary cancers) using bioinformatics' software and web-based tools. The search in databases and genome browsers will allow the interpretation of the detected mutations and the discussion of disease diagnosis, prognosis, and therapeutics.





## Desafios éticos emergentes na escrita científica, fotografia e produção/edição de imagens

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**Keywords:** ética, má-conduta, fraude, literacia científica, plágio, manipulação de imagens

São inúmeras as questões éticas na experimentação com animais e seres humanos, daí a importância do conhecimento e respeito pelas normas internacionais, como o princípio dos 3Rs (Redução, Refinamento e Substituição) e a Declaração de Helsínquia. Serão discutidas as boas práticas na escrita de artigos científicos, incluindo a transparência metodológica, a citação adequada de fontes e a responsabilidade dos autores na comunicação de resultados. Serão ainda abordados alguns aspetos relativos a atividades antiéticas a serem evitadas na investigação, como o plágio, a falsificação e a fabricação de dados, o viés na interpretação dos resultados e o conflito de interesses não declarado. Será ainda abordado o perigo das revistas predatórias, que cobram taxas de publicação sem realizar uma revisão por pares adequada, comprometendo a credibilidade científica.

A publicação cada vez mais rápida e intensiva de artigos científicos associado ao aumento do número de revistas científicas aumenta o risco de má-conduta/fraude relacionada com o uso e a edição de imagens. Até onde pode ir à manipulação digital de imagens? Qual a responsabilidade dos autores que utilizam ferramentas de IA para a escrita dos manuscritos? Qual o limite do uso das ferramentas de IA? Todos estas questões levantam

problemas éticos, alguns clássicos, outros emergentes devido a crescente disponibilidade de ferramentas IA e a crescente deteção de problemas de má-conduta e também de conduta fraudulenta por investigadores juniores (ansia de publicar) e séniores (por ansia de fama e estatuto) devido á pressão crescente e a ânsia de publicar.

Os dados mostram que tem ocorrido um aumento destas más práticas na manipulação de imagens, na produção de imagens via IA (com erros grosseiros), etc. confirmada pelo número crescente de artigos retratados. Quais são os limites éticos e quem os define? Todos estas questões devem ser analisadas de forma crítica e sérias, e devem ser tomadas medidas adequadas para que estas novas tecnologias não venham impactar negativamente e denegrir a investigação científica que é feita com rigor e qualidade.

Cabe às universidades e instituições aumentar a literacia sobre a ética na investigação científica estimulando o rigor, a seriedade dos trabalhos realizados e publicados e divulgados junto do público em geral sem esquecer a moral e a ética.

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## Relevance of Biosensors for the One Health Approach

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**Keywords:** One Health Approach, Point-of-Care Diagnostics, Biosensors, Nanoparticles

Biosensors have emerged as powerful tools in the field of healthcare, revolutionizing the way diseases are diagnosed, monitored, and managed. These devices, which combine biological components with analytical systems, enable the detection of specific biomolecules or pathogens in real-time, offering rapid, sensitive, and cost-effective solutions for health-related applications. In clinical diagnostics, biosensors provide a non-invasive and highly accurate means of detecting diseases such as diabetes, cancer, and infectious diseases, enhancing early detection and treatment outcomes. Additionally, biosensors play a critical role in personalized medicine by facilitating continuous monitoring of patients' health conditions, leading to more tailored therapeutic interventions. The integration of biosensors with wearable technologies and mobile health platforms has further expanded their accessibility, providing patients with the ability to track vital health data remotely. With ongoing advancements in nanotechnology, microfabrication, and molecular biology, the future of biosensors holds great promise in improving global healthcare through enhanced diagnostics, disease prevention, and real-time health monitoring.

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## Descomplica a Histopatologia: a ciência por detrás do diagnóstico

**Pires M., Travassos F., Martins L.**

A histopatologia é uma área da medicina que efetua diagnóstico em tecidos/células animais. Como ferramenta de diagnóstico, temos o microscópio óptico, que permite visualizar no material biológico alterações celulares e extracelulares que se relacionam com os sintomas e sinais clínicos das doenças. As amostras analisadas são provenientes de animais vivos (como as citologias, biópsias e exéreses cirúrgicas) ou mortos, recolhidas durante as necropsias. Pretende-se neste workshop demonstrar os diferentes passos de colheita do material biológico, o processamento, coloração e leitura das preparações histológicas. Pretende-se mostrar exemplos da utilidade deste exame complementar de diagnóstico de rotina nos hospitais por todo o mundo, tão importante em lesões inflamatórias, degenerativas, mas também no cancro. O diagnóstico histológico determina muitas vezes o tratamento, bem como o prognóstico. Dado ser a base para a investigação, bem como para a clínica, propomos demonstrar a utilidade desta metodologia nos diferentes projetos em curso no nosso laboratório.



## Skincare Formulation Workshop: innovating with upcycled ingredients for sustainable beauty

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**Keywords:** skincare; by-products; sustainability

This workshop aims to deepen knowledge on the use of plant extracts in skincare formulation, highlighting the crucial role of the specific genetic characteristics of plant species in obtaining high-efficacy natural ingredients. As the cosmetic industry shifts towards more sustainable and ethical practices, the valorization of plant by-products and the replacement of synthetic compounds with natural alternatives become essential for developing innovative and effective formulations.

Participants will explore the bioactive potential of plant extracts, understand how genetic variability influences ingredient composition and functionality, and learn advanced formulation techniques to create high-performance skincare products. Additionally, the workshop will address circular economy principles in cosmetics, trends in the sustainable beauty market, and regulatory challenges associated with the use of natural ingredients.



## Physiological Responses of Fruit Trees to Climate Change: Challenges and Innovative Mitigation Strategies

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**Keywords:** Abiotic Stress; Biostimulants; Fruit Tree Physiology; Climate Change Adaptation; Omics Technologies

The latest climate projections indicate a decline in water availability, rising air temperatures, and an increase in extreme weather events, such as heavy rainfall near harvest periods. These changes contribute to significant economic losses by reducing the commercial value of fruits. Abiotic stresses, including extreme temperatures, drought, salinity, and UV-B radiation, are among the most critical limiting factors for crop productivity.

In this context of climate change, coupled with the growing global trade in fruits to meet consumer demand for a continuous supply of high-quality produce, it is essential to understand the impact of pre-harvest biostimulant treatments on the physiological behaviour of fruit trees. Although there is limited literature on the effects of compounds such as glycine betaine (GB) and algae-based biostimulants (ABB) on fruit tree physiology, these substances may provide innovative solutions to enhance crop resilience under stressful environmental conditions. GB, a quaternary ammonium compound, acts as an osmolyte that stabilizes cellular structures and maintains membrane integrity, mitigating the damaging effects of abiotic stresses through osmoregulation and osmoprotection. Similarly, ABB contain plant hormones, proteins, sugars, vitamins, humic substances, and phenolic compounds, which have been reported to enhance plant productivity by improving nutrient

assimilation, increasing photosynthetic activity, reducing transpiration rates, and decreasing fruit-cracking incidence.

This workshop will provide an up-to-date overview of recent studies examining the physiological responses of fruit trees to changing environmental conditions. Specifically, we will explore how new technologies can be leveraged to assess plant stress responses by integrating physiological measurements with omics approaches, paving the way for innovative strategies to mitigate the impacts of climate change on fruit production.

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## The use of Forensic Science in solving crimes against animals

**Pires, I., Maia, G., Prada, J.**

**Keywords:** forensic, necropsy, samples collection

Animal cruelty is not only a serious ethical and legal issue but also a recognized indicator of broader patterns of violence. Research has established a strong correlation between animal abuse and crimes such as domestic violence, child abuse, and other violent offenses. Individuals who harm animals often pose a risk to vulnerable human populations, highlighting the importance of forensic science in investigating such cases. By applying scientific knowledge to legal proceedings, forensic investigations help ensure justice for animal victims while identifying and preventing further acts of violence within society.

A post-mortem examination is crucial in forensic investigations to determine the cause and circumstances of death. However, for forensic evidence to be admissible in court, strict protocols must be followed in collecting, handling, and preserving samples. A key challenge for forensic investigators is preventing evidence contamination, as improperly collected material can be rendered inadmissible in legal proceedings.

Addressing crimes against animals requires a multidisciplinary forensic approach, incorporating pathology, genetics, toxicology, ballistics, and histopathology. In this workshop, we will perform necropsies of various animal species to collect samples for further forensic analysis, including genetics, toxicology, ballistics, and histopathology. By applying forensic techniques to animal investigations, we contribute to animal welfare and broader crime prevention and justice efforts.



## Forensic Genetics Applied to the Food Sector

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**Keywords:** Molecular Markers; High Resolution Melting; Biosensors; Wine; Olive oil; Microsatellites; Single Nucleotide Polymorphism

The food sector is a very challenging and demanding area, with an intensive search for control measures that can build consumer's confidence and the application of fair trade among the producers. Among the consumer's worries stands food authenticity, as high-quality food products are normally more prone to fraudulent practices due to their high market value. The detection of such events can be achieved using different methodologies, but when dealing with varietal/species detection the use of molecular markers is one of the most suitable mean, as it is not dependent on environmental conditions, processing technologies, etc.

The choice of the molecular markers is one of the crucial points when using such a strategy. In this workshop we will explain the several considerations that are imperative when designing an authenticity scheme, giving an inside view to the long experience of the DNA & RNA Biosensing Lab in this type of approach. The process from food matrix to the platform design (HRM and Biosensor) will be discussed.

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## SSRs for genotyping plant genetic resources

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**Keywords:** Genotyping; microsatellites; grapevines; cultivars identification.

This workshop intends to provide an overview of the application of the nuclear and chloroplast SSR molecular markers for the plant genetic diversity assessment, specifically in *Vitis vinifera* cultivars. During the workshop will be addressed the theoretical concepts of SSR molecular marker, its applications to plant breeding and management of germplasm collections. In the practical part, the participants will have the opportunity to analyse SSR amplicons to genotype grapevines samples with specific software's, identify cultivars, determine parent/offspring relationships and prepare dendrograms and networks.

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## Antimicrobial resistance in a One Health context: isolation and characterization of pathogenic bacteria

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**Keywords:** Antimicrobial resistance, pathogenic bacteria, One Health, Antibiotic susceptibility testing, Bacterial isolation, epidemiology, Public health

Antimicrobial resistance (AMR) is a growing global health threat impacting human, animal, and environmental health. The One Health approach recognizes the interconnectedness of these sectors and emphasizes the need for a multidisciplinary response to combat AMR. The widespread use and misuse of antibiotics in human medicine, veterinary practices, and agriculture contribute to the emergence and dissemination of resistant bacterial strains, posing a significant challenge to public health. Understanding the dynamics of AMR is crucial for developing effective strategies for surveillance, control, and mitigation.

This workshop aims to provide hands-on experience in the isolation and identification of pathogenic bacteria from various sources relevant to the One Health framework. Participants will learn microbiological techniques to culture and identify bacteria using selective and differential media, followed by biochemical and molecular methods for species

confirmation. The practical session will also include antibiotic susceptibility testing to characterize resistance profiles, employing standard methodologies such as disk diffusion or broth microdilution. Additionally, participants will explore the interpretation of results and discuss the implications of AMR in a broader epidemiological context. By integrating theoretical knowledge with laboratory-based training, this workshop will equip participants with essential skills to investigate and understand antimicrobial resistance patterns. Ultimately, it aims to foster awareness and encourage collaborative efforts in addressing this global challenge through a *One Health* perspective.

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## Eco-Friendly Fungus: Harnessing Fungi to Combat Fungi and Parasites

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**Keywords:** Biological Pest Control; Antagonistic Fungi; Biological Pest Control

Fungi offers a sustainable alternative to chemical fungicides and insecticides. Certain fungi, known as antagonistic fungi, effectively combat other fungi and parasitic organisms through mechanisms like mycoparasitism, antibiosis, and competition for nutrients and space. Several fungal species suppress plant pathogens, such as *Trichoderma* spp., which degrade the cell walls of harmful fungi like *Fusarium*, *Rhizoctonia*, and *Sclerotinia* using hydrolytic enzymes. Similarly, *Gliocladium* spp. serves as a soil biocontrol agent, while *Ampelomyces quisqualis* is a hyperparasite that targets powdery mildew. These fungi contribute to disease suppression and enhance plant resilience. Some fungi also act as natural enemies of agricultural pests. *Beauveria bassiana* and *Metarhizium anisopliae* infect and kill insect pests like locusts, whiteflies, and beetles, making them valuable for pest management. Additionally, *Lecanicillium* spp. attacks aphids and whiteflies while also exhibiting antifungal properties, reducing the need for synthetic pesticides and promoting eco-friendly pest control. Beyond agriculture, fungi also contribute to human and animal health. Research suggests that *Candida oleophila* could act against *Candida albicans*, a common opportunistic pathogen. In amphibians, beneficial skin fungi from the families *Chaetomiaceae* and *Lasiosphaeriaceae* have shown the ability to inhibit *Batrachochytrium dendrobatidis*, a

deadly pathogen causing chytridiomycosis. Scientists are also exploring innovative antifungal therapies, such as nanotechnology, immunotherapy, and vaccine development, to address challenges posed by drug resistance. Antagonistic fungi represent a promising tool in agriculture, pest control, and medicine. Their ability to target pathogens and parasites reduces reliance on chemical treatments, promoting more sustainable biological control strategies. Further research and innovation are essential to maximizing their potential in diverse ecosystems. In this workshop, we will explore how fungi can be used to combat other fungi and parasites in a natural and sustainable way.





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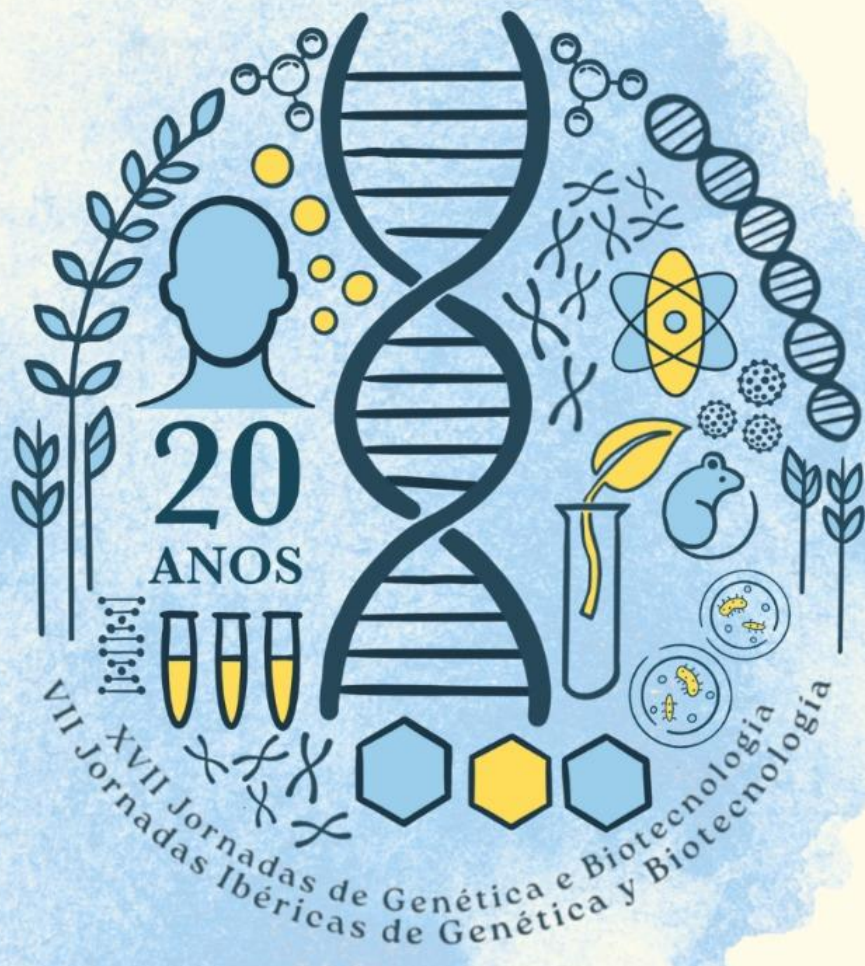
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