

ECM  
**2025**  
Conference

# The 3rd International Electronic Conference on Microbiology

01–03 April 2025 | Online



Program and Abstract Book



1–3 April 2025  
Online

The 3rd International Electronic  
Conference on Microbiology

# **The 3rd International Electronic Conference on Microbiology**

**1–3 April 2025 | Online**



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## Welcome from the Chair

Dear Colleagues,

We are delighted to invite you to the 3rd International Electronic Conference on Microbiology (ECM 2025). ECM 2025 will showcase the latest research on microorganisms, covering a diverse range of topics, including the following:

- S1. Gut Microbiota and Health Disease;
- S2. Foodborne Pathogens and Food Safety;
- S3. Antimicrobial Agents and Resistance;
- S4. Emerging Infectious Diseases;
- S5. Microbiome and Soil Science;
- S6. Microbial Characterization and Bioprocess;
- S7. Microbe–Plant Interactions.

Participation in ECM 2025 is free of charge for both authors and attendees. This conference provides an excellent platform for researchers to present their latest findings, engage in direct discussions, and participate in online Q&A sessions.

All submitted abstracts will be reviewed by the conference committee. Upon acceptance, authors will be asked to contribute an extended abstract for the conference proceedings and a slide presentation of their work. Authors of the most outstanding contributions will be invited to submit a full manuscript for potential publication in *Microorganisms*.

I look forward to your participation in this exciting conference and encourage you to share your latest ideas and research in the field of microbiology.

Kind regards,

Dr. Nico Jehmlich

Department of Molecular Toxicology, Helmholtz-Centre for Environmental Research - UFZ GmbH, Leipzig, Germany.



**Dr. Nico Jehmlich**

Department of Molecular  
Toxicology, Helmholtz-Centre for  
Environmental Research - UFZ  
GmbH, Leipzig, Germany.



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Impact Factor: 4.1 (2023); 5-Year Impact Factor: 4.5 (2023)

## Keynote Speakers



**Prof. Dr. Laurent Dufossé**

University of Réunion island,  
Reunion, France



**Prof. Dr. Thomas Brück**

Dept. of Chemistry, Technical  
University of Munich (TUM),  
Garching bei München,  
Germany



**Prof. Dr. Theodoros Varzakas**

Department of Food Science  
and Technology, University of  
Peloponnese, Kalamata, Greece



**Prof. Dr. Hesham Enshashi**

Institute of Bioproduct  
Development (IBD), Universiti  
Teknologi Malaysia (UTM), Johor  
Bahru, Malaysia



**Prof. Dr. Benno H. Ter Kuile**

Swammerdam Institute of Life  
Sciences, University of  
Amsterdam, Amsterdam,  
Netherlands



**Dr. Edward Fox**

Department of Applied  
Sciences, Northumbria  
University, Newcastle, UK



**Dr. Spiros Paramithiotis**

Department of Biological  
Applications and Technology,  
University of Ioannina, Ioannina,  
Greece



**Dr. Manita Guragain**

U.S. Department of Agriculture,  
Agricultural Research Service,  
Wyndmoor, USA.



**Prof. Dr. Mark Gomelsky**

Department of Molecular  
Biology, University of Wyoming,  
Laramie, USA.

## Invited Speakers



**Dr. Ramesh Chatragadda**

National Institute of  
Oceanography, Council of  
Scientific and Industrial  
Research (CSIR), Dona Paula,  
India



**Dr. Debjyoti Ghosh**

Helmholtz-Centre for  
Environmental Research - UFZ  
GmbH, Leipzig, Germany



**Dr. Ahmed Elbakush**

Department of Molecular  
Biology, University of Wyoming,  
Laramie, USA.

## Program at a Glance

|           | Day 1                                                       | Day 2                                                                                              | Day 3                                                 |
|-----------|-------------------------------------------------------------|----------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| Morning   | <b>Session 1.</b> Microbial Characterization and Bioprocess | <b>Session 3.</b> Antimicrobial Agents and Resistance                                              | <b>Session 5.</b> Foodborne Pathogens and Food Safety |
| Afternoon | <b>Session 2.</b> Gut Microbiota and Health Disease         | <b>Session 4.</b> Emerging Infectious Diseases and<br><b>Session 7.</b> Microbe-Plant Interactions | <b>Session 6.</b> Microbiome and Soil Science         |

# ECM 2025 Program

1st April 2025 (Tuesday)

## Session 1. Microbial Characterization and Bioprocess

**Time: 8:50 (CEST, Basel) | 02:50 (EDT, New York) | 14:50 (CST Asia, Beijing)**

| Time (CEST) | Speaker                                                                   | Title                                                                                                                                                                 |
|-------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 08:50-09:00 | Dr. Nico Jehmlich                                                         | Welcome and Opening Remarks<br>Event Chair                                                                                                                            |
| 09:00-09:10 | Prof. Dr. Laurent Dufossé<br>and Prof. Dr. Thomas Brück<br>Session Chairs | Welcome by Session Chairs                                                                                                                                             |
| 09:10-09:30 | Prof. Dr. Laurent Dufossé<br>Keynote Speaker                              | Biotechnological colorants from microorganisms:<br>worldwide overview of private companies and start-ups                                                              |
| 09:30-09:50 | Prof. Dr. Thomas Brück<br>Keynote Speaker                                 | Circularity in the food and cosmetic industry –<br>Biocatalytic routes for generation<br>of high value cosmetic and food ingredients from<br>industrial waste streams |
| 09:50-10:05 | Dr. Ramesh Chatragadda<br>Invited Speaker                                 | The Future of Bioentrepreneurship: Exploring the<br>Potential of Microbes from Coastal and Deep-Sea<br>Environments                                                   |
|             | Contributed Oral<br>Presentations                                         | Session 1. Microbial Characterization and Bioprocess                                                                                                                  |
| 10:05-10:20 | Alejandra de la Cruz<br>Munguía<br>Selected Speaker                       | The bacterial microbiota of knees affected by<br>Gonarthrosis                                                                                                         |
| 10:20-10:35 | Catarina Miranda<br>Selected Speaker                                      | Shifts in Aerobic Granular Sludge Bacteriome Driven by<br>Seawater Intrusion in Wastewater                                                                            |
| 10:35-10:50 | Péter Szabó<br>Selected Speaker                                           | Spectral Efficacy: The Role of UV-C Light Frequency in<br>Viral Inactivation and Disinfection                                                                         |
| 10:50-11:05 | Antonio Peña-Fernández<br>Selected Speaker                                | Assessing the potential association between pigeons<br>and the presence of Acanthamoeba spp. in the River<br>Soar and public parks in Leicester, England              |
| 11:05-11:20 | Lucia Fernandez Goya<br>Selected Speaker                                  | Interplay of symbiotic bacteria in Wolbachia density<br>modulation among Naupactini weevils (Coleoptera,<br>Curculionidae)                                            |
| 11:20-11:35 | Beatrice Farda<br>Selected Speaker                                        | Exploring Cellulose Fibers as Support for a Microbial<br>Inoculant Immobilization                                                                                     |
| 11:35-14:00 | Break                                                                     | Break                                                                                                                                                                 |

## Session 2. Gut Microbiota and Health Disease

**Time: 14:00 (CEST, Basel) | 08:00 (EDT, New York) | 20:00 (CST Asia, Beijing)**

| Time (CEST) | Speaker                                         | Title                                                                                                   |
|-------------|-------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| 14:00-14:10 | Prof. Dr. Theodoros Varzakas<br>Session Chair   | Welcome by Session Chair                                                                                |
| 14:10-14:30 | Prof. Dr. Theodoros Varzakas<br>Keynote Speaker | Foods and probiotic colonization                                                                        |
| 14:30-14:50 | Prof. Hesham Enshashi<br>Keynote Speaker        | Gut Microbiome: From Lab. To Market                                                                     |
|             | Invited and Contributed Oral<br>Presentations   | Session 2. Gut Microbiota and Health Disease                                                            |
| 14:50-15:05 | Joana Monteiro Marques<br>Selected Speaker      | Dissemination of Enterococcus spp. across One<br>Health Settings: Insights from fingerprinting analysis |
| 15:05-15:20 | Oumaima Anachad<br>Selected Speaker             | Evaluation of Molecular Hydrogen in the Treatment of<br>Intestinal Inflammation in a Murine Model       |
| 15:20-15:35 | Natalia G. Bednarska<br>Selected Speaker        | Microbial Signatures in High- and Low-Blood-Glucose<br>Participants of the Generation 100 Study         |



|             |                                               |                                                                                                                 |
|-------------|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| 15:35-15:50 | <b>Maria Getsina</b><br>Selected Speaker      | Decreased levels of aromatic microbial metabolites as a reflection of microbiota dysfunction in cancer patients |
| 15:50-16:05 | <b>Ekaterina Sorokina</b><br>Selected Speaker | Comparative analysis of gut microbiota metabolism in healthy and pathological conditions                        |

## 2nd April 2025 (Wednesday)

### Session 3. Antimicrobial Agents and Resistance

**Time: 9:00 (CEST, Basel) | 03:00 (EDT, New York) | 15:00 (CST Asia, Beijing)**

| Time (CEST) | Speaker                                           | Title                                                                                                                                                                                     |
|-------------|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 09:00-09:10 | Prof. Dr. David Rodríguez-Lázaro<br>Session Chair | Welcome by Session Chair                                                                                                                                                                  |
| 09:10-09:30 | Prof. Dr. Benno H. Ter Kuile<br>Keynote Speaker   | Development of antimicrobial resistance: Mechanisms, pathways and driving forces                                                                                                          |
|             | <b>Contributed Oral Presentations</b>             | Session 3. Antimicrobial Agents and Resistance                                                                                                                                            |
| 09:30-09:45 | Qinmao Zhou<br>Selected Speaker                   | Deciphering microbiome dynamics and antibiotic resistome profiles in anaerobic bioreactors treating swine wastewater containing different sulfonamides                                    |
| 09:45-10:00 | Francesca Coppola<br>Selected Speaker             | Antibiofilm activity of Rosaceae honeys against <i>Acinetobacter baumannii</i> and their prebiotic effect on <i>Lactiplantibacillus plantarum</i> and <i>Lactocaseibacillus rhamnosus</i> |
| 10:00-10:15 | Elkadaoui Soukaina<br>Selected Speaker            | Study of the antimicrobial activity of chitosan from insects against Gram-positive and Gram-negative bacteria                                                                             |
| 10:15-10:30 | Badr-Edine Sadoq<br>Selected Speaker              | Targeting the Resistant F533L Mutant in PBP3 of <i>Pseudomonas aeruginosa</i> : A Computational Approach Using ZnO and CuO Nanoparticles                                                  |
| 10:30-10:45 | Maciej Grzegorzczak<br>Selected Speaker           | <i>Staphylococcus aureus</i> biofilm gene analysis: risks for patients with chronic lymphocytic leukemia (CLL)                                                                            |
| 10:45-11:00 | Jin Ma<br>Selected Speaker                        | RecA Inhibitor Mitigates Bacterial Antibiotic Resistance                                                                                                                                  |
| 11:00-11:15 | Mahmoud Naser Aldine<br>Selected Speaker          | Botocidin: A novel bacteriocin produced by a nontoxic <i>C. botulinum</i> strain, HA5                                                                                                     |
| 11:15-14:00 | Break                                             | Break                                                                                                                                                                                     |

### Session 4. Emerging Infectious Diseases and Session 7. Microbe-Plant Interactions

**Time: 14:00 (CEST, Basel) | 08:00 (EDT, New York) | 20:00 (CST Asia, Beijing)**

| Time (CEST) | Speaker                                           | Title                                                                                                                                 |
|-------------|---------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| 14:00-14:10 | Prof. Dr. Mohamed Hijri<br>Session Chair          | Welcome by Session Chair                                                                                                              |
|             | <b>Invited and Contributed Oral Presentations</b> | Session 4. Emerging Infectious Diseases                                                                                               |
| 14:10-14:25 | Daniel Hanna<br>Selected Speaker                  | Rapid Benchtop Purification of SARS-CoV-2 for Immunological Studies: Minimizing Cytotoxic Effects on Monocytes                        |
| 14:25-14:40 | Gabriela Pereira Macelaro<br>Selected Speaker     | Impact of the COVID-19 Pandemic on Visceral Leishmaniasis Reporting in Brazil: A Retrospective Analysis of Epidemiological Trends     |
| 14:40-14:55 | Ikram Joubair<br>Selected Speaker                 | Design and Pilot Scale Production of an Alternative Non-Live Attenuated BTV Vaccine in Yeast                                          |
| 14:55-15:10 | Susanne Reichinnek<br>Selected Speaker            | ULTimate Y2H: A Powerful Platform to decipher Host-Pathogen Protein Interactions and Biological Pathways                              |
| 15:10-15:25 | Khadija Muhammad Kawu<br>Selected Speaker         | Detection and Antifungal Susceptibility of Fungal Pathogens Associated with Ootomycosis in a Tertiary Health Center in Kano, Nigeria. |
| 15:25-15:40 | Isabela Vilas Boas<br>Selected Speaker            | Gestational toxoplasmosis: An analysis of cases over the last 5 years in Brazil according to clinical evolution                       |
|             | <b>Invited and Contributed Oral Presentations</b> | Session 7. Microbe-Plant Interactions                                                                                                 |

|             |                                                |                                                                                                                                                                                      |
|-------------|------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 15:40-15:55 | <b>Dr. Ahmed Elbakush</b><br>Invited Speaker   | Maple Compounds Prevent Biofilm Formation in the Foodborne Pathogen, <i>Listeria monocytogenes</i>                                                                                   |
| 15:55-16:10 | <b>Mian Muhammad Ahmed</b><br>Selected Speaker | Harnessing Antioxidant-Producing Rhizobacteria to Boost Loquat Oxidative Stress Defense Under Adverse Environmental Conditions                                                       |
| 16:10-16:25 | <b>Clarisse Brígido</b><br>Selected Speaker    | Proteomic Insights into Bacterial Endophyte Responses to Chickpea Root Exudates Highlight the Role of GacS                                                                           |
| 16:25-16:40 | <b>Roukaya Ben Gaied</b><br>Selected Speaker   | Treasures of Tunisian Wild Legumes: Diversity of Root Nodule Endophytes                                                                                                              |
| 16:40-16:55 | <b>Steven Orito</b><br>Selected Speaker        | Impact of Co-Inoculating <i>Bradyrhizobium japonicum</i> with <i>Bacillus subtilis</i> or <i>Priestia megaterium</i> on Nitrate Inhibition of Symbiotic Nitrogen Fixation in Soybean |
| 16:55-17:10 | <b>Rajani Pijakala</b><br>Selected Speaker     | Biocontrol of plant pathogenic fungi by endophytic <i>Trichoderma harzianum</i> through production of volatile organic compounds                                                     |

### 3rd April 2025 (Thursday)

#### Session 5. Foodborne Pathogens and Food Safety

**Time: 9:00 (CEST, Basel) | 03:00 (EDT, New York) | 15:00 (CST Asia, Beijing)**

| Time (CEST) | Speaker                                     | Title                                                                                                                                                                                                                                    |
|-------------|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 09:00-09:10 | Prof. Dr. Ute Römling<br>Session Chair      | Welcome by Session Chair                                                                                                                                                                                                                 |
| 09:10-09:30 | Dr. Edward Fox<br>Keynote Speaker           | Aeromonas species - a potential emerging food safety concern?                                                                                                                                                                            |
| 09:30-09:50 | Dr. Spiros Paramithiotis<br>Keynote Speaker | The title would be 'Integration of next generation sequencing in food safety, are we there yet?'                                                                                                                                         |
| 09:50-10:10 | Dr. Manita Guragain<br>Keynote Speaker      | Non-diarrheagenic pathotypic E. coli in food                                                                                                                                                                                             |
| 10:10-10:30 | Prof. Dr. Mark Gomelsky<br>Keynote Speaker  | Antibiofilm compounds protecting fresh produce from Listeria                                                                                                                                                                             |
|             | <b>Contributed Oral Presentations</b>       | <b>Session 5. Foodborne Pathogens and Food Safety</b>                                                                                                                                                                                    |
| 10:30-10:45 | Joana Martins<br>Selected Speaker           | Natural Extracts as Antimicrobial Agents: A Comparative Study of <i>Castanea sativa</i> , <i>Hibiscus sabdariffa</i> L., and <i>Punica granatum</i> L.                                                                                   |
| 10:45-11:00 | Boluwatife Esther Ajayi<br>Selected Speaker | Evaluation of Lactose-Sulfite-Phenol Red-Rifampicin (LSPR) Agar for the Co-Enumeration of <i>Salmonella enterica</i> and Shiga-toxigenic <i>Escherichia coli</i> in sausage batter                                                       |
| 11:00-11:15 | Joana Paiva<br>Selected Speaker             | Microbiological assessment and acceptability of minced meat from hypermarkets: pathogenic and spoilage microorganisms                                                                                                                    |
| 11:15-11:30 | Antonio Peña-Fernández<br>Selected Speaker  | Strategies for the decontamination of <i>Acanthamoeba</i> spp. and <i>Naegleria fowleri</i> in different water systems in Bombali and Tonkolili districts (Sierra Leone): lessons from the UK Recovery Handbook for Biological Incidents |
| 11:30-11:45 | Raúl Emilio Iza<br>Selected Speaker         | <i>Listeria monocytogenes</i> isolated from fresh pork meat commercialised in La Plata, Buenos Aires, Argentina                                                                                                                          |
| 11:45-14:00 | Break                                       | Break                                                                                                                                                                                                                                    |

#### Session 6. Microbiome and Soil Science

**Time: 14:00 (CEST, Basel) | 08:00 (EDT, New York) | 20:00 (CST Asia, Beijing)**

| Time (CEST) | Speaker                                           | Title                                                                                                                                        |
|-------------|---------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| 14:00-14:10 | Dr. Nico Jehmlich<br>Session Chair                | Welcome by Session Chair                                                                                                                     |
| 14:10-14:25 | Dr. Debjyoti Ghosh<br>Invited Speaker             | Cover crop monocultures and mixtures enhance bacterial abundance and functionality in the maize root zone                                    |
|             | <b>Invited and Contributed Oral Presentations</b> | <b>Session 6. Microbiome and Soil Science</b>                                                                                                |
| 14:25-14:40 | Laura Sala-Comorera<br>Selected Speaker           | Phage-based biocontrol of nitrification in agricultural soil                                                                                 |
| 14:40-14:55 | Éva Abod<br>Selected Speaker                      | Analysis of Soil Biological Activity in the Surroundings of Sfântu Gheorghe in Covasna                                                       |
| 14:55-15:10 | Romain Kouakou Fossou<br>Selected Speaker         | Comparative evaluation of 16S rRNA and housekeeping gene-specific primer pairs for rhizobia and agrobacteria community metagenomics          |
| 15:10-15:25 | Maria Andrea Reyes Reyes<br>Selected Speaker      | The microbial and physicochemical interactions of soils in the degradation of buried polyethylene pipes in Colombia                          |
| 15:25-15:40 | Savanah Senn<br>Selected Speaker                  | Preliminary data on biodiversity effects of replacing plastic row covers with legume and non-leguminous covers in organic artichoke cropping |

## **Session 1. Microbial Characterization and Bioprocess**

# sciforum-110886: Evaluation of in vivo and in vitro pathogenicity of selected *Staphylococcus aureus* strains isolated from humans and animals.

Filip Jacek Kot \*, Marta Książczyk and Gabriela Bugla-Płoskońska

Department of Microbiology, Faculty of Biological Sciences, University of Wrocław, Poland

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*Staphylococcus aureus* is a major pathogen responsible for numerous human and animal diseases, including Staphylococcal scalded skin syndrome, folliculitis, and mastitis. Known for its virulence factors, it can evade host immune responses and enhance colonization. Recent research has focused on alternative in vivo models, such as the *Galleria mellonella* larval model, which offers a cost-effective, less harmful, and accessible approach to studying pathogens and testing therapies. This model is compatible with the 3R principle (replacement, reduction, and refinement).

In this study, the virulence of 35 *S. aureus* strains isolated from humans, dogs, and cats was assessed both in vivo and in vitro. The bactericidal effect of a commercial human serum reagent on these strains was evaluated, and selected isolates were tested in the *G. mellonella* model. Biofilm formation was also analyzed using the crystal violet assay. Most strains were resistant to the human serum reagent, with only three being susceptible. In the larval model, varying levels of virulence were observed. Biofilm formation was detected in only five out of thirty-five strains.

No significant differences in serum resistance were observed between strains from different hosts. The Gram-positive structure of *S. aureus* likely explains this. Strains exhibiting the MRS (Methicillin-resistant *Staphylococcus*) phenotype caused higher mortality in the larvae, and those capable of forming biofilms displayed both low and high virulence levels in vivo on the larval model.

This study was partially financed by an internal grant of the University of Wrocław IDUB titled "*Galleria mellonella* - the use of larvae in the optimization and improvement of the *in vivo* insect model for bacterial pathogenicity studies.", No. BPIDUB.7.2024

The tested strains were graciously provided by the Wrocław University of Environmental and Life Sciences, Department of Epizootiology and Clinic of Birds and Exotic Animals.



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# sciforum-114299: Meat Processing Wastewater Valorised through Microalgae Production

Ana S.S. Sousa, Ana T. Oliveira, Paula M.L. Castro and Catarina L. Amorim \*

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal

---

The treatment of meat processing wastewater (MPWW) is crucial to mitigating its environmental impact but remains challenging due to its high organic content and contents of nitrogen and solids.

The novelty of this work lies in evaluating the treatment and valorization of real MPWW with variable composition, as well as the process scalability, in a fed-batch raceway reactor while exploring the synergistic microbial interactions within the system. A microalgae-based consortium with proven bioremediation capacity was used as biomass. In batch assays, the microalgae-based biomass thrived in raw MPWW, demonstrating synergistic interactions with the native wastewater microbiome that facilitated organic carbon and total nitrogen removal. In sieved and sedimented MPWW, the COD removal rates were higher ( $1097.5 \pm 22.2 \text{ mg L}^{-1} \text{ day}^{-1}$ ) than in raw MPWW ( $828.5 \pm 60.5 \text{ mg L}^{-1} \text{ day}^{-1}$ ), and the microalgae growth rate also improved. In a laboratory-scale raceway reactor, in fed-batch mode, COD removal rates ranging from  $181.3$  to  $806.3 \text{ mg L}^{-1} \text{ day}^{-1}$  were achieved, despite variable wastewater composition. The nitrogen mass balance indicated that ammonium, nitrate, and nitrite were consumed. The increase in photosynthetic pigments' concentration throughout operation, from  $0.4$  to  $17.9 \text{ } \mu\text{g mL}^{-1}$ , demonstrated robust microalgae growth under variable wastewater compositions. The biomass microbiome, initially dominated by microalgae from the *Chlorophyta* and *Ciliophora* phyla, evolved to include other eukaryotic taxa (including yeast and fungi) and likely bacteria. The microbiome's adaptability and its microbial diversity appeared to be key drivers in enhancing biological removal processes.

This work advances microalgae-based processes for treating and valorising MPWW by highlighting microbial ecology's role in system resilience and efficiency and giving insights in process scalability, showcasing its robustness for real-world applications.

**Acknowledgments:** This work was supported by National Funds from FCT—Fundação para a Ciência e a Tecnologia—through the project MobFood (POCI-01-0247-FEDER-024524/SI-47-2016-10). The authors thank the CBQF scientific collaboration under FCT project UIDB/50016/2020.



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# sciforum-111005: An innovative electrochemical nanogenosensor for the detection of *Candida* species

**Michelle Shereen Alves Castanheira**<sup>1,2,3\*</sup>, **Stephanie Morais**<sup>1</sup>, **Luís Lima**<sup>3</sup>, **Isabel Seguro**<sup>1</sup>, **Marlene Santos**<sup>2</sup>, **João Pacheco**<sup>1</sup> and **M. Fátima Barroso**<sup>1</sup>

<sup>1</sup> REQUIMTE/LAQV-Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Portugal

<sup>2</sup> REQUIMTE/LAQV, Escola Superior de Saúde, Instituto Politécnico do Porto, Portugal

<sup>3</sup> Centro de Investigação, Instituto Português de Oncologia do Porto Francisco Gentil, E.P.E., Portugal

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Despite tremendous progress in the prevention and treatment of fungal infections, invasive fungi like *Candida* species remain the major cause of morbidity and mortality worldwide [1]. In fact, the Global Action Fund for Fungal Infections estimates that each year, almost 300 million individuals contract a fungal infection, and over 1.5 million of them died as a result [1,2]. *Candida* can enter the bloodstream and spread to internal organs, but it can also commonly cause surface infections, like those in the skin or mucous membranes, which can be readily and effectively treated [3]. This fungal infection has been observed in high-risk patients, such as those receiving allogeneic stem-cell transplants and those receiving high-dose chemotherapy for acute leukemia [4]. These patients are more vulnerable to infections since their immune systems are weakened during the transplant process. Systemic fungal infections are still difficult to diagnose. Therefore, developing early diagnosis methods that are more precise, sensitive, and effective is required. A quick, easy, and accurate method for identifying fungal infections in patients following hematopoietic stem cell transplantation (HSCT) was devised in this study. To overcome this problem, an electrochemical nanogenosensor for the detection of *Candida albicans* was developed. This nanogenosensor was assembled in an innovative low-cost electrochemical paper-based analytical device (ePAD). The electrochemical signal's sensitivity was increased through the application of a sandwich hybridization procedure. Preliminary findings indicate that these nanogenosensors can be used to identify *Candida* spp. in a synthetic fungus sample. Despite these findings, work is being performed to enhance the nanogenosensor for the quantification of *Candida albicans*, and this procedure will be verified through more study. The application in a hospital context will be covered in future studies in terms of sensitivity, accuracy, response time, issues, and potential.



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## sciforum-110070: Antifungal activity of some rhizospheric actinomycetes

**Ratchanon Thongpheap\*, Prapanrat Manota, Anillada Nettakul and Ekachai Chukeatirote**

School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

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Rhizosphere soil is one of the unique microbial habitats, especially the actinobacteria. This study was carried out to screen and isolate actinomycetes exhibiting antifungal activity. Initially, the soil samples collected from *Allium ascalonicum* L., *Alpinia galangal*, *Amorphophallus* sp., *Clausena harmandiana*, and *Cymbopogon citratus* were pretreated by air-drying and subsequent heat incubation. By using three different media (international Streptomyces project 1 and 2 (ISP-1 and ISP-2) and starch casein agar (SCA)), 79 isolates were obtained and identified as actinomycetes by morphology. Ten actinomycete isolates were then randomly selected to assess their antifungal activity against five pathogenic fungi: *Aspergillus* sp., *Colletotrichum* sp., *C. acutatum*, *C. gloeosporioides*, and *Lasiodiplodia theobromae*. Initial screening showed that there were three most active strains, namely CN1, CN4, and LG5, exhibiting a strong and broad spectrum. The bacterial culture supernatants were then prepared and evaluated for their stability under various pH and temperature conditions. The data obtained showed that the antifungal activity of the three culture supernatants was varied depending on the bacterial source and testing fungal strains. The present work implies that rhizospheric soil samples are an attractive source for the discovery of numerous actinomycetes with activity against phytopathogenic fungi. Identification of the bacterial species and determination of the active compounds are essential for further analysis for use in fungal control.



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# sciforum-113579: Assessing the potential association between pigeons and the presence of *Acanthamoeba* spp. in the River Soar and public parks in Leicester, England

Umar Anjum and Antonio Peña-Fernández \*

<sup>1</sup> Leicester School of Allied Health Sciences, De Montfort University, Leicester, LE1 9BH, UK.

<sup>2</sup> Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Ctra. Madrid-Barcelona, Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain.

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*Acanthamoeba* spp. are free-living amoebae that can cause life-threatening brain infections and can also infect the eyes and skin. Amoebas/cysts of this species have previously been isolated from the River Soar, which runs through the city of Leicester, England, and is frequently used by the public. It has also been found in grass samples collected from public parks and green spaces across the city. *Acanthamoeba* can host endosymbionts, which can include important human microbes, acting as Trojan horses. Although their zoonotic role is poorly understood, cysts of *Acanthamoeba* spp. and other free-living amoebae have been found in the faeces and/or the gastrointestinal tract of various animals, including domestic livestock (pigs, cows) and wildlife (squirrels, pigeons), suggesting a potential source of transmission. To investigate their zoonotic potential in pigeons, we analysed fresh droppings from 628 pigeons collected from various urban parks and green spaces in the city of Leicester and surrounding rural areas as follows: 136 samples in summer 2017, 140 in autumn, 124 in winter 2017/18, 135 in spring, and 94 in summer 2018. After appropriate pre-concentration, thin smears were stained with trichrome and examined by light microscopy. DNA was extracted using a Fast DNA® Spin Kit. PCR inhibitors were removed using the QIAamp® micro DNA extraction kit. The extracts were screened for *Acanthamoeba* spp. using a triplex real-time TaqMan PCR assay with an appropriate positive control. All samples tested microscopically and molecularly were negative. Although their zoonotic role is not well understood, a potential and unknown animal reservoir could explain the moderate to high distribution found in both the grass samples and the river, posing a potential risk to people involved in sports such as rowing, football, or rugby. Culture techniques should be used to better investigate the potential interaction of *Acanthamoeba* with urban pigeons.



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# sciforum-110656: Characteristics of Biofilms Formed by *Bacillus subtilis* subsp. *spizizenii* Growing in a Simple Culture Medium with two Different Carbon Sources

Gabriela Cristina Sarti<sup>1,2\*</sup>, Josefina Ana Eva Cristóbal-Miguez<sup>3</sup>, Antonio Paz-González<sup>2</sup>, Ivanna Lorena Avram<sup>3</sup>, Elizabeth García-Guzmán<sup>3</sup>, Andrea Belén Alegre<sup>3</sup>, Ana Rosa García<sup>3</sup> and Mirta Esther Galelli<sup>4</sup>

<sup>1</sup> Inorganic and Analytical Chemistry Cathedra, Department of Natural Resources and Environment, Faculty of Agronomy, University of Buenos Aires

<sup>2</sup> AQUATERRA Research Group, Interdisciplinary Center for Chemistry and Biology, CICA, As Carballeiras, s/n Campus de Elviña, University of A Coruña, 15008 Coruña, Spain

<sup>3</sup> Inorganic and Analytical Chemistry Cathedra, Department of Natural Resources and Environment, Faculty of Agronomy, University of Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina

<sup>4</sup> Agrofood Area, Department of Applied Biology and Food, Faculty of Agronomy, University of Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina

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Biofilms represent a key survival strategy, providing bacteria with effective protection against abiotic and biotic stress. In the laboratory, *Bacillus subtilis* subsp. *spizizenii* forms a biofilm at the liquid–air interface, the matrix of which is primarily composed of polysaccharides, proteins, and nucleic acids. The aims of this study are as follows: 1) to evaluate how the chemical composition of the matrix and the stability of the biofilm are modified when the bacteria grow using either glucose or glycerol as carbon sources; 2) to study the effect of temperature on biofilm production and planktonic growth. The bacteria were cultured in a saline medium with 55 mM L-glutamic acid and 1% glucose or glycerol at different temperatures. Biofilm was produced under static conditions and planktonic cells by shaking at 150 rpm. Biofilms were analyzed by high-resolution chromatography. In the presence of glucose, polysaccharide monomers at the biofilm matrix showed substitutions that conferred acidic properties. This biofilm disintegrated after 5 days at 20 °C and 15 days at 4 °C. In contrast, when cultured with glycerol, the polysaccharide sugars were neutral, and the biofilm disintegrated after 20 days at 20 °C and 60 days at 4 °C; visually, this biofilm was more compact. Planktonic growth of *B. subtilis* was highest at 45 °C, whereas the maximum biofilm formation (1.2 mg/mL of culture) was achieved between 30 °C and 37 °C. In the static cultures, the planktonic form of *B. subtilis* disappeared within 24 hours after biofilm synthesis. In conclusion, the biofilms obtained from different carbon sources show differences, suggesting that the substituents of the polysaccharide monomers might be crucial for their structural properties. Moreover, in cases where the biofilm was formed at the liquid–air interface, the planktonic cells developed in this culture migrated toward the biofilm and as a result, the medium appeared clear; this was likely due to nutrient depletion, which does not support planktonic growth.



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# sciforum-115135: Development of new tools for sustainable management olive trees crop

**Clara Izquierdo Jiménez<sup>1,\*</sup>, Cecilia Recuero García<sup>1</sup>, Sergi Maicas Prieto<sup>2</sup> and Inmaculada del Castillo Madrigal<sup>1</sup>**

<sup>1</sup> R&B Department; Microbiology Area; SEIPASA. L'Alcúdia (Valencia). 46250. Spain

<sup>2</sup> Department of Microbiology and Ecology; Faculty of Biological Sciences, Universitat de València

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## 1. Introduction

Climate change significantly impacts agrarian systems, compromising the quality of agricultural production. This situation has led farmers to demand sustainable tools to enhance crop performance while maintaining soil fertility and product quality. Microorganisms capable of interact with plants represent an opportunity for cultivating healthy crops through growth promotion and biostimulation. Numerous studies point to native microbiomes in agricultural soils as a primary source of well-adapted microorganisms to develop new tools for crop treatment. This study was focused on the olive tree, a high-value Mediterranean crop. Microbial diversity was analyzed at two different phenological stages of the crop and both, the growth promotion and inhibitory capability against phytopathogens of isolated microorganisms were also evaluated.

## 2. Material and methods

A metagenomic analysis was performed using high-throughput sequencing. Additionally, culturable microorganisms were selected based on morphological criteria. Diversity data, combining molecular analysis and variability on isolated microorganisms, were studied to determine the effect of the crop stage on the soil microbiome structure.

The isolated strains were characterized for their plant growth-promoting properties (PGPR), such as nitrogen fixation, phosphate solubilization, siderophores, and phytohormones production, to identify and select candidates to be used for the development of biotechnological tools.

## 3. Results

The number of morphotypes isolated during the fruit development stage was twice that obtained during the flowering stage. This increment is associated with greater root activity and the presence of exudates richer in organic compounds. Furthermore, microorganisms with remarkable PGPR characteristics were found, granting their strong potential as biocontrol and biofertilization agents.

## 4. Conclusions

This study demonstrates that soil is a valuable reservoir of microorganisms, that can be used to create bio-tools for sustainable agriculture.



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# sciforum-111350: Evaluation of antioxidant stability of jinicuil (*Inga paterno*) probiotic beverages fermented by *Lactobocillus delbrueckii* ssp *bulgaricus* and *Streptococcus thermophilus* during refrigeration storage

Lizbeth Rosas-Ordoñez<sup>1,\*</sup>, Oscar Jiménez-González<sup>1</sup> and Taisa S.S. Pereira<sup>2</sup>

<sup>1</sup> Department of Chemical, Food and Environmental Engineering, Universidad de las Américas Puebla, Puebla, 72810, Mexico

<sup>2</sup> Department of Health Sciences, Universidad de las Américas Puebla, Puebla, 72810, Mexico

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*Inga paterno* produces seeds (Se) covered by a sarcotesta (Sa), both of which remain understudied. Meanwhile, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (L-S) are commonly used in fermented products. This study investigated the potential of Se and Sa as substrates for probiotic fermentation using L-S.

Three formulations were prepared: 5%(w/v) Sa, 5%(w/v) Se, and a 1:1 mixture (Ae). These were sterilized, inoculated with L-S ( $7.67 \times 10^6$  CFU/ml), fermented (72 hours at 35°C), and stored at refrigeration temperature (5°C). Antioxidant activity (AA), total phenolic content (TPC), condensed tannins (CT), flavonoids (FC), and phytic acid content (PAC) were measured at 0, 4, 11, and 25 days of storage. One-way ANOVA and Tukey's test ( $p < 0.05$ ) were used to analyze the data.

L-S counts ranged from 6.69 to 9.41 log CFU/ml, with higher stability in Se (8.39 to 7.62 log CFU/ml) and Sa (8.44 to 8.01 log CFU/ml). AA ranged from 27.50 to 115.92 µg Trolox equivalents/ml, with Sa showing the highest and stable levels (84.07 to 113.46 log CFU/ml). PAC increased in Sa (from 34.22 to 42.54 µg of phytic acid /ml) but decreased in Se (from 40.13 to 37.73 µg of phytic acid /ml). CT decreased in all formulations (ranging from 1.53 to 0.70 mg of catechin equivalents/ml), and Sa presented a higher amount of FC (from 0.53 to 1.04 mg of quercetin equivalents/ml) than Se (from 0.03 to 0.08 mg of quercetin equivalents/ml) and Ea (from 0.09 to 0.21 mg of quercetin equivalents/ml). AA was mainly correlated with FC ( $R^2 = 0.6$ ). L-S remained viable throughout storage in all formulations, while Sa provided a rich source of TPC and FC. These findings suggest that *I. paterno* sarcotesta can serve as promising substrates to produce a functional fermented beverage with enhanced antioxidant properties.



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# sciforum-105068: Evaluation of the sensitivity of sulfate-reducing bacteria and indicators of the microbiologically influenced corrosion of steel under the influence of dimethyl sulfoxide

Nataliia Tkachuk<sup>1,\*</sup>, Liubov Zelena<sup>2</sup> and Yaroslav Novikov<sup>3</sup>

<sup>1</sup> T.H. Shevchenko National University "Chernihiv Colehium"

<sup>2</sup> Department of Virus Reproduction, Danylo Zabolotny Institute Microbiology and Virology NAS of Ukraine, Kyiv, Ukraine

<sup>3</sup> Department of Biology, T.H. Shevchenko National University "Chernihiv Colehium"

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Anaerobic sulfate-reducing bacteria (SRB) play an important role in the process of microbiologically influenced corrosion (MIC). In the study of corrosion processes without bacteria with the participation of DMSO, an increase in the rate of corrosion of metals was shown. However, the bactericidal properties of DMSO against SRB, the indicators of MIC of steel in the presence of SRB and DMSO, have not been investigated; thus, this forms the aim of this study. The sensitivity of SRB *Desulfovibrio oryzae* strain NUChC SRB1 (accession number in GenBank is MT102713.1) to DMSO at concentrations of 1%-100% (v/v) was investigated by the method of dilution in liquid Postgate's "C" medium. The corrosion activity of *D. oryzae* strain NUChC SRB1 against steel 3 with the addition DMSO (final concentration of 45%) was investigated by the biofilm formation ability based on biofilm biomass on the surface of steel samples (crystal violet method) and the effect on corrosion rate (gravimetric method). SRB and DMSO were not added in the control. Corrosion research took place for 35 days in Eppendorf-type tubes (50 mL) under anaerobic conditions and at a temperature of  $29 \pm 2$  °C. Statistical analysis was carried out. It was found that DMSO with a concentration of 10% to 100% exhibits antibacterial properties against the studied SRB. Adding DMSO to the medium with bacteria ensured a significant reduction in biofilm biomass. Therefore, DMSO, despite its bactericidal properties and ability to inhibit biofilm formation, does not demonstrate inhibiting activity against microbiologically influenced corrosion in the presence of SRB.



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# sciforum-114091: Exploring Cellulose Fibers as Support for a Microbial Inoculant Immobilization

**Beatrice Farda<sup>1,\*</sup>, Amedeo Mignini<sup>2</sup>, Fabrizia Pasquarelli<sup>3</sup>, Luz de-Bashan<sup>4</sup>, Iris Beatriz Vega Erramuspe<sup>5</sup>, Rihab Djebaili<sup>1</sup> and Marika Pellegrini<sup>1,2</sup>**

<sup>1</sup> Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, 67100, Italy

<sup>2</sup> Agroqualis Srl, Academic Spin off of University of L'Aquila, L'Aquila, Italy

<sup>3</sup> SPAA s.r.l., Via delle Industrie 11-13, Città Sant'Angelo, 65013 PE, Italy

<sup>4</sup> The Bashan Institute of Science, 1730 Post Oak Court, Auburn, 36830 AL, USA

<sup>5</sup> Department of Biosystems Engineering, College of Agriculture, Auburn University, Auburn (AL), USA

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Creating robust and long-lasting storage solutions presents a major challenge in developing microbial inoculants for eco-friendly farming. Well-developed techniques for anchoring bacteria onto solid supports are available, and growing interest is focusing on the implementation of sustainable carrier materials. This work aims to examine the utilization of cellulose functional fibers, chemically altered by the integration of natural polymers derived from the pulp industry. The fibers were assessed as a carrier for a consortium of plant growth-promoting bacteria. The consortium was immobilized on fibers culturing the strains in a combined fermentation with the addition of fibers (1% w/v) to allow for microbial self-adhesion to the surface. The fibers were added at three specific stages of the bioreaction (0, 24 hours, and 48 hours), utilizing two separate culture mediums. Desiccation was executed using freeze-drying and heat-drying techniques. Cell viability was assessed in never-dried functional fibers and dried fibers until one month from inoculation, after drying. Immobilized bacteria were also assessed for plant growth-promoting (PGP) traits, encompassing indoles, ammonia production, and phosphate solubilization. Never-dried fibers demonstrated a favorable microbial load (between 6.78 and 8.22 Log CFU g<sup>-1</sup>), although the cell viability after drying diminished more than 1 Log CFU g<sup>-1</sup> relative to the never-dried matter. Notable findings were achieved when comparing the two distinct growth conditions and the timing of functional fiber inoculation. Post-drying disparities between unmodified fibers and functional fibers were observed, underscoring the possible role of natural polymers in enhancing cellular protection. Plant growth-promoting testing exhibited advantageous results relative to the non-immobilized consortia. These results establish a foundation for additional research, concentrating on shelf-life assessments and suitable applications in plant and greenhouse investigations.



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## sciforum-115158: Identification of a strain of the genus *Janibacter* isolated in a pharmaceutical industry using molecular and proteomic techniques.

**Andressa Sbano S. Ferreira \***, Maria Luiza Souza Galvão Dacio, Maria Giovanna de Araújo Ferreira, Pamella de Sousa Silva, Talita Bernardo Valadão, Rebeca Vitória da Silva Lage de Miranda, Joyce Modesto de Andrade, Marcelo Luiz Lima Brandão and Luciana Veloso da Costa

DEQUA, Bio-Manguinhos, Rio de Janeiro, 21040-900, Brazil

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The production of medicines and vaccines must comply with strict Good Manufacturing Practice standards to ensure the safety of the manufactured products. Consequently, environmental monitoring of pharmaceutical production areas is essential to ensure compliance with recommended limits for microbiological contamination, and identifying microorganisms isolated from these areas supports preventive and corrective actions. This study aimed to identify at the species level strain B1373/24, which was isolated during air monitoring in a viral vaccine production area of a pharmaceutical industry in Rio de Janeiro. The strain was initially analyzed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the MALDI Biotyper® (Bruker); however, it could not be identified. Therefore, the 16S rRNA gene of B1373/24 was sequenced, and the resulting data were compared with the EzBioCloud database, using the  $\geq 98.7\%$  threshold for species-level identification. The following species were proposed: *Janibacter anophelis* (100.0%), *Janibacter hoylei* (98.82%), and *Janibacter cremeus* (98.75%). The species was confirmed as *Janibacter anophelis* through phylogenetic analysis using the following software: MegaX, jModelTest, Mafft-online, and FigTree. This confirmation allowed the spectrum of strain B1373/24 to be incorporated into the MALDI Biotyper® system, contributing to the expansion of its database (DB). A subsequent reanalysis of B1373/24 using MALDI-TOF MS successfully identified it as *Janibacter anophelis*, confirming the validity of the newly added spectrum within the system's DB. The identification of B1373/24 as *Janibacter anophelis* highlights the importance of combining molecular tools to expand the MALDI-TOF MS database, thereby improving its capacity to identify environmental microorganisms. This study underscores its significant technological and scientific value for the pharmaceutical industry.



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# sciforum-111364: Interplay of symbiotic bacteria in *Wolbachia* density modulation among Naupactini weevils (Coleoptera, Curculionidae)

Lucia Fernandez Goya <sup>1,\*</sup>, Alejandra Carla Scannapieco <sup>2</sup>, Panagiota Stathopoulou <sup>3</sup>, Naima Bel Mokhtar <sup>3</sup>, Silvia Beatriz Lanzavecchia <sup>2</sup>, Claudia Alejandra Conte <sup>2</sup>, Diego Fernando Segura <sup>2</sup>, George Tsiamis <sup>3</sup> and Marcela Silvina Rodriguez <sup>1, 4</sup>

<sup>1</sup> Instituto de Ecología, Genética y Evolución de Buenos Aires (CONICET-UBA), Argentina.

<sup>2</sup> Instituto de Genética "E. A. Favret", Instituto Nacional de Tecnología Agropecuaria (INTA) – Grupo vinculado al Instituto de Agrobiotecnología y Biología Molecular (IABIMO) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Hurlingham

<sup>3</sup> Laboratory of Systems Microbiology and Applied Genomics, Department of Sustainable Agriculture, University of Patras, Agrinio, Greece.

<sup>4</sup> Departamento de Ecología, Genética y Evolución de la Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

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*Wolbachia pipientis* is the most widespread endosymbiotic bacterium, known for its ability to alter host reproduction. In Naupactini weevils, their reproductive mode appears to be linked to *Wolbachia* density, whereby species with low levels or an absence of *Wolbachia* reproduce sexually, while high-density infections would promote parthenogenesis. Although the factors driving high *Wolbachia* loads remain unclear, evidence suggests that the diversity of the host's microbiota may influence *Wolbachia* abundance. This study explored the bacterial microbiota of Naupactini species with contrasting reproductive modes and *Wolbachia* densities using targeted metagenomics. The species analysed included *Naupactus dissimulator* and *Naupactus xanthographus* (sexually reproducing), as well as *Naupactus versatilis* and *Pantomorus postfasciatus* (parthenogenetic), all of which were infected with the dominant strain within the tribe, i.e., wNau1. Notably, *P. postfasciatus* comprises both parthenogenetic (PpP) and sexually reproducing populations (PpS), providing unique insights into factors influencing *Wolbachia* colonization.

A total of 1,630,618 high-quality reads yielded 923 operational taxonomic units (OTUs) across 106 bacterial genera. The four most abundant genera, *Wolbachia* (26.47%), *Spiroplasma* (20.35%), *Enterobacter* (13.88%), and *Rickettsia* (11.49%), accounted for 72% of all reads. Parthenogenetic populations showed a marked predominance of *Wolbachia* and *Rickettsia*, whereas *Spiroplasma* and *Enterobacter* were the most prevalent symbionts in sexual populations, which also exhibited a richer microbiota in terms of the genera identified. Principal Coordinate Analysis based on Bray–Curtis distances clearly distinguished populations by reproductive mode. PpS' bacterial microbiota differed significantly from both PpP and other sexually reproducing species, exhibiting a profile that combined features of both groups. The low relative abundance of *Wolbachia*, coupled with the increased levels of *Enterobacter* and *Spiroplasma*, distinguished it from parthenogenetic populations, while elevated *Rickettsia* levels set it apart from other sexually reproducing species. These observations suggest that *Enterobacter* and *Spiroplasma* may inhibit *Wolbachia* proliferation, whereas *Rickettsia* might promote its growth.



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# sciforum-115174: Photobioreactors for Atmospheric Carbon Dioxide Fixation (Liquid trees): A Conceptual Approach Using *Chlorella vulgaris* Cultivation

Henry Adolfo Lambis Miranda <sup>1,\*</sup>, Juliana Puello-Mendez <sup>2</sup>, Ildefonso Baldiris-Navarro <sup>3</sup>, Jorgelina C. Pasqualino <sup>4</sup>, Miguel Cuesta-Peña <sup>5</sup> and Shirley Osorio-Herrera <sup>6</sup>

<sup>1</sup> Research Professor, Processes and Systems Engineering. Fundación Universitaria Tecnológico Comfenalco. CIPTEC Research Group.

<sup>2</sup> GICI Research Group, Chemical Engineering Department, Universidad de San Buenaventura Cartagena, Diag. 32 # 30-966, Cartagena, Colombia. ORCID: 0000-0003-0185-1053

<sup>3</sup> Universidad de Cartagena, Departamento de Ing. Química. Campus Piedra de Bolívar, Cartagena. ibaldiris@gmail.com ORCID: 0000-0001-9189-3710

<sup>4</sup> GISAH Research Group, Environmental Engineering Program, Universidad Tecnológica de Bolívar, Campus Tecnológico, km 1 vía Turbaco Cartagena, Cartagena, Colombia. ORCID: 0000-0002-8731-762X. jpasqualino@utb.edu.co

<sup>5</sup> Processes and Systems program, Fundación Universitaria Tecnológico Comfenalco, Cartagena, Colombia. mcuesta@tecnologicocomfenalco.edu.co; sosorih@tecnocomfenalco.edu.co

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Liquid trees are photobioreactors cultivating microalgae such as *Chlorella vulgaris* and represent a promising innovation in the fight against environmental pollution. These systems leverage the photosynthetic capacity of microalgae to capture atmospheric carbon dioxide (CO<sub>2</sub>), thereby contributing to the mitigation of climate change effects. Furthermore, the produced algal biomass can be utilized for diverse applications, ranging from biofuel production to animal feed.

Construction Methodology:

**Reactor:** The core of the system is a reactor, typically a transparent glass or plastic vessel, which provides a controlled environment for microalgae cultivation, including adequate illumination, mixing, and aeration of the culture.

**Light Source:** A solar panel serves as the primary energy source for the system. During daylight hours, the solar panel provides the necessary energy for microalgae photosynthesis. Additionally, a battery can store excess energy generated during the day to power the system at night.

**Aeration System:** A compressor, powered by the solar panel, supplies CO<sub>2</sub>-enriched air to the reactor. CO<sub>2</sub> is essential for the photosynthetic process and enables efficient microalgae growth.

**Control System:** A control system, which can be electronic or mechanical, monitors and regulates culture parameters, such as temperature, pH, nutrient concentration, and light intensity.

**Recirculation Pump:** A recirculation pump ensures a homogeneous distribution of nutrients and oxygen within the culture.

Expected Outcomes:

**Fix Large Amounts of CO<sub>2</sub>:** Microalgae, due to their high growth rate and photosynthetic efficiency, can capture significant amounts of atmospheric CO<sub>2</sub>.

**Produce Algal Biomass:** The produced algal biomass can be used for various applications, such as the production of biofuels, animal feed, fertilizers, and chemical products.

**Improve Air Quality:** By removing CO<sub>2</sub> from the atmosphere, liquid trees improve air quality.

**Be Energy Self-Sufficient:** Through the use of solar panels, liquid trees can operate autonomously, without requiring connection to the electricity grid.



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# sciforum-115154: Preliminary genome assembly of the *Wolbachia* endosymbiont of the South American fruit fly *Anastrepha fraterculus* sp. 1.

Claudia Alejandra Conte<sup>1\*</sup>, Máximo Rivarola<sup>2</sup>, Sergio Gonzalez<sup>2</sup>, María Romina Russo<sup>1</sup>, Alejandra Scannapieco<sup>1</sup>, Juan Pedro Wulff<sup>3</sup>, Alfred M. Handler<sup>4</sup>, Ioannis Ragoussis<sup>5</sup>, Konstantinos Bourtzis<sup>6</sup> and Silvia Beatriz Lanzavecchia<sup>1</sup>

<sup>1</sup> Instituto de Genética "E. A. Favret", Instituto Nacional de Tecnología Agropecuaria (INTA) – Grupo vinculado al Instituto de Agrobiotecnología y Biología Molecular (IABIMO), Hurlingham, Buenos Aires, Argentina.

<sup>2</sup> Instituto de Biotecnología IABIMO-CONICET, Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Buenos Aires, Argentina.

<sup>3</sup> North Carolina State University, NCSU, USA.

<sup>4</sup> U.S. Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608, USA.

<sup>5</sup> McGill Centre, Montreal, Canada.

<sup>6</sup> Insect Pest Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Seibersdorf, Austria.

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*Wolbachia* sp., an alphaproteobacterium associated with reproductive phenotypes, has previously been characterized in the fruit fly pest *Anastrepha fraterculus* (Diptera: Tephritidae). Two *Wolbachia* strains (wAfraCast1\_A and wAfraCast2\_A) have been identified using MLST and HVR as molecular markers. Mating assays have revealed a female-biased sex ratio in flies carrying wAfraCast1\_A, while behavioral tests have suggested a protective effect against parasitoids in *A. fraterculus* larvae harboring wAfraCast2\_A. Here, we assembled the wAfraCast2\_A genome from a whole-genome project of *A. fraterculus* morphotype 1 (Argentina).

High-molecular-weight DNA was extracted from a single adult female, and Oxford Nanopore high-throughput genome sequencing was performed. After quality control and filtering of raw reads, a metagenomic approach was followed to obtain de novo assemblies of *A. fraterculus* and *Wolbachia* sp. genomes, and a *Wolbachia*-assigned contig was further analyzed. Quast and BUSCO were used to check the integrity and completeness of a preliminary *Wolbachia* genome assembly. Annotation was carried out with Prokka and compared to the reference genome of the *Wolbachia* endosymbiont of *Drosophila melanogaster* (wMel).

A total of 15,810,325 reads were obtained after adapter removal and quality filtering (10% of the reads were discarded). From these, a total of 1,360 contigs were generated de novo, and 1 contig was assigned to the *Wolbachia* sp. taxon. A preliminary assembly of the wAfraCast2\_A genome revealed a circularized contig of 1,463,312 bp with 93.1% completeness, 0% duplicated genes, and 6.9% missing genes (rickettsiales\_odb10 database, n:364). A genome comparison with wMel showed an Average Nucleotide Identity of 99.1% and structural annotation identified 1,701 CDSs, including 633 genes, 907 hypothetical proteins, 3 rRNAs, and 34 tRNAs. These results will enhance our understanding of the *Wolbachia*-*A. fraterculus* interaction, including mechanisms influencing host behavior, physiology, and ecological functions, thereby supporting novel pest management strategies.



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## sciforum-111356: Proteomic characterization of *Legionella micdadei* membranes

**Bożena Kowalczyk<sup>1,\*</sup>, Jacek Tarasiuk<sup>1</sup>, Katarzyna Pastuszak<sup>2</sup>, Małgorzata Jurak<sup>2</sup> and Marta Palusińska-Szys<sup>1</sup>**

<sup>1</sup> Department of Genetics and Microbiology, Institute of Biological Sciences, Faculty of Biology and Biotechnology, Maria Curie-Skłodowska University, 20-033 Lublin, Poland

<sup>2</sup> Department of Interfacial Phenomena, Institute of Chemical Sciences, Faculty of Chemistry, Maria Curie-Skłodowska University, 20-031 Lublin, Poland

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*Legionella* spp. are pathogens of free-living protozoa that can cause a respiratory disease called legionellosis. Pneumonia caused by *L. micdadei* is mainly associated with hospital infections and primarily affects immunosuppressed patients. The infection of host cells by *L. micdadei* is facilitated by specialized mechanisms through which the bacteria modulate host cell physiology to create a replicative niche. Central to this process is the Dot/Icm Type IV secretion system (T4SS), complemented by the Type II secretion system (T2SS) and other host-manipulation strategies involving specific proteins.

This study aimed to analyze the composition of the outer (OM) and inner membrane (IM) proteins isolated from *L. micdadei* bacteria.

The bacterial mass collected from BCYE plates was digested with DNase and RNase and then disintegrated in a French press. A sucrose density gradient was used to isolate the outer and inner membranes of *L. micdadei*. Proteins isolated from these membranes were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS-MS). The *L. micdadei* proteome was investigated through diverse subtractive proteomics approaches, e.g., identification of pathogen-specific essential proteins, and druggability.

A detailed analysis of the *L. micdadei* membrane proteome allowed for the identification of 1784 proteins, including virulence factors, proteins associated with the transport of substances across cell membranes and the synthesis of cell membrane components, proteins related to bacterial mobility, and signal transduction pathways. The virulence factors present in *L. micdadei* membranes included protein building type IVB and II secretion systems, as well as major outer membrane protein (MOMP), macrophage infectivity potentiator (Mip), periplasmic protein EnhC, RND efflux system, outer membrane lipoprotein, the NodT family, and phosphatidylglycerol-prolipoprotein diacylglycerol transferase.

The identification of *L. micdadei* membrane proteins and the elucidation of their functions provide deeper insights into the mechanisms underlying the pathogenesis of these microorganisms.



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# sciforum-115003: Shifts in Aerobic Granular Sludge Bacteriome Driven by Seawater Intrusion in Wastewater

Catarina Miranda \*, Paula M. L. Castro and Catarina L. Amorim

Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, 4169-005 Porto, Portugal

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High salinity levels in wastewater treatment plants (WWTPs) can be attributed to multiple sources, including seawater intrusion in wastewater streams. In coastal WWTPs, this phenomenon is becoming more frequent, causing transient salinity shocks on the microbial populations involved in the treatment process. Aerobic granular sludge (AGS) has emerged as a revolutionary technology that has been adopted worldwide for treating several types of wastewater. Much of its success is related to its great tolerance to extreme environments, including high-saline wastewater. In this study, a laboratory-scale AGS reactor was exposed to different salinity stresses over 286 days. First, over 131 days, the seawater content in wastewater was gradually increased in the feeding regime (1.5 – 15 g/L). For the remainder of the operation, the AGS had to deal with daily salinity oscillations, ranging from high (7.5 g/L) to very high (22.5 g/L) seawater levels in wastewater. Throughout the operation, the removal performance of organic carbon, ammonium, and phosphate was consistently effective, despite the daily fluctuations in the seawater content of the wastewater. This was likely ensured by the nutrient removal-related taxa present in the AGS core microbiome, which was highly diverse and resilient to changes in wastewater composition. Over time, enrichment of the core microbiome with halotolerant taxa and extracellular polymeric substance producers proved crucial for maintaining the integrity and stability of the reactor's performance. The findings of this work underscore the flexibility and robustness of AGS communities in thriving under diverse environmental challenges and adapting to sustain AGS reactor performance.

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# sciforum-110892: Spectral Efficacy: The Role of UV-C Light Frequency in Viral Inactivation and Disinfection

**Péter Szabó<sup>1,2,3,\*</sup>, Roland Hetényi<sup>1,2,3</sup>, Dániel Hanna<sup>1,2,3</sup>, Zoltán Kopasz<sup>2</sup> and József Szentpéteri<sup>3,4</sup>**

<sup>1</sup> Szentágothai Research Centre, University of Pécs, Pécs, Hungary

<sup>2</sup> National Virology Laboratory, University of Pécs, Pécs, Hungary

<sup>3</sup> RoLink Biotechnology Kft., Pécs, Hungary

<sup>4</sup> Medical School, Institute of Transdisciplinary Discoveries, University of Pécs, Pécs, Hungary

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## Introduction

Our systematic review and meta-analysis address gaps in UV-C viral inactivation protocols in liquid solutions to understand both the influencing characteristics of viruses and related experimental setups. Key issues include inconsistent reporting of experimental setups, virus identification, terminology, and dose-effectiveness relationships, which have all been examined in the literature (Chiappa et al., 2021; Freeman et al., 2022; Masjoudi et al., 2021; Raeiszadeh & Adeli, 2020). A clear distinction between "inactivation" and "disinfection" is crucial. We propose defining complete inactivation as an undetectable viral load while preserving viral and protein structures to improve research applications.

## Methods

A PICO search (April 10, 2024) across five databases (N=2814) yielded 1,686 records after duplicates were removed adhering to the PRISMA, GRADE, and MECIR protocols. We reviewed 309 full texts, with 33 studies meeting our inclusion criteria for strain-specific viruses, defined UV-C setups, and quantifiable viral reductions. Random-effects meta-regression of 18 studies analyzed strain-specific inactivation, while Spearman correlation was used to examine terminology usage.

## Results

UV-C effectively (254nm being the most effective) reduced viral loads but faced inconsistencies in experimental setups, viral conditions, and dosages. Virus-specific dose-response relationships varied, leading us to focus on only SARS-CoV-2 strains for further calculations. Our semantic analysis revealed the interchangeable use of "inactivation" and "disinfection". The issues identified in our meta-analysis—O-O distortions, dose saturation, and PFU/TCID inconsistencies—necessitate non-linear models and data refinement. Despite the variability, the preliminary regression ( $R^2 = 0.49$ ) suggested predictable outcomes.

## Conclusions

We propose a reporting framework to improve reliability and cross-study comparability. Inactivation studies must start with higher viral loads to account for plaque assay and TCID<sub>50</sub> detection limits, and the "delivered dose" should only be reported when measured; otherwise, "calculated dose" is correct. Reports must include TCID/plaque assay details, log-transformed data (e.g., log<sub>3</sub> = base 3), and at least five data points for inactivation curves. "Inactivation" should be used for laboratory studies maintaining viral structures, while "disinfection" suits hospitals and water treatments.



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# sciforum-115051: The bacterial microbiota of knees affected by Gonarthrosis.

**Alejandra de la Cruz Munguía<sup>1,\*</sup>, Juan Manuel Vélez Ixta<sup>1</sup>, Tizziani Benítez Guerrero<sup>1</sup>, Mirna Liliana López Pérez<sup>1</sup>, Alberto Piña Escobedo<sup>1</sup>, Jossue Castañeda Garduño<sup>2</sup> and Jaime García Mena<sup>1</sup>**

<sup>1</sup> Departamento de Genética y Biología Molecular, Cinvestav, Mexico City, Mexico.

<sup>2</sup> Centro Médico ISSEMYM Toluca/ Hospital Ángeles Lomas, Estado de México, México.

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## Introduction

Gonarthrosis, or osteoarthritis located in the knee, is a joint disease that causes the tissues of the affected joint to progressively degenerate. When there is a wear away of the synovial membrane due to this disease, its permeability increases, causing bacteria to thrive. The objective of this study is to identify whether Gonarthrosis is associated with a microbiota profile dominated by proinflammatory bacteria in the synovial space.

## Methods

Synovial fluid samples were extracted from 57 patients diagnosed with stage 4 Gonarthrosis and were subsequently stored at -80 C. The extraction of total DNA was carried out with a specific kit for microbiome measurement. Gene libraries were created by PCR of the V3-V4 region of the 16S ribosomal gene to subsequently characterize the diversity of the microbiota using next-generation sequencing. The analysis of the results was conducted using the QIIME version 2 pipeline together with the GreenGenes database for taxonomic assignment and the R programming environment to determine the comparison of the relative abundance of taxa.

## Results

The results determined that more than half of the samples contained bacteria of the genera *Blautia\_A\_141781*, *Bacteroides\_H*, *Lacticaseibacillus*, and *Akkermansia*, among others. This information reveals that there are bacteria in the synovial space, contributing to the field of research on microbiota in spaces that are considered sterile, such as, in this case, joints affected by the degenerative disease Gonarthrosis.

## Conclusion

In conclusion, it was detected that there are bacteria in the synovial space in people with Osteoarthritis. This work was financed by CONACyT 163235 INFR-2011-01 and SECTEI/249/2019–CM-SECTEI/109/2020–CM-SECTEI/124/2021, Convocatoria 2019.



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# sciforum-111168: The effect of Light and temperature on the accumulation of $\beta$ -carotene in *Rhodotorula* yeasts

Regina Losinska-Siçiūnienė \*, Živilė Strazdaitė-Žielienė and Elena Servienė

Nature Research Centre

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Yeasts of the genus *Rhodotorula* have pink, orange, and red colors, indicating their ability to synthesize carotenoids. Due to their biological properties, carotenoids are widely used in the phytomedicine, chemical, pharmaceutical, cosmetic, food, and feed industries. One of the most important carotenoids is  $\beta$ -carotene, which exhibits interesting biological activities, such as antioxidant, anticancer, and antimicrobial. *Rhodotorula* yeasts were isolated from the natural environment and analyzed using morphological and molecular methods. Species were identified based on the restriction fragment length polymorphism analysis and sequencing of the rDNA internal transcribed spacer region (ITS). In this work, the  $\beta$ -carotene content of *Rhodotorula* yeasts was investigated when grown on a solid YEPD medium at different temperatures (4 °C and 26 °C) using various light intensities (8-10  $\mu\text{mol}/\text{m}^2\text{s}$ ). The biomass was collected, and carotenoids were extracted using dimethylformamide (DMF). The amount of  $\beta$ -carotene was calculated based on absorption at 440 nm. The highest  $\beta$ -carotene contents were found in *R. babjevae*, *R. glutinis*, *R. graminis*, and *R. kratochilovae* after 14 days of incubation at 26 °C in the dark. The level of  $\beta$ -carotene was 30 to 55% lower depending on the yeast species when the yeast culture after 2 days of cultivation at 26 °C was transferred to 4 °C, followed by 12 days of incubation in the dark. When yeasts were kept in the light at 4 °C, out of the eight yeast species tested, *R. mucilaginosa* and *R. babjevae* accumulated about 20-30% more  $\beta$ -carotene than under dark conditions. Our results show that yeasts that are cultured in the dark at 26 °C accumulate  $\beta$ -carotene better than yeasts that are held under the same conditions in the light. The synthesis and accumulation of  $\beta$ -carotene are decreased at a low temperature. The obtained data suggest that isolated *Rhodotorula* yeast species can be promising for carotenoid production under appropriate conditions.



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## **Session 2. Gut Microbiota and Health Disease**

# sciforum-115783: Dissemination of *Enterococcus* spp. across One Health Settings: Insights from fingerprinting analysis

**Joana Monteiro Marques**<sup>1,2,\*</sup>, Nicolau Fialho<sup>1,2</sup>, Maria do Mar Duarte<sup>1,2</sup>, Maria Teresa Barreto Crespo<sup>3</sup>,  
and Teresa Semedo-Lemsaddek<sup>1,2</sup>

<sup>1</sup> Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Av. da Universidade Técnica de Lisboa, 1300-477 Lisbon, Portugal

<sup>2</sup> Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal

<sup>3</sup> iBET - Institute of Experimental Biology and Technology, 2781-901 Oeiras, Portugal

<sup>4</sup> ITQB NOVA – Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, 2780-157 Oeiras, Portugal

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*Enterococcus* spp. are ubiquitous Gram-positive bacteria present in the gastrointestinal tract of humans and animals, as well as in various environmental niches. Over recent decades, *Enterococcus* spp. have emerged as significant opportunistic pathogens in healthcare-associated infections, with vancomycin-resistant *E. faecium* identified as a critical health threat. In this context, the One Health concept underscores the interconnection of human, animal, and environmental health, highlighting the importance of intervention fields such as antimicrobial resistance and food safety.

This study aimed to isolate and analyse the genetic diversity of enterococci from a diverse range of samples across One Health domains: sick (n=18) and healthy (n=17) humans, sick (n=13) and healthy (n=14) animals, canteen food (n=11), public transportation surfaces (n=8), and surface water (n=8). Samples were cultured on enterococci-selective media, with and without vancomycin supplementation. Approximately 20% of characteristic colonies were selected and confirmed at the genus level, followed by DNA extraction and RAPD-PCR using primers OPC15 and GTG5 in independent reactions. Amplification analysis and dendrogram construction were performed using the BioNumerics software to obtain relationship patterns within and across One Health sectors.

A total of 148 isolates were obtained from selective media, comprising 81 environmental, 27 animal-, and 35 human-derived isolates, including 24 from vancomycin-supplemented media. Overall, no distinct clustering by sample type or One Health sector was observed, suggesting the widespread dissemination of genetically diverse enterococci across these settings. However, isolates from surface water and clinical samples showed high genetic similarity ( $\geq 80$ –95%), with some clinical clusters also containing isolates from healthy humans.

These findings confirm the widespread dissemination of enterococci across One Health sectors, including presumptive vancomycin-resistant enterococci, and underscore the need for further biosurveillance studies.



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# sciforum-110897: Comparative Analysis Of Gut Microbiota Metabolism In Healthy And Pathological Conditions

Ekaterina Sorokina \*, Ekaterina Chernevskaya and Maria Getsina

Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russia

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Metabolite analysis is a promising method and is increasingly being used to identify dysfunctions of various organs in patients. One of the sources of metabolites in the body is the gut microbiota. An increase in sepsis-associated aromatic microbial metabolites is a prognostically unfavorable factor and increases the risk of death in patients with sepsis.

The aim of this work was to evaluate the composition and function of the gut microbiota in patients with sepsis compared to healthy donors using chromatograph mass spectrometry.

The study included 10 patients with sepsis and 9 healthy donors, comparable in gender and age. The incubation of feces samples was carried out in thioglycollate medium (TG (at 37 °C)) with the addition of sepsis-associated microbial metabolites (25 µM phenyllactic acid (PLA) or 25 µM 4-hydroxyphenyllactic acid (4-HPLA)). Metabolite concentrations were determined using a GC-2010 Plus gas chromatograph and a GCMS-QP2020 mass spectrometer (Shimadzu, Japan). The proportion of sepsis-associated aromatic metabolites in patients with sepsis was significantly higher than normal with a significance of 40%, while in donors, it did not exceed 5%. Comparison of the metabolomic profiles of normobiota and pathobiota in an experiment showed that when loaded with sepsis-associated metabolites of PLA and 4-HPLA, the microbiota of a healthy subject biotransforms them into the end products of microbial metabolism, whereas the pathobiota of a septic patient is unable to perform this function. Thus, in sepsis, the normobiome is transformed into a pathobiome, which reflects the dysfunction of the gut microbiota.



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# sciforum-114450: Decreased levels of aromatic microbial metabolites as a reflection of microbiota dysfunction in cancer patients

**Maria Getsina \*** and Ekaterina Chernevskaya

Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, 25-2 Petrovka Str., 107031 Moscow, Russia

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## Introduction

There is growing evidence that cancer patients have altered metabolism due to disruption of the microbiome. Serum concentrations of aromatic microbial metabolites may reflect microbiota dysfunction.

## Aim

The aim of this study was to evaluate the serum concentrations of aromatic microbial metabolites in patients undergoing treatment for malignant oncological diseases.

## Methods

Two cohorts of patients were studied: patients with pancreatic cancer before surgery (n=64) and children with various malignant oncological diseases (leukemia, lymphoma, nephroblastoma, ependymoma, etc.) (n=40). The study used two comparison groups: healthy donors (n=18) and practically healthy children referred for preventive examination (n=18). Aromatic microbial metabolites such as phenyllactic acid, hydroxyphenyllactic acid and hydroxyphenylacetic acid were identified by GC-MS.

## Results

The concentration of metabolites in the serum of patients with cancer in both studies is statistically significantly lower than that of healthy subjects. The sum of concentrations of the aromatic microbial metabolites was 1.9 (1.5; 2.2)  $\mu\text{mol/L}$  in healthy donors and 1.4 (0.9; 2.0)  $\mu\text{mol/L}$  in pancreatic cancer patients before surgery ( $p\text{-value}=0.001$ ). The sum of concentrations of the aromatic microbial metabolites in children was 2.2 (1.5; 2.6)  $\mu\text{mol/L}$  in the control group vs 1.5 (1.1; 1.8)  $\mu\text{mol/L}$  in the cancer group ( $p\text{-value}=0.001$ ).

## Conclusions

Our study reveals the profound metabolic dysfunction of microbiota in cancer, as serum aromatic microbial metabolites were significantly different in patients compared to healthy subjects. It is promising to study the relationship between metabolic profiles and gut microbiota composition for a deep understanding of cancer pathogenesis.



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# sciforum-115202: Evaluation of Molecular Hydrogen in the Treatment of Intestinal Inflammation in a Murine Model

Oumaima Anachad \*, Wafaa Taha, Imad Fenjar, Hicham Wahnou, Faiza bennis and Fatima Chegdani

Laboratory of Immunology and Biodiversity, Faculty of Sciences Ain Chock, Hassan II University of Casablanca, Morocco

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Intestinal inflammation is central to the pathogenesis of various gastrointestinal disorders, making the exploration of effective treatment strategies essential. This study investigates the effects of molecular hydrogen in alleviating intestinal inflammation induced in a murine model. We aim to assess its therapeutic potential through histopathological analysis, gene expression evaluation, and bacterial load quantification.

Intestinal inflammation was induced in 15 female mice using kappa-carrageenan over 3 months. Following induction, the mice were assigned to four treatment groups: diclofenac (20 mg/kg), molecular hydrogen, negative control, and positive control. Treatment lasted 15 days. Colon length was measured as an indicator of inflammation, and histopathological analysis was conducted on the colon, liver, and intestines. RNA was extracted from the colon for quantitative reverse transcription PCR to assess the expression of inflammation-related genes (TNF $\alpha$ , IL1 $\beta$ , IL6, IL10, and SOD1), while DNA from fecal samples was analyzed for *E. coli* and *Bifidobacterium* presence. Bacterial load quantification is being optimized using qPCR, with a focus on refining the melting temperature protocol for specificity.

The histopathological analysis revealed significant tissue damage in the inflammation-induced group, with partial recovery observed in the diclofenac- and molecular hydrogen-treated groups. The qRT-PCR results showed a more pronounced inflammatory response in the untreated group, while IL10 was lower in the treated groups, suggesting the modulation of the inflammatory response. IL6 was moderately reduced by diclofenac, while molecular hydrogen had only mild effects. Interestingly, IL1 $\beta$  was elevated in both treatment groups, indicating possible pro-inflammatory effects. Additionally, SOD1 levels were lower in treated groups, indicating reduced antioxidant activity.

The preliminary findings suggest that both diclofenac and molecular hydrogen have therapeutic potential in reducing intestinal inflammation. Diclofenac primarily acts through anti-inflammatory pathways, while molecular hydrogen may modulate oxidative stress and gut microbiota. Further studies are needed to clarify the mechanisms of action for these treatments in gastrointestinal diseases.



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# sciforum-111358: Gut microbiota-supporting beverage: Kombucha's activity against Gram-positive bacteria

**Maria Leszczyńska<sup>1\*</sup>, Paulina Lenart<sup>1</sup>, Maciej Grzegorzczak<sup>1</sup>, Agnieszka Grzegorzczak<sup>2</sup>, Martyna Kasela<sup>2</sup> and Sylwia Andrzejczuk<sup>2</sup>**

<sup>1</sup> Student's Scientific Association "mikroGRAM" at the Department of Pharmaceutical Microbiology, Medical University of Lublin, 20-093 Lublin, Poland

<sup>2</sup> Department of Pharmaceutical Microbiology, Medical University of Lublin, 20-093 Lublin, Poland

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Fermented foods have been rapidly gaining popularity recently due to their use in medicine and dietetics. Kombucha is a fermented beverage containing numerous probiotic bacteria and their metabolites, which have beneficial effects on the digestive system. The purpose of this study was to determine the antimicrobial activity of kombucha containing probiotic strains against bacteria that cause gastrointestinal diseases.

The activity of kombucha was tested before fermentation and during fermentation on days 1, 2, 3, 7, 8, and 14 against reference strains of Gram-positive bacteria from the American Type Culture Collection (ATCC) such as methicillin-sensitive *Staphylococcus aureus* (MSSA) ATCC 29213, two methicillin-resistant *Staphylococcus aureus* (MRSA) strains (ATCC 43300 and ATCC BAA-1707), *Bacillus cereus* ATCC 10876, and *Enterococcus faecalis* ATCC 29212. The well-diffusion method was used for this assessment, and the antimicrobial activity of this beverage was evaluated by the zone of growth inhibition (mm) around the kombucha well.

The highest activity was observed after 14 days of kombucha fermentation against almost all tested bacteria with a zone of growth inhibition of 18-21 mm. The highest activity was observed against *S. aureus* ATCC 29213 and *S. aureus* ATCC 43300.

These data show that kombucha is a beverage that can support the treatment of food poisoning caused by pathogenic microorganisms such as methicillin-sensitive *S. aureus* (MRSA), methicillin-resistant *S. aureus* (MRSA), *B. cereus*, and *E. faecalis*.



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# sciforum-114939: Microbial Signatures in High- and Low-Blood-Glucose Participants of the Generation 100 Study

Natalia G Bednarska <sup>1,\*</sup> and Asta Kristine Håberg <sup>2</sup>

<sup>1</sup> London School of Hygiene and Tropical Medicine, Department of Infectious Tropical Diseases

<sup>2</sup> NTNU, Department Neuromed & Movement Science, Norwegian University of Science & Technology (NTNU), 7034 Trondheim, Norway

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## Introduction

Type 2 diabetes mellitus (T2DM) is an age-related metabolic disease that is often considered inflammatory, as various cytokine profiles are associated with its progression. T2DM typically follows a prediabetic stage, during which insulin resistance develops. This stage can often be corrected early in life, potentially preventing or delaying the onset of T2D. Intestinal dysbiosis has been linked to metabolic disorders, including insulin resistance and T2D. A healthy, diverse gut microbiome, composed primarily of four phyla—*Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Proteobacteria*—is essential for maintaining the gut epithelial barrier. Phylogenetically related bacterial groups, such as *Proteobacteria* and *Enterobacteriaceae*, have been linked to poor glycaemic control and negative metabolic outcomes, including obesity, insulin resistance, and an impaired lipid profile.

## Methods

This study involved 73 older adults (ages 76–83) from the randomized controlled trial entitled the Generation 100 Study. We performed high-throughput sequencing of the bacterial 16S rRNA gene to obtain metagenomic microbial profiles for all participants. These profiles were then correlated with clinical measures.

## Results

We observed distinct patterns of microbial beta diversity between the high- and normal-glucose groups. Overall, the microbial diversity was significantly reduced in the high-glucose group. At the highest taxonomic level (Phylum), we found that *Synergistes*, *Elusimicobia*, *Euryarchaeota*, *Verrucomicrobia*, and *Proteobacteria* were all significantly decreased in participants with high blood glucose. Additionally, *P. copri* was significantly elevated in the high-glucose (10-fold increase) and high-CRP (777-fold increase) groups, suggesting that it may serve as an early inflammatory and diabetic marker. These findings are consistent with previous research identifying *P. copri* as a pro-inflammatory pathogen. We also found that the *Fusobacterium* genus was significantly increased in the normal glucose group, with a 151-fold increase compared to the high-glucose group (p 0.005).

## Conclusions

Our results indicate significant changes in the microbiome that may provide valuable insights for early intervention in pre-diabetic states.



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# sciforum-115811: The role of personalized nutrition to modulate gut microbiota for disease prevention

**A. Perez-Vazquez<sup>1,\*</sup>, P. Barciela<sup>1</sup>, M. Carpena<sup>1</sup>, F. Chamorro<sup>1</sup>, A.O.S Jorge<sup>1,2</sup>, P. Donn<sup>1</sup>, Rafael Nogueira-Marques<sup>1</sup> and M.A. Prieto<sup>1</sup>**

<sup>1</sup> Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Instituto de Agroecoloxía e Alimentación (IAA) – CITE XVI, 36310 Vigo, Spain.

<sup>2</sup> REQUIMTE/LAQV, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

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The human body hosts a diverse community of microorganisms, with the gastrointestinal tract being the most densely colonized, hosting a thousand microbial species collectively referred to as the gut microbiota. Recent studies have demonstrated that the gut microbiota maintains multidirectional and communicational connections with various organs through metabolic, endocrine, neural, humoral, and immunological pathways. Alterations in this gut–organ axis can lead to a wide range of health issues beyond gastrointestinal disorders which affect other organ systems. Emerging evidence highlights the intricate relationship between diet and microbiota in the onset and progression of diseases. Personalized nutrition has gained attention as a strategy to identify specific microbiome traits that predict responses to dietary components. This approach holds promise for designing targeted dietary interventions that promote favorable health outcomes by modulating the gut microbiota. This systematic review explores current insights into the mechanisms underlying gut–organ interactions and examines the potential of personalized nutrition for disease prevention taking advantage of these mechanisms. Furthermore, it addresses existing limitations in understanding the mechanistic pathways and the implementation of personalized nutrition as a preventive strategy. Finally, by summarizing the latest findings, this review aims to provide a clearer understanding of the gut microbiota's role in systemic health and its modulation through dietary strategies, paving the way for innovative approaches in preventive medicine.



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# sciforum-114166: Vertical transmission of Microbiota and Metabolites: Impact of Maternal obesity on Neonates

Aparna Krishnakumar<sup>1,\*</sup>, Juan Manuel Vélez Ixta<sup>1</sup>, Carmen Josefina Juárez Castelán<sup>1</sup>, Tizziani Benítez Guerrero<sup>1</sup>, Alberto Piña Escobedo<sup>1</sup>, Enrique Rico Arzate<sup>2</sup>, Jose Javier Castro Arellano<sup>2</sup>, Silvia Romero Maldonado<sup>3</sup>, Yair Cruz Narváez<sup>2</sup>, María Luisa Pizano-Zárate<sup>3</sup> and Jaime García-Mena<sup>1</sup>

<sup>1</sup> Departamento de Genética y Biología Molecular, Av Instituto Politécnico Nacional 2508, San Pedro Zacatenco, Gustavo A. Madero, 07360 Ciudad de México, CDMX.

<sup>2</sup> ESIQIE-IPN, Av Instituto Politécnico Nacional s/n, Lindavista, Gustavo A. Madero, 07700 Ciudad de México, CDMX.

<sup>3</sup> Instituto Nacional de Perinatología, C. Montes Urales 800, Lomas - Virreyes, Lomas de Chapultepec IV Secc, Miguel Hidalgo, 11000 Ciudad de México, CDMX.

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**Introduction:** This study investigates the effects of maternal obesity on microbiota composition and metabolite profiles in neonates and breast milk. Maternal obesity can alter the bacterial landscape and bioactive compounds in breast milk, increasing the risk of obesity in infants. The microbiota and metabolites in breast milk and neonatal fecal samples from obese and normal-weight Mexican mothers were compared. **Methodology:** Fecal and milk samples were collected from binomials. DNA extraction was performed, followed by the PCR amplification of the V3-16S ribosomal RNA gene for next-generation sequencing (NGS) analysis. Taxonomic microbiota profiling was conducted, and metabolic pathway analysis was carried out using PICRUST to identify pathways associated with different bacterial taxa. Metabolites were extracted and analyzed via FT-ICR mass spectrometry. Comprehensive bioinformatics analyses were conducted to assign taxonomy and assess bacterial diversity. **Results:** The findings suggest that the vertical transmission of bacteria and metabolites from mother to infant exists. Patescibacteria and Firmicutes were identified as dominant phyla in human milk. Fecal samples from neonates born to obese mothers exhibited a lower abundance of *Bifidobacterium* and *Lactobacillus* than those born to normal-weight mothers. In addition, fecal samples from neonates and milk from obese mothers were characterized by an increased abundance of *Lactobacillus* and *Staphylococcus*. **Conclusion:** This study highlights breastfeeding's crucial role in transferring maternal microbiota and metabolites to infants, showing strong microbial and metabolic connections between breast milk and meconium. Maternal BMI does not affect overall bacterial composition, but distinct metabolites linked to maternal obesity were found, suggesting potential neonatal health impacts. The research suggests a pathway for intergenerational obesity risk transmission via breastfeeding, emphasizing the need for further investigation into the long-term health effects on infants of obese mothers. This work was financed by CONACyT 163235 INFR-2011-01 and CONACyT FORDECYT-PRONACES/6669/2020\_Programa Presupuestario F003-Ciencia de frontera 2019.



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## **Session 3. Antimicrobial Agents and Resistance**

# sciforum-111351: *Staphylococcus aureus* biofilm gene analysis: risks for patients with chronic lymphocytic leukemia (CLL)

Maciej Grzegorzczuk<sup>1,\*</sup> and Agnieszka Grzegorzczuk<sup>2</sup>

<sup>1</sup> Students Scientific Association “mikroGRAM” at Department of Pharmaceutical Microbiology, Medical University of Lublin, 20-093 Lublin, Poland

<sup>2</sup> Department of Pharmaceutical Microbiology, Medical University of Lublin, 20-093 Lublin, Poland

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Biofilms have a complex multicellular structure composed of bacteria and extracellular polymeric substances, which collectively display adhesive properties and allow bacterial cells to stick to each other and to given surfaces. Biofilms may be formed on the abiotic surfaces of medical instruments such as catheters or implants, as well as on biotic surfaces, e.g., living tissues. Biofilm structures are formed in order to increase bacteria's chances of survival in unfavorable environmental conditions, prevent immune cells from eliminating bacterial cells, and to provide antibiotic resistance. *Staphylococcus aureus* is one of the most common sources of upper respiratory tract infections and is a procaryote with the ability to form biofilm structures. Biofilm is considered to be, among others, a virulence factor that contributes to difficulties and therapeutic failures in treating antibiotic-resistant staphylococcal infections. This can pose a huge risk for individuals with an impaired function of the immune system, such as patients with CLL.

A total of 51 *S. aureus* strains were isolated from 34 patients with CLL treated in the Saint Jan of Dukla Oncology Centre of the Lublin Region (Poland). DNA from bacteria was extracted using the spin-column method and was later used to perform PCR reactions in order to detect the *icaA*, *icaD*, *icaB*, and *icaC* genes responsible for the formation of biofilm.

An analysis of the electrophoretic separation of amplification products in the studied *S. aureus* strains showed the presence of *icaA* genes in 49 (96.08%) isolates, while *icaD*, *icaB*, and *icaC* genes were detected in 45 (88.24%), 47 (92.16%), and 41 (80.4%) *S. aureus* isolates, respectively.

Our study found a very significant prevalence of all genes responsible for biofilm formation among isolated *S. aureus* strains, which, in turn, may contribute to chronic staphylococcal infections, difficulties in antibiotic therapies, and may also delay anticancer treatment, undermining its efficiency and even threatening the lives of patients.



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# sciforum-115721: Effects of environmental antimicrobial residues on the conjugative transfer of antimicrobial resistance genes in the gut microbiota

Amer Abdelgany<sup>1,2,\*</sup> and Jiewen Guan<sup>1</sup>

<sup>1</sup> Canadian Food Inspection Agency

<sup>2</sup> Biology department, Carleton University

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Antimicrobial resistance (AMR) poses a significant public health challenge, as AMR bacteria and their genes persist in environments such as farms and water systems. AMR bacteria can infect humans through contaminated food, water, or direct contact. We hypothesize that both intermittent exposure to clinical antimicrobials and environmental residues might exacerbate this colonization, leading to gut dysbiosis and facilitating the spread of resistance genes. This could enhance the survival and persistence of AMR bacteria and promote AMR gene transfer among bacterial communities in the gut.

To investigate this, we utilized a mouse model to examine the conjugative transfer of a mobile AMR plasmid from a *Salmonella* Heidelberg donor to Enterobacteriaceae in the gut under treatments of intermittent clinical doses of ampicillin and sub-clinical levels of tetracycline in drinking water. Fecal samples were collected from mice at regular intervals to isolate AMR bacteria on selective agar media. DNA was extracted from these samples for future 16S rRNA sequencing to analyze microbial community shifts and qPCR targeting ARGs to examine plasmid transfer dynamics.

Preliminary results show that the donor *Salmonella* Heidelberg persisted for four months in all treatment groups except the control, which had no antimicrobial exposure. The donor reached a high density immediately after each ampicillin treatment and gradually became undetectable. The AMR plasmid was transferred to commensal *E. coli* and to an introduced recipient *Salmonella* Heidelberg. While detailed sequencing and bioinformatics analyses are pending, initial findings highlight the significant impacts of antimicrobial exposure on the persistence of AMR bacteria and the transfer of AMR plasmids within the gut microbiota.



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# sciforum-115809: From Surface to Infection: Biofilm Formation of Coagulase Negative Staphylococci

**Catarina Ribeiro Freitas**<sup>1,2,\*</sup>, **Vanessa Silva**<sup>1,2,3,4</sup>, **Eliana Costa**<sup>5</sup>, **Ana Fontes**<sup>5</sup>, **Ana Bento-Pinto**<sup>5</sup>, **Gilberto Igrejas**<sup>2,3,4</sup> and **Patrícia Poeta**<sup>1,6,7</sup>

<sup>1</sup> Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

<sup>2</sup> Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

<sup>3</sup> Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

<sup>4</sup> LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, University NOVA of Lisbon, Caparica, Portugal

<sup>5</sup> Hospital Centre of Trás-os-Montes and Alto Douro, Clinical Pathology Department, Vila Real, Portugal

<sup>6</sup> CECAV—Veterinary and Animal Research Centre, University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

<sup>7</sup> Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

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Biofilm formation by Coagulase Negative Staphylococci (CoNS) represents a critical challenge in device-associated infections, often leading to treatment failures. While *Staphylococcus epidermidis* has been extensively studied, the biofilm-forming potential of other CoNS species remains underexplored. This study analysed 152 isolates belonging to 11 CoNS species obtained from individuals with infections, with the aim of assessing interspecies variability in biofilm formation and its potential clinical implications.

The 152 isolates were evaluated for their capacity to form biofilms using the microtiter biofilm assay. Statistical analyses were conducted to ascertain associations between biofilm formation in the various CoNS species.

The majority of CoNS isolates (64,47%) demonstrated a high capacity for biofilm production, while only 7,24% were classified as non-producers. Despite the variability observed between the isolates, no statistically significant differences were identified in the ability to form biofilms between the different species.

The results obtained in this study offer significant insights into the biofilm forming capacity of CoNS, which may have substantial ramifications for the formulation of novel therapeutic strategies aimed at mitigating biofilm formation and, by extension, the reduction in the prevalence of infections within hospital environments.

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## sciforum-115750: Isolation of fungal endophytes from *Monsonia angustifolia* and screening for their antimicrobial and extracellular enzymatic activities

Maruping Hoffman Frost \*, Khumiso Dithebe, Elbert Jansen Van Rensburg and Raymond Makola

Department of Biochemistry Microbiology and Biotechnology, University of Limpopo, Private Bag X1106, Sovenga, 0727, South Africa

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Endophytes reside in healthy plant tissue without causing visible harm. Fungal endophytes produce structurally and chemically diverse secondary metabolites, including those similar to the host plant, with antimicrobial and anti-inflammatory activities. Fungal endophytes also produce various extracellular enzymes with applications in various industries including the pharmaceutical industry. Despite this knowledge, the diversity and bioactivities of fungal endophytes of South African medicinal plants are relatively underexplored. Thus, the aim of this study was to isolate fungal endophytes from the medicinal plant *Monsonia angustifolia* and evaluate their antimicrobial and extracellular enzymatic activities. A total of 29 morphologically distinct fungal endophytes with 21 isolates related to filamentous fungi and 8 to yeasts were isolated from the surface-sterilised leaves, stems and spikes. The majority of the isolates (59%) were obtained from the stems. The sequencing of the internal transcribed spacer region revealed that all the filamentous fungi and four yeast isolates belonged to the Phylum Ascomycota, while the other four yeast isolates reside with Basidiomycota. The broth micro-dilution assay revealed that the ethyl acetate crude extracts of the endophytes possessed varying inhibitory activity, with minimum inhibitory concentrations ranging from 0.02 to 2.50 mg/mL against six clinically significant pathogens. Agar-plate screening for amylase, cellulase, laccase, lipase, pectinase, protease and xylanase enzymes revealed that the isolates had the ability to produce at least one of the tested enzymes, with four filamentous fungi exhibiting the ability to produce all the screened enzymes. This study highlights the significance of fungal endophytes as sources of antimicrobial secondary metabolites and industrially significant enzymes.



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## sciforum-115788: Achieving Natural control of the phytopathogenic fungus *Cylindrocladium* sp. using two different thyme essential oils applied under in vitro conditions

**María Cecilia Prieto <sup>1,\*</sup>, Nicolas Ortiz <sup>2</sup>, María José Duarte <sup>2</sup>, Pedro Sansberro <sup>2</sup> and Ernestina Galdeano <sup>2</sup>**

<sup>1</sup> Departamento de Agroalimentos, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina.

<sup>2</sup> Instituto de Botánica del Nordeste, Consejo Nacional de Investigaciones Científicas y Técnicas, Corrientes, 3400, Argentina

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*Ilex paraguariensis* (*Ip*) is a crop with economic and cultural importance in South America, where its leaves are used to prepare an infusion called “mate”. This crop is affected by different phytopathogenic microorganisms. *Cylindrocladium* (*Cy*) are the causal agent of the black spot of *Ip*. The objective of this work was to evaluate the use of two thyme (*Thymus vulgaris*) essential oils (EOs) as control agents of *Cy*. The thyme EOs (T1 and T2) were characterized by means of GC-MS. *Cy* was isolated from infected leaves of *Ip*. *Cy*, and a 14-day culture was used to obtain the work solution (100,000 spores/ml). The EOs were diluted in dimethyl sulfoxide at a rate of 1:4, and nine two-fold serial dilutions were performed. Treatments consisted of the addition of 15 µl of work solution to 165 µl broth with *Ip* and 20 µl of each EO dilution, which were then incubated for 72 h. Four replications were carried out. Negative (*Ip* broth) and positive controls (without EOs) were performed. The minimum inhibitory concentration (MIC) was determined as the minimal concentration in which no micelial growth was observed. Tubes whitout micelial growth were sowed in *Ip* medium and incubated for 48 h. The minimum fungicide concentration (MFC) was defined as the minimal EO concentration at which no *Cy* colonies were grown. T1 was mainly composed of thymol (40.32%) and p-cymene (36.43%), while T2 comprised p-cymene (59.65%), thymol (11.78%), and eucalyptol (5.35%). Both EOs exhibited fungistatic and fungicidal activity, with MIC values of 2.35 µl/ml for T1 and 31.25 µl/ml for T2, and MFCs of 2.93 µl/ml and 50 µl/ml, respectively. Despite both EOs showing good antifungic activity, T1 showed a greater bioactivity, which was correlated with the higher relative amount of thymol compared with T2. These results demonstrate the effectiveness of thyme EOs as natural control agents against *Cy*.



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## sciforum-111156: Antibacterial and antifungal properties of aqueous and alcoholic extracts of *Arnica montana* L.

Sylvia Andrzejczuk<sup>1</sup>, Magdalena Sozoniuk<sup>2</sup> and Martyna Kasela<sup>2</sup>

<sup>1</sup> Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland

<sup>2</sup> Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Poland

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*Arnica montana* L. is renowned for its biological properties, including its antimicrobial effects, which are attributed to the presence of sesquiterpene lactones or flavonoids. The aim of this study was to assess the antimicrobial activity of ethanolic (EtE) and aqueous (AqE) extracts of *Arnica montana* L. against a reference strains of microbes, employing a double broth dilution method (concentration of 7.81-30000 µg/mL); identifying MICs and MBC/MFCs. Amoxicillin, nystatin, and 70% ethanol were used as positive controls. The MBC(MFC)/MIC ratios were used to assess the activity of EtE/AqE (4 bactericidal/fungicidal; ≥4 -static). As a result, a differential antimicrobial activity was demonstrated in MIC=1000-2000 µg/mL and MIC=8000-16000 µg/mL for Gram(+) bacteria for EtE and AqE, respectively. EtE was more effective against *Salmonella* Typhimurium, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* (MIC=1000-2000 µg/mL), compared to AqE (MIC>16000 µg/mL). EtE was bactericidal against staphylococci and *Enterobacteriaceae* rods, while AqE was bactericidal against *Bacillus cereus*. Unlike EtE, which was effective to most *Candida* spp. (MIC=500-800 µg/mL), AqE had no antifungal properties.

This study suggests that the extracts and essences of *A. montana* have several beneficial properties such as antibacterial and antifungal effects. It highlights that essence extracts (EtEs) are more potent than aqueous extracts (AqEs) and can serve as natural antimicrobial agents. The raw material is mainly used in topical preparations due to its high toxicity, but can also be taken orally in low concentrations as a homeopathic remedy. The results of this study may pave the way for further research into activity against other microbial strains, and the development of new formulations for different applications. However, the challenge is to optimise solvent concentrations that are most suitable for biological experiments, particularly for alcohol extracts, which are good for solubilisation but can be cytotoxic if not carefully controlled.



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# sciforum-115287: Antibiofilm activity of *Rosaceae* honeys against *Acinetobacter baumannii* and their prebiotic effect on *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*

Francesca Coppola <sup>1,2,\*</sup>, Manar Abdalrazeq <sup>3</sup>, Florinda Fratianni <sup>1</sup>, Maria Neve Ombra <sup>1</sup>, Gokhan Zengin <sup>4</sup> and Filomena Nazzaro <sup>1</sup>

<sup>1</sup> Institute of Food Science, CNR, Avellino, Italy

<sup>2</sup> Department of Agricultural Sciences, University of Naples "Federico II", 80055 Portici, Italy

<sup>3</sup> An-Najah National University, Nablus, Palestine

<sup>4</sup> Department of Biology, Faculty of Science, Selcuk University, Konya, Turkey

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## Introduction

Natural products are an important source of therapeutic agents for their antimicrobial and prebiotic properties in an era of increasing antibiotic resistance. The antimicrobial and prebiotic properties of honeys from hawthorn, cherries, raspberries, almonds and apples were investigated.

## Methods

The antimicrobial activity of the honey, diluted 1:1 w: vol with deionized water and filtered, was assessed (20 mg/ml) by evaluating, through the crystal violet test, its ability to inhibit the biofilm of the high-risk pathogen *Acinetobacter baumannii*, and, through the MTT test, its capacity to affect the metabolism of the *A. baumannii* sessile cells. The prebiotic potentiality of the honey was assessed on *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*, to evaluate the capacity to affect their growth and to inhibit the biofilm of that pathogen.

## Results

The honeys inhibited the biofilm of *A. baumannii* with percentages ranging from 43.49% (cherry) to 59.43% (apple). The honeys did not affect the metabolism of the sessile cells of the pathogen (except cherry honey, 27.16%), so other inhibition mechanisms should be investigated. *Rosaceae* honey positively affected the growth of the two lactobacilli, with an increase ranging from 8-fold to even 13-fold (when *L. rhamnosus* grew in the presence of cherry honey), compared to the control, grown in MRS medium. The biofilm inhibitory activity of the supernatants of lactobacilli showed that all types of honey were more effective than the control, with inhibition percentages of up to 34.24% (supernatant of *L. plantarum* grown with apple honey), and was especially evident on the metabolism of sessile cells, as demonstrated by the high inhibition percentages observed (up to 61.31%), higher than the supernatant of the two strains conventionally grown.

## Conclusions

Future work will focus on the effect of *Rosaceae* honey on other pathogenic bacteria and probiotics, with the aim of expanding knowledge of its biological properties and assessing the consequent possibilities of its use.



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## sciforum-111567: Bark extract of *Melia azedarach* inhibits motility and viability of *Ralstonia solanacearum* growing in humid substrate

**María Cecilia Prieto**<sup>1,\*</sup>, Silvia Schaller<sup>2</sup>, Verónica Obregón<sup>3</sup>, Nelson Rubén Grosso<sup>4</sup> and Ernestina Galdeano<sup>2</sup>

<sup>1</sup> Departamento de Agroalimentos, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina.

<sup>2</sup> Instituto de Botánica del Nordeste; Consejo Nacional de Investigaciones Científicas y Técnicas; Corrientes; 3400; Argentina

<sup>3</sup> EEA Bella Vista; Instituto Nacional de Tecnología Agropecuaria; Bella Vista; 3432; Argentina

<sup>4</sup> Departamento de Fundamentación Biológica, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, 5000, Argentina

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Bacterial wilt of tomato is caused by the soilborn bacterium *Ralstonia solanacearum* (Rs). The disease is difficult to manage and produces serious yield losses. Natural products, such as plant extracts represent new environmentally sustainable control strategies. The aim of this study was to evaluate the antibacterial efficiency of *Melia azedarach* bark extracts against the phytopathogenic bacteria Rs. Three bark extracts were performed using water and ethanol as solvents. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of bark extract against Rs were defined using the broth microdilution method. Motility inhibition and changes in colony morphology were assessed, and bacterial survival was evaluated on humid substrate added with different bark extract amounts. Bark extract affected the viability, colony morphology (where were observed changes in the shape, cell density and edge morphology of the colony), and motility (with swarming motility reduced in 47.61% with ½ bark extract MIC, and 66.67% with bark extract MIC) on Rs. The application of the extract on humid substrate controlled Rs in lesser amounts than MIC (0.176 g/l). Bacterial motility has been described as a key feature for pathogenicity in Rs. In this context, the inhibitory effect of *M. azedarach* bark extract on bacterial motility, and its bactericidal activity when is applied on substrate (where Rs survives between growing seasons), showed that this natural product is a promising tool to be used as an efficient sustainable strategy for the control of bacterial wilt of tomato.



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# sciforum-115007: Botocidin: A novel bacteriocin produced by a nontoxic *C. botulinum* strain, HA5

**Mahmoud Naser Aldine**<sup>1,2,\*</sup>, Ehtesham Hashmi<sup>1</sup>, Joleen Masschelein<sup>2</sup> and Chris Michiels<sup>1</sup>

<sup>1</sup> Department of Microbial and Molecular Systems, Faculty of Bioscience Engineering, KU Leuven, Kasteelpark Arenberg 23—bus 2457, 3001 Leuven, Belgium

<sup>2</sup> Department of Biology, KU Leuven, VIB, Center for Microbiology, Kasteelpark Arenberg 31 - Bus 2438, 3001 Leuven, Belgium Email address: mahmoud.naseraldine@kuleuven.be

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The rise in antimicrobial resistance underscores the urgent need for novel antimicrobial compounds. Bacteria, including *Clostridium botulinum*, offer a promising natural reservoir for such discoveries. While *C. botulinum* is best known for its neurotoxin production, its potential as a source of antimicrobial compounds is largely unexplored. This study integrates next-generation sequencing (NGS), bioinformatics, and antimicrobial assays to identify novel antimicrobial compounds, focusing on bacteriocins—ribosomally synthesized and post-translationally modified peptides (RiPPs) targeting closely related species. The nontoxic *C. botulinum* HA5 strain exhibited antimicrobial activity against clostridial species, including toxigenic *C. botulinum*, *C. difficile*, and *C. perfringens*. Bioinformatics revealed multiple biosynthetic gene clusters (BGCs). Knockout experiments identified a spontaneous mutant lacking antimicrobial activity due to the loss of a plasmid (pHA5) encoding the botocidin biosynthetic gene cluster (a ranthipeptide or sactipeptide). This confirmed the role of botocidin in antimicrobial activity. The botocidin BGC comprised two operons: one encoding the core peptide, modification enzymes, an ABC transporter, and proteases, and the other, oriented oppositely, encoding an MFS transporter and a potential modification enzyme. The Spo0A transcription factor regulated the production operon, while the MFS transporter conferred immunity without affecting production. Botocidin production was confirmed via TCA precipitation and LC-MS, revealing peptide masses consistent with the predicted compound. Stability tests showed botocidin's heat stability (1 h at 90 °C), pH stability (pH 4–9), moderate UV sensitivity, and protease sensitivity. Time-lapse microscopy revealed bactericidal activity, causing cell lysis by targeting the membrane, as confirmed by propidium iodide staining. While botocidin did not inhibit spore germination, it blocked spore outgrowth sporestatically, as shown by plate counts and microscopy. These findings identify botocidin as the active antimicrobial in *C. botulinum* HA5, highlighting its potential to address antimicrobial resistance.



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## sciforum-115490: Celery (*Apium graveolens*) seed extracts: A natural and effective strategy against *Helicobacter pylori* infection.

Esther Rodríguez González <sup>1,\*</sup>, Mónica Rodríguez García Risco <sup>1</sup>, Jose Manuel Silvan Jimenez <sup>2</sup> and Adolfo Martinez Rodriguez <sup>2</sup>

<sup>1</sup> Department of Production and Characterization of Novel Foods. Institute of Food Science Research (CIAL, CSIC-UAM), Cantoblanco Campus, Autonomous University of Madrid, C/Nicolás Cabrera 9, 28049 Madrid, Spain.

<sup>2</sup> Microbiology and Food Biocatalysis Group (MICROBIO), Department of Biotechnology and Food Microbiology, Institute of Food Science Research (CIAL, CSIC-UAM), Cantoblanco Campus, Autonomous University of Madrid, C/Nicolás Cabrera 9, 28049 Madrid, Spain.

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*Helicobacter pylori* (*H. pylori*) is a significant human pathogen that infects approximately 50-70% of the global population. It colonizes the gastric epithelium, causing histological alterations that can lead to diseases ranging from chronic gastritis to gastric cancer. Recent research has focused on exploring bioactive compounds from natural sources as potential therapeutic strategies against *H. pylori* due to their promising biological properties. This study investigated bioactive extracts from celery seeds (*Apium graveolens*) to determine their potential antibacterial, antioxidant, and anti-inflammatory effects against *H. pylori*.

The extracts were obtained using various solvents through the solid-liquid extraction technique assisted by ultrasound, and both their chemical composition and biological activities, including antioxidant, anti-inflammatory, and antibacterial effects, against a specific strain of *H. pylori*, were evaluated.

The results showed that the chemical composition of the extracts varied depending on the solvent polarity employed, which also influenced their biological activity. Ethanolic extracts exhibited the highest phenolic content ( $42.8 \pm 0.9$  mg GAE/g extract) and were found to be rich in luteolin and apigenin derivatives, which are known for their potent antioxidant properties. In contrast, hexane extracts contained higher levels of essential oil compounds. Regarding biological activities, the ethanolic extract demonstrated the highest antioxidant activity (IC<sub>50</sub> 2 mg/mL), while aqueous and ethanolic extracts showed the strongest anti-inflammatory activity (90% NO production inhibition). Both ethanolic and hexane extracts exhibited the greatest antibacterial activity against *H. pylori* (MIC 0.005 mg/mL).

Based on these results, the ethanolic extract of celery seeds appears to be the most promising for use against *H. pylori* due to its chemical composition and potent antioxidant, anti-inflammatory, and antibacterial activities against this pathogen.



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# sciforum-115444: Deciphering microbiome dynamics and antibiotic resistome profiles in anaerobic bioreactors treating swine wastewater containing different sulfonamides

**Qinmao Zhou<sup>1,\*</sup>, Hebin Liang<sup>2</sup>, Jin Huang<sup>3</sup>, Uli Klümper<sup>4</sup>, Peiju Fang<sup>5</sup>, Zehui Yu<sup>5</sup> and Bing Li<sup>5</sup>**

<sup>1</sup> Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen City, Guangdong Province, China

<sup>2</sup> College of Resources and Environment, Anhui Agricultural University, Hefei City, Anhui Province, China

<sup>3</sup> Central South University, Changsha City, Hunan Province, China

<sup>4</sup> Institute for Hydrobiology, Technische Universität Dresden, Germany

<sup>5</sup> Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen city, Guangdong Province, China

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Residual antibiotics in swine wastewater (SW) promote antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs), threatening the environment and human health. Anaerobic digestion (AD) is a promising green technology for treating SW, but the impact of antibiotics on the microbial community and resistome during AD is unclear.

To address this gap, anaerobic batch experiments treating SW containing different sulfonamides (SMs) were conducted, with samples collected for DNA extraction and analysis of the microbial community and antibiotic resistome. ARG-carrying mobile genetic elements (MGEs) were detected, and ARG hosts were identified using metagenomic binning.

The results showed a significant negative correlation between the Abundance-based Coverage Estimator (ACE) index and the batches, with notable variability in the Shannon index. *Enterococcus* emerged as the dominant genus under SMs stress. A total of 24 types and 440 subtypes of ARGs were identified, with SMs stress significantly increasing the abundance of *sul1*, *sul2*, and *sul3*. The highest ARG abundance was observed in the sulfamonomethoxine (SMM) group during the initial stage and in the sulfamerazine (SMR) group after long-term anaerobic culture. Plasmid identification revealed that ARGs on chromosomes are much higher than those on plasmids. However, ARGs are more likely to co-occur on plasmids. Hybrid assembly identified two complete integrons and two composite transposons. Binning reconstructed 34 and 33 medium- to high-quality ARGs host genomes from all samples, determining their taxonomic classification and potential pathogenicity.

In conclusion, SMs stress intermittently disrupts the microbial community structure during AD. ARG composition and distribution are influenced by antibiotic pressure and initial ARGs in seed sludge. Prolonged antibiotic exposure increases the risk of ARG mobilization via MGEs. Specific ARB without ARGs but with inherent resistance also require attention. This study offers new insights into the occurrence and impact of antibiotics during the AD treatment of SW.



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# sciforum-112035: Dual-Action Antibacterial Mechanisms: Exploring Horseshoe-Crab AMPs and Phage Synergy Against Resistant Pathogens

Alaleh Valiallahi

Department of Biology, Alzahra University, Tehran, 16511393313, Iran

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The alarming rise of multidrug-resistant bacteria has intensified the search for alternative therapies capable of overcoming these global challenges. Antimicrobial peptides (AMPs) derived from horseshoe crabs represent a promising avenue due to their potent ability to disrupt bacterial membranes and dismantle protective biofilms. Simultaneously, bacteriophages, natural bacterial predators, have demonstrated remarkable precision in targeting and lysing specific bacterial pathogens. This study investigates the potential for combining these two agents to enhance their efficacy against MDR bacteria.

AMPs effectively weaken bacterial defense mechanisms, creating vulnerabilities that allow bacteriophages to penetrate biofilms and access deeper bacterial populations. This interaction not only enhances the bactericidal activity of phages but also reduces the likelihood of bacterial resistance, as pathogens must counteract multiple distinct mechanisms simultaneously. Preliminary findings indicate that such a synergistic approach could prove particularly effective in addressing biofilm-associated infections and chronic conditions that remain unresponsive to conventional antibiotics.

By exploring the interplay between horseshoe-crab-derived AMPs and bacteriophages, this research highlights a novel strategy to combat antibiotic resistance. The integration of these agents offers a promising therapeutic framework, with the potential to redefine infection management and provide new hope in the fight against MDR pathogens. Future investigations should focus on optimizing these combinations for clinical application, ensuring both safety and efficacy in diverse medical settings.



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# sciforum-115271: Effect of atmospheric pollution on the taxonomic composition, diversity, and antibiotic resistance of the culturable bacterial community of the aerobiome in the Región Metropolitana of Chile.

**Madeline Mejías<sup>1,2,\*</sup>, Romina Madrid<sup>1</sup>, Karina Díaz<sup>1</sup>, Diego Varas<sup>1</sup>, Williams López<sup>2</sup>, Javiera Pozo<sup>2</sup>, Rodrigo Pulgar<sup>2</sup> and Dinka Mandakovic<sup>1</sup>**

<sup>1</sup> Laboratorio de Microbiología Molecular Ambiental, GEMA Center for Genomics, Ecology and Environment, Universidad Mayor, Santiago, 8580745, Chile

<sup>2</sup> Laboratorio de Genómica y Genética de Interacciones Biológicas (LG2IB), Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, 7830490, Chile

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The bacteriome varies according to environmental conditions, such as pollution. These atmospheric variables also generate genetic and phenotypic changes in them, including antibiotic resistance. One way to study the bacteriome is using culturable communities, allowing the evaluation of changes in the community through controlled interventions. Our objective was to characterize and analyze the antibiotic resistance profile of the cultivable bacteriome of the Región Metropolitana. For this purpose, active air collections were obtained in Petri dishes with culture media from two areas of the Región Metropolitana that were differentially contaminated. After 7 days of growth, the composition and diversity were evaluated by high-throughput sequencing of the bacterial members. In addition, the resistant bacteriome was sampled in culture media supplemented with antibiotics at selective concentrations, and the multi-resistance quality of the isolates was evaluated. Finally, kanamycin-resistant isolates were selected and grown in a liquid medium supplemented with particulate matter, and their tolerance to antibiotics and the abundance of resistance gene transcripts were evaluated. The sequencing of the bacterial community allowed us to detect 170 bacterial ASVs. The most abundant bacterial phyla in the samples were Proteobacteria and Firmicutes. Although there were no significant differences in the alpha diversity of the cultivable bacteriome samples, there were significant differences in beta diversity. Also, we obtained 59 bacterial isolates resistant to antibiotics, of which more than 37% were multi-resistant. We detected resistance to aminoglycosides, beta-lactams, phenicols, glycopeptide antibiotics, and quinalones. Bacterial isolates subjected to particulate matter significantly increased their tolerance to kanamycin, which could be explained by an increase in the relative abundance of the antibiotic-resistance genes *qacH* and *ampC*. This study allowed us to determine the effects of pollution on the air bacterial community and antibiotic resistance, contributing to the generation of knowledge in the areas of microbial ecology and environmental sciences.



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## sciforum-115844: Evaluating the bioactive and extracellular enzymatic potential of fungal endophytes isolated from the leaves of *Asparagus densiflorus*

Mogoshadi Moshapo, Raymond Tshepiso Makola and Khumiso Dithebe \*

Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Private Bag X1106, Sovenga, 0727

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The emergence of new pathogens and the increased rise in antimicrobial resistance warrants the search for sources of novel therapeutic agents. Even though secondary metabolites with notable biological activities have been isolated from medicinal plants, it has been shown that some of these metabolites originate from the endophytic fungi associated with the plants. The capabilities of endophytic fungi are not limited to the production of secondary metabolites, these microorganisms are able to produce industry-significant enzymes with broad applications. In this study, four (4) endophytic fungi isolated from *Asparagus densiflorus* leaves were screened for their antimicrobial, antioxidant and extracellular enzymatic activities. Sequencing of the ITS region revealed that all four isolates belong to the phylum Ascomycota. The antimicrobial activity of the fungal endophytes crude extracts was tested against *Psuedomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* where MIC values ranged from 0.63 to 1.25 mg/ml. The ferric reducing antioxidant power assay revealed that the crude extracts demonstrated minimal antioxidant activity. All the isolates except AspL-3 were observed to possess laccase activity, however only AspL-3 possesses the protease activity. All the isolates were observed to possess peroxidase activity. The results obtained in this study further highlights that medicinal plants are an excellent source of fungal endophytes significant biological and biotechnological potential.



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# sciforum-115779: Evaluating the Efficacy of Dielectric Barrier Discharge Plasma Against Planktonic and Biofilm Cultures of *Staphylococcus aureus*

Irena Maliszewska \* and Anna Zdubek

Department of Organic and Medicinal Chemistry, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

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## Background

It is well-documented that numerous pathogenic microorganisms present in hospital settings exhibit diminished susceptibility to various biocidal agents, including but not limited to hydrogen peroxide, alcohols, chlorhexidine, iodophors, triclosan, and benzalkonium compounds. Previous reports have also highlighted the cross-resistance between biocide exposure and bacterial resistance to antibiotics. Therefore, it is necessary to search for effective methods of pathogen eradication. One of such effective methods is nonthermal plasma. It seems that due to the complex and diverse mechanisms of pathogen destruction, the development of bacterial resistance/tolerance to this eradication method is unlikely.

The aim of this study was to evaluate the effect of sublethal exposures of *S. aureus* to nonthermal plasma on some cell surface properties, biofilm formation efficiency, and the possibility of inducing tolerance of pathogenic bacteria to this biocidal technique.

## Methods

Plasma inactivation of *S. aureus* inoculated on the surface of glass plates was performed using a DBD reactor operating at atmospheric pressure with air as working gas. The effects of multiple exposures to sublethal doses of plasma on the ability of cells to auto-aggregate, surface hydrophobicity, the ability to form biofilm on different surfaces, and changes in decimal reduction time (D) were assessed.

## Results

Exposure of planktonic bacteria to nonthermal plasma for 2 min and 11 s results in 90% bacterial mortality (D). Destruction of the biofilm formed on the glass surface turned out to be more difficult. It was shown that the auto-aggregation properties and the hydrophobicity of cells significantly decreased after plasma treatments compared to control cells and the degree of these changes increased with the number of exposures to plasma. These changes in the surface structures of bacteria suggested that the ability of these pathogens to form biofilms may be limited. Our results showed that DBD plasma remained an effective biocidal method even after multiple exposures.



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# sciforum-111321: Exploring Beer By-products as Novel Antibacterial Ingredients for Health Care Products

**Carolina Benera<sup>1,\*</sup>, Patrícia Branco<sup>2,3</sup>, Adília Charmier<sup>2</sup>, Catarina Pinto Reis<sup>4,5</sup> and Elisabete Muchagato Maurício<sup>2,6,7</sup>**

<sup>1</sup> Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal

<sup>2</sup> BIORG—Bioengineering and Sustainability Research Group, Faculdade de Engenharia, Universidade Lusófona, Av. Campo Grande 376, 1749-024 Lisbon, Portugal

<sup>3</sup> Linking Landscape, Environment, Agriculture and Food (LEAF), Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>4</sup> Instituto de Biofísica e Engenharia Biomédica (IBEB), Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal

<sup>5</sup> Nanomedicine and Biomedical Imaging Group, Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal

<sup>6</sup> CBIOS—Research Center for Biosciences & Health Technologies, Universidade Lusófona, Campo Grande 376, 1749-024 Lisbon, Portugal

<sup>7</sup> Elisa Câmara, Lda, Dermocosmética, Centro Empresarial de Talaíde, n°7 e 8, 2785-723 Lisbon, Portugal

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Beer is a widely consumed carbonated beverage made from natural ingredients, including malted cereal, hops, yeast and water. It is rich in nutrients and contains carbohydrates, minerals, vitamins, amino acids and polyphenols. The brewing process, however, generates a significant amount of solid waste, including hot trub, a slurry of entrained wort, hop particles and mainly unstable high-molecular-weight colloidal proteins that coagulate during the boiling of the wort. Given the environmental impact of agro-industrial waste, finding sustainable methods to reuse this waste by transforming it into bio-products is crucial.

The aim of this study was to assess the potential of converting these by-products into biologically active extracts suitable for use as functional ingredients in cosmetic and pharmacological formulations. The extracts consisted of one alcoholic and one hydroalcoholic extract prepared by Soxhlet extraction and one hydroalcoholic extract prepared by maceration. The extracts' antibacterial activity against the Gram-positive bacteria *S. aureus*, *S. aureus* (MRSA), *S. epidermidis*, *B. cereus*, *E. faecalis*, *S. mitis*, *S. pyogenes*, and *S. mutans*, as well as against the Gram-negative bacteria *E. coli* and *P. aeruginosa* and against the yeast *C. albicans*, was determined by the agar diffusion method and the minimum inhibitory concentration (MIC) by the microdilution method. The minimum inhibitory concentration and inhibition zones values obtained showed significant inhibitory effects against the Gram-positive bacteria tested, with the Soxhlet extracts presenting the best inhibitory results for both hydroalcoholic and alcoholic extracts. In both methods, the extracts showed the best antibacterial activity against the microorganisms *S. epidermidis* and *B. cereus*, with an MIC of 0.63 and 0.31 mg/mL, respectively. The tested extracts seem to be promising, low-cost antibacterial agents that can be incorporated in cosmetic and pharmacological formulations. Additional studies will be conducted to assess their antioxidant activity and safety by using in vitro and in vivo models and investigating the chemical composition of the extracts.



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# sciforum-115519: Exploring the biopotential of secondary metabolites derived from fungal endophytes of *Kirkia acuminata* stems for drug discovery

**Mfundo Magagula<sup>1,\*</sup>, Thabiso Eric Motaung<sup>2,3</sup>, Zukile Mbita<sup>1</sup> and Khumiso Dithebe<sup>1</sup>**

<sup>1</sup> University of Limpopo, Department of Biochemistry, Microbiology and Biotechnology, Private Bag X1106, Sovenga 0727, Polokwane, Limpopo, South Africa

<sup>2</sup> University of Pretoria, Department of Biochemistry, Genetics and Microbiology, Private Bag X20, Hatfield 0028, Pretoria, Gauteng, South Africa

<sup>3</sup> University of Pretoria, Forestry and Agricultural Biotechnology Institute, Hatfield 0083, Pretoria, Gauteng, South Africa  
University of Pretoria, Forestry and Agricultural Biotechnology Institute, Hatfield 0083, Pretoria, Gauteng, South Africa

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Antimicrobial resistance (AMR) is a global threat that has led to nearly incurable ailments, with prolonged disease occurrences being linked to cancer development. Fungal endophytes from medicinal plants produce diverse natural compounds that can combat the progression of AMR pathogens. *Kirkia acuminata* is an overharvested African medicinal plant that is used to treat various ailments, such as diarrhoea, toothache and thrush; however, there is little knowledge on the biological potential of its fungal endophytes. Thus, the current study aimed to assess the antimicrobial, cytotoxicity and anticancer properties of fungal endophytes associated with *K. acuminata* stems. Culturable fungal endophytes from surface-sterilised stems of *K. acuminata* were identified through sequencing of the internal transcribed spacer (ITS) region. Ethyl acetate extracts of the isolates were assessed for their antimicrobial activity using the broth microdilution assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxic profiles of the crude extracts against non-cancerous human embryonic kidney (HEK-293) cells. Moreover, the alamarBlue assay was used to screen the anticancer activity of the non-cytotoxic extracts against cervical cancer ME-180 and melanoma A375 cancerous cells. A total of 18 morphologically distinct isolates distributed among the genera *Diaporthe*, *Neofusicoccum* and *Pseudofusicoccum* were obtained. Seventeen out of the eighteen isolates possessed inhibitory activity against *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with overall MIC values between 0.31 and 2.5 mg/mL. However, only four isolates, *D. macadamiae* KaS-4, *D. neotheicola* KaS-6, *N. parvum* KaS-3 and *P. olivaceum* KaS-5, were non-cytotoxic against the non-cancerous HEK-293 cells and exhibited moderate inhibitory activity against the ME-180 and A375 cancerous cells. Thus, this study highlights the potential of fungal endophytes from *K. acuminata* in producing safe natural compounds with antimicrobial and anticancer action for drug discovery.



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# sciforum-115421: Extended-spectrum $\beta$ -lactamase-encoding genes in the pig production chain in Brazil

Gracielle Rodrigues Pereira <sup>1,\*</sup>, Marcelo Luiz Lima Brandão <sup>2</sup>, Ana Beatriz Portes <sup>3,4</sup> and Bernardete Ferraz Spisso <sup>5</sup>

<sup>1</sup> Oswaldo Cruz Foundation

<sup>2</sup> Instituto de Tecnologia em Imunobiológicos/ Fundação Oswaldo Cruz (BioManguinhos/Fiocruz), Rio de Janeiro, Brazil

<sup>3</sup> Instituto de Microbiologia Paulo de Góes/ Universidade Federal do Rio de Janeiro (IMPG/UFRJ), Rio de Janeiro, Brazil

<sup>4</sup> Laboratory of Advanced Analysis in Biochemistry and Molecular Biology (LAABBM), Department of Biochemistry, Federal University of Rio de Janeiro (UFRJ), RJ, Brazil

<sup>5</sup> Instituto Nacional de Controle de Qualidade em Saúde/ Fundação Oswaldo Cruz (INCQS/Fiocruz), Rio de Janeiro, Brazil

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Animal husbandry is a key promoter of antibiotic resistance (AMR) and contributes to the spread of AMR genes throughout the environment and food supply. The pig industry is one of the leading sectors in animal-based food production worldwide, with Brazil ranking fourth in terms of pork production and export. The emergence of extended-spectrum  $\beta$ -lactamase (ESBL)-producing bacteria in this sector represents a significant public health concern. These enzymes confer resistance to a broad range of  $\beta$ -lactams, threatening the effectiveness of antibiotic therapy for infections in both humans and animals. A systematic review was conducted to address the current knowledge of AMR within the Brazilian pig production chain. The presence of ESBL-encoding genes in bacteria isolated from pig feces, intestine, carcass, and urine, as well as pork-based food, was investigated. TEM-type genes were detected in 11 of the 12 studies, including blaTEM-1A (n=33 strains) and blaTEM-1B (n=44 strains). Both genes were found in *Salmonella* isolated from all sample categories. Additionally, blaTEM-1 was detected in *E. coli* (n=4 strains from feces) and *Salmonella* spp. (n=2 from carcass; n=5 from feces). CTX-type genes were detected in *Proteus mirabilis* (blaCTX-M-65; n=6 from food) and *Salmonella* (blaCTX-M-8; n=1 from feces; n=1 from pig carcass). blaCMY-2 was detected in *Salmonella* from feces (n=1) and carcass (n=1), whereas blaCMY-M2 was detected in *E. coli* (n=5) from pig feces. blaPSE-1 and blaSHV-1 genes were investigated using PCR in *E. coli* strains isolated from pig feces; however, these were not detected. In conclusion, the pig industry may contribute to the spread of ESBL-encoding genes, posing a public health risk. Data reveal a significant presence of blaTEM genes across various samples, alongside other ESBL-encoding gene types in the Brazilian pig production chain. These findings highlight the need for integrated strategies to control AMR, particularly to reduce risks that may arise from animal -based food production systems.



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# sciforum-115345: From Desert to Lab: Evaluating Camel Urine's Antibacterial Efficacy Against Carbapenem-Resistant Gram-Negative Bacilli

**Amina Ressmi<sup>1,\*</sup>, Habiba Raqraq<sup>1</sup>, Rafiq Aniba<sup>1</sup>, Asmaa Dihmane<sup>1</sup>, Nabila Soraa<sup>2</sup> and Abouddihaj Barguigua<sup>1</sup>**

<sup>1</sup> Team of Biotechnology and Sustainable Development of Natural Resources, Biology-Geology Department, Polydisciplinary Faculty, Sultan Moulay Slimane University, Beni Mellal 23023, Morocco

<sup>2</sup> Faculty of Medicine and Pharmacy of Marrakech Cadi Ayyad University, Microbiology Department, Mohamed VI University Hospital Center, Marrakech, 40000, Morocco

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Antimicrobial resistance currently contributes to over seven million deaths annually worldwide, and that number is projected to rise to ten million by 2050. The increasing prevalence of multidrug-resistant (MDR) bacteria, compounded by the widespread misuse of antibiotics, underscores the urgent need for alternative treatment strategies. Natural products, including camel urine, are emerging as promising candidates to combat this growing public health crisis. By exploring these alternative agents, we may be able to effectively address multidrug resistance and improve global health outcomes.

Using the well diffusion method, sterilized camel urine from male and female camels was tested against eight multidrug-resistant bacterial strains isolated from clinical samples. The strains were first identified through phenotypic characterization on selective media, followed by confirmation using MALDI-TOF mass spectrometry and the BD Phoenix system.

Our findings revealed that female camel urine exhibited a stronger antibacterial effect than male camel urine. Specifically, the largest inhibition zones were observed on Mueller–Hinton agar, with diameters of 32 mm for NDM-1-producing *Enterobacter cloacae*, 30 mm for NDM-1-producing *Klebsiella oxytoca*, 29 mm for VIM-producing *Acinetobacter baumannii* and *Citrobacter freundii*, 25 mm for OXA-48-producing *Pseudomonas aeruginosa* and *Escherichia coli*, and 22 mm for NDM-1-producing *Klebsiella pneumoniae*. The minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were ranging from 0.08 to 12.5mg/l and from 0.8 to 25 mg/l, respectively.

This investigation demonstrated that sterilized female camel urine exhibits significant in vitro antibacterial activity against various carbapenem-resistant bacteria isolated from clinical samples which may serve as a promising source of new compounds to combat bacterial infections.



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# sciforum-115041: Gut dysbiosis promotes dissemination of antimicrobial resistance genes

**Jiewen Guan<sup>1,\*</sup>, Mingsong Kang<sup>1</sup>, Calvin Lau<sup>2</sup>, Catherine Carrillo<sup>2</sup> and Edward Topp<sup>3</sup>**

<sup>1</sup> Ottawa Laboratory (Fallowfield), Canadian Food Inspection Agency, Ottawa, Ontario, Canada

<sup>2</sup> Ottawa Laboratory (Carling), Canadian Food Inspection Agency, Ottawa, Ontario, Canada

<sup>3</sup> Agroecology research unit, INRAE, University of Burgundy, Dijon, France

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**Introduction:** The global spread of antimicrobial resistance (AMR) genes (ARGs) poses a major challenge to bacterial infection treatment. Antibiotic use, while targeting pathogens, also disrupts commensal gut bacteria, with effects varying by antibiotic type. The impact of gut dysbiosis on ARG mobility from foodborne bacteria, especially with or without food matrix microbes, remains unclear.

**Methods:** Using a murine model, we investigated ARG dissemination via mobile AMR plasmids. Mice were pre-treated with streptomycin, ampicillin, or sulfamethazine to induce varying levels of gut dysbiosis. They were then inoculated with beta-lactam-resistant *Salmonella* Heidelberg (donor) and beta-lactam-susceptible *Salmonella* Typhimurium (recipient) with or without additional food matrix microbes. Fecal samples were cultured to detect ARG transfer among *Salmonella*, *E. coli*, and other gut bacteria, which were confirmed using whole-genome sequencing. Changes in gut microbiota were assessed using 16S rRNA sequencing.

**Results:** Without background food matrix microbes, streptomycin caused severe gut dysbiosis, enhancing AMR plasmid transfer from *S. Heidelberg* to *S. Typhimurium* and *E. coli*. Ampicillin induced moderate dysbiosis, allowing *S. Heidelberg* colonization and plasmid transfer to *E. coli*. Sulfamethazine caused mild dysbiosis, hindering both *Salmonella* colonization and plasmid transfer. In contrast, food matrix microbes reduced AMR plasmid transfer, except in streptomycin pre-treated mice, where Enterobacteriaceae enrichment enabled AMR plasmid transfer to *Escherichia*, *Enterobacter*, *Citrobacter*, and *Proteus*.

**Conclusions:** Pre-existing gut microbiome disturbances from antibiotics significantly affect ARG dissemination. These findings support more judicious antibiotic use to mitigate resistance spread.



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# sciforum-111354: Potential sustainable antagonistic biocontrol strategy against *Xanthomonas vesicatoria*

Belén Álvarez<sup>1,2,\*</sup>, Isabel Salas<sup>1</sup>, Thais Castellón<sup>1</sup> and Elena G. Biosca<sup>1</sup>

<sup>1</sup> Departamento de Microbiología y Ecología, Universitat de València, 46100 Valencia, Spain.

<sup>2</sup> Área de Investigación Aplicada y Extensión Agraria, IMIDRA, 28805 Madrid, Spain.

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The use of microorganisms with antagonistic activity against one or more pathogens can be an alternative to chemical control in agroecosystems. In this work, the potential for biocontrol by antagonism of a collection of environmental bacterial isolates against a phytopathogenic bacterium of *Solanaceae*, *Xanthomonas vesicatoria*, which causes bacterial spot in tomato and pepper, was evaluated. Control of this disease is usually limited by the lack of resistant hosts, the threat to the environment and the global health risks posed by the continued use of chemical agents.

Bacteria were isolated from different environmental samples from disease-free areas and tested for their in vitro antagonistic capacity against a reference strain of *X. vesicatoria*. The isolates with the greatest antagonistic activity were selected for initial ex vivo assays to determine this activity in plant material against the reference strain, using leaves from tomato plants of a variety sensitive to bacterial spot. The isolates with the greatest activity were further characterized for their capacity to produce hydrolytic enzymes, such as proteases, lipases, amylases and DNases, and for their nitrogen fixation capacity.

Overall, the results revealed the presence of bacterial isolates able to inhibit the growth of *X. vesicatoria* in vitro and reduce the severity of bacterial spot symptoms ex vivo, in addition to producing different hydrolases related to biological control and biotechnological use, and fixing molecular nitrogen. The prospects for improving biological control strategies against bacterial spot in tomatoes and peppers caused by *X. vesicatoria* are promising, thus promoting sustainable agricultural production that favors ecosystem balance.

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# sciforum-111092: RecA Inhibitor Mitigates Bacterial Antibiotic Resistance

**Jin Ma\***, Keke Shang, Liwen Xu, Qing-Yu He and Gong Zhang

Key Laboratory of Functional Protein Research of Guangdong Higher Education Institutes and MOE Key Laboratory of Tumor Molecular Biology, Institute of Life and Health Engineering, Jinan University, Guangzhou, China

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Bacterial antibiotic resistance (AR) has become a critical global health threat. AR is mainly driven by adaptive resistance mutations and the horizontal gene transfer of resistance genes, both of which are enhanced by genome recombination. We have previously discovered that genome recombination-mediated tRNA up-regulation is important for AR especially in the early stages. RecA is the most important genome recombination factor. Therefore, RecA inhibitors should be effective in reducing AR. In this study, we found that BRITE338733 (BR), a RecA inhibitor, can prevent ciprofloxacin (CIP) resistance in subculturing *Escherichia coli* strain BW25113 in the early stages (up to the 7<sup>th</sup> generation). In the presence of BR, the tRNA was decreased, so the bacteria cannot evolve resistance via the tRNA up-regulation-mediated AR mechanism. The RecA expression level was also not increased when treated with BR. Transcriptome sequencing revealed that BR causes a down-regulation of ATPase activity and therefore suppresses the energy state of bacteria. Also, the effective concentrations of BR do not harm human cell viability, indicating its clinical safety. These findings demonstrate that BR effectively delays the emergence of spontaneous antibiotic resistance by targeting RecA-mediated pathways. Our findings shed light on a new strategy to counteract the clinical AR: applying BR with the antibiotics together at the beginning.



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## sciforum-110651: Silent mupirocin resistance in methicillin-resistant *Staphylococcus aureus*

**Martyna Kasela**<sup>1,\*</sup>, Weronika Słotwińska<sup>2</sup>, Sylwia Andrzejczuk<sup>1</sup> and Agnieszka Grzegorzczuk<sup>1</sup>

<sup>1</sup> Department of Pharmaceutical Microbiology, Medical University of Lublin, 20-093 Lublin, Poland

<sup>2</sup> Student's Scientific Circle at the Department of Pharmaceutical Microbiology, Medical University of Lublin, 20-093 Lublin, Poland

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*Staphylococcus aureus*, including methicillin-resistant strains (MRSA), is a bacterium responsible for multiple types of human infections. The discrepancy between phenotypic and genotypic resistance is called silencing of antibiotic resistance by mutation (SARM), while the genes are called silent or cryptic. The presence of SARM bacteria carries a risk of implementing an ineffective antimicrobial drug in therapy, as the gene would not be activated until therapy. This study aimed to determine the frequency of SARM and its genetic mechanisms. The virulence and sequence type of the SARM strains were also characterized.

In total, 334 *S. aureus* strains were investigated for phenotypic resistance to cefoxitin, erythromycin, tetracycline, gentamycin, and mupirocin. PCR was used to screen for the presence of corresponding antibiotic-resistance genes. SARM isolates were further characterized by the detection of genes encoding toxins, adhesins, types of *SCCmec* cassette, and genotyping with MLST. Silenced genes were sequenced to determine the genetic defect and its consequences for protein translation.

The analysis showed the presence of SARM in 0.6% of *S. aureus* strains (2/334). In both cases, they were strains harbouring the *mupA* gene (resistance to mupirocin). Sequencing showed the presence of a deletion, resulting in the incorrect translation of the nucleotide into an amino acid sequence, shortening the amino acid chain and inhibiting the synthesis of the protein responsible for mupirocin resistance.

Mupirocin is an antibiotic applied in the treatment of staphylococcal infection of the skin, including the eradication (the removal of a microorganism from the body) of *S. aureus* from the nasal cavity. Despite the fact that the analysis showed a low share of SARM (1%), there is still a risk of antimicrobial therapy failure and reinfection. Further studies should also focus on determining factors that increase the probability of activation of silent genes responsible for resistance to mupirocin.



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# sciforum-115801: Study of the antimicrobial activity of chitosan from insects against Gram-positive and Gram-negative bacteria

Elkadaoui Soukaina <sup>1,2</sup>

<sup>1</sup> Interdisciplinary Research Laboratory in Bioresources Environment and Materials (LIRBEM), ENS, Cadi Ayyad University, Hay Hassani, 40000 Marrakech, Morocco

<sup>2</sup> Bioresource and Food Safety Laboratory, Faculty of Sciences and Technologies, Cadi Ayyad University, 112 Boulevard Abdelkrim Al Khattabi, 40000 Marrakech, Morocco

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This work studies the antibacterial activity of chitosans prepared with controlled physicochemical characteristics against Gram-positive and Gram-negative reference bacteria. The optimal conditions in acetic acid and chitosan concentration were determined, and the disk diffusion method was used to evaluate the antimicrobial activity. All prepared chitosans, P CHTs, show an inhibition zone that varies between 14mm and 18mm depending on the strain and the physicochemical characteristics of the chitosan (degree of acetylation, DA; molar mass, Mv; and surface structure). These chitosans demonstrate enhanced antibacterial activity compared to the tested commercial chitosans, C CHTs. The effect of DA is particularly interesting. P CHTs exhibit significantly better antibacterial activity than C CHTs, especially against Gram-negative bacteria, where the improvement can reach 100%. Totally deacetylated chitosan (DA=0%) provided the highest inhibition diameter and the lowest MIC/MBC values. Also, PCHTs with Mv>80000 g/mol showed relatively high antimicrobial activity compared to Mv 20000 g/mol. Interference of the surface structure with the effects of other parameters (Mv, DA, and strain) was observed. The antibacterial activity of the prepared chitosan (DA=0%) against *E. faecalis* was improved by 21% and 6% compared to Gentamicin and Ciprofloxacin. For the other bacteria, this chitosan provides 68 to 86% of the activity of Gentamicin and 49 to 80% of that of Ciprofloxacin.



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# sciforum-115413: Targeting the Resistant F533L Mutant in PBP3 of *Pseudomonas aeruginosa*: A Computational Approach Using ZnO and CuO Nanoparticles

**Badr-Edine Sadoq**<sup>1,\*</sup>, **Mohamed Sadoq**<sup>2</sup>, **Yassir Boulaamane**<sup>1</sup>, **Mohammed Reda Britel**<sup>1</sup>, **Adel Bouajaj**<sup>1</sup> and **Amal Maurady**<sup>1,3</sup>

<sup>1</sup> Laboratory of Innovative Technologies, National School of Applied Sciences of Tangier, Abdelmalek Essaadi University, Tetouan, Morocco

<sup>2</sup> Laboratory of Chemistry and Biology Applied to the Environment, URL-CNRST-N°13, Faculty of Sciences, Moulay Ismail University, 50050 Meknes, Morocco

<sup>3</sup> Faculty of Sciences and Techniques of Tangier, Abdelmalek Essaadi University, Tetouan, Morocco.

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**Introduction:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is a multidrug-resistant opportunistic pathogen frequently encountered in clinical settings, posing substantial challenges for effective treatment. Its resistance often arises from mutations in critical drug targets, such as penicillin-binding protein-3 (PBP3), a key  $\beta$ -lactam target essential for bacterial survival. The F533L mutation is linked to resistance against multiple antibiotics, underscoring the need for alternative therapeutic strategies. **Methods:** This study employed molecular docking to analyze the interactions between zinc oxide (ZnO) and copper oxide (CuO) nanoparticles (NPs) and the mutated PBP3 (F533L) of *P. aeruginosa*. Additionally, computational approaches were used to assess the impact of the F533L mutation on the structural stability of PBP3. Binding energy and interaction analyses were conducted to evaluate the PBP3-nanoparticle complexes. **Results:** The F533L mutation was found to alter the stability of PBP3, potentially contributing to resistance mechanisms. Molecular docking revealed that ZnONPs and CuONPs demonstrated binding energies of -2.15 kcal/mol and -1.48 kcal/mol, respectively. Both nanoparticles formed hydrogen bonding interactions with the mutated residue 533L, highlighting their potential to mitigate resistance. **Conclusions:** The findings suggest that ZnO and CuO nanoparticles could serve as promising therapeutic agents against antibiotic resistance in *P. aeruginosa*, particularly targeting the F533L mutation in PBP3. Further experimental and in vitro studies are essential to validate their therapeutic efficacy and broader applications.



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# sciforum-115637: The use of metal ions as potential inhibitors of ferrochelatase

**Anna Zdubek**\* and Irena Maliszewska

Department of Organic and Medicinal Chemistry, Wrocław University of Science and Technology

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## Introduction

The need for alternative techniques of microbial control has been determined by the rapid spread of antibiotic-resistant bacteria. One approach that is gaining popularity is antimicrobial photodynamic therapy (aPDT). This method involves the use of a chemical compound (photosensitizer), which is activated by light in the presence of molecular oxygen. The activated photosensitizer induces the production of reactive oxygen species (ROS), which leads to cell destruction. Significant attention has been directed toward the application of 5-aminolevulinic acid (5-ALA) in aPDT. This compound occurs naturally in cells, where it is converted mainly to the intracellular photosensitizer protoporphyrin IX (PpIX). Over time, PpIX is transformed into heme (which does not possess photosensitizing properties) by the ferrochelatase enzyme [1]. An increased concentration of PpIX in cells can be achieved by inhibiting ferrochelatase with divalent metal ions (e.g.,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ) [2].

## Methods

The aim of this study is to evaluate the combined effect of 5-ALA (2.5 mM), light, and metal ions on the photosensitization of bacteria. The microorganism tested was antibiotic-resistant *Proteus mirabilis* (PCM 543). Irradiation was performed with a 404 nm diode laser (the light dose was 23.5 J cm<sup>-2</sup>). For the research, ions of manganese (1 mM), zinc (1 mM), calcium (10 mM), and magnesium (10 mM) were used.

## Results

The most effective approach was found to involve the use of 5-ALA with zinc ions, resulting in the destruction of almost 99% of bacterial cells, while the use of 5-ALA alone led to the eradication of approximately 90% of cells after the same exposure time.

## Conclusions

The results obtained suggest that metal ions, especially zinc, may compete with iron ions for the ferrochelatase binding site, leading to the inhibition of the enzyme and, at the same time, the accumulation of PpIX in cells.

## References

- [1] doi:10.3390/ijms25073590
- [2] doi:10.1074/jbc.M803372200



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# sciforum-109978: Thymoquinone as a Potential Antimicrobial Agent against Resistant *Pseudomonas aeruginosa*: A Computational Chemistry Approach

Goran Slivšek<sup>1,2,3,\*</sup>, Sandra Mijač<sup>3,4</sup>, Nives Matković<sup>5</sup>, Marija Fabijanec<sup>3</sup>, Ivan Dolanc<sup>1</sup>, Marin Marinović<sup>2</sup>, Silvija Petković<sup>2</sup>, Renato Mautner<sup>2</sup>, Donatella Verbanac<sup>3</sup> and Miran Čoklo<sup>1</sup>

<sup>1</sup> Institute for Anthropological Research, Zagreb, Croatia

<sup>2</sup> Faculty of Medicine, University of Rijeka, Rijeka, Croatia

<sup>3</sup> Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

<sup>4</sup> Children's Hospital Srebrnjak, Zagreb, Croatia

<sup>5</sup> University Hospital of Split, Split, Croatia

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## Introduction

Antimicrobial resistance is a significant public health challenge worldwide, especially in Gram-negative bacteria such as *Pseudomonas aeruginosa*. In recent years, *P. aeruginosa* has shown increasing multi- and pan-drug resistance, even to colistin, which is considered to be the last line of defence against resistant bacteria. Black cumin or *Nigella sativa* L., globally used as a spice, contains the bioactive compound thymoquinone, which is promising as a natural antibacterial agent due to its chemical properties. The high cost and complexity of developing new antibiotics is a significant obstacle and often leads to high attrition rates. However, computational chemistry is a promising approach that optimises the selection of candidates and supports the development of targeted antibacterial agents. This can speed up the discovery process and reduce costs in the ongoing fight against antimicrobial resistance.

## Methods

The pharmacokinetic properties of thymoquinone and its pharmacological potential and bioavailability were computationally evaluated using the SwissADME tool. The topologies of the receptor proteins were analysed using the CASTp web server, and triple molecular docking simulations were performed using AutoDock Vina 1.1.2. Molecular visualisation and analysis were performed with PyMol and DS Visualizer.

## Results

Thymoquinone fulfils all of Lipinski's rules of five criteria, which indicates favourable pharmacokinetic properties for potential therapeutic use. Molecular docking simulations show a strong binding affinity to the MvfR protein in *P. aeruginosa*, which is an important regulator of virulence and quorum sensing. Seven (out of nine) models showed consistent interactions with a root mean square deviation (RMSD) of less than 5 Å, often involving the amino acid Ile236.

## Conclusions

Computational chemistry can enhance the development of new antibiotics. Thymoquinone shows favourable pharmacokinetics and a strong binding affinity to the MvfR protein in *P. aeruginosa*. Thymoquinone shows potential as an antimicrobial agent against *P. aeruginosa*, but further in vitro and in vivo tests, including toxicological studies, are needed to evaluate its cytotoxicity.



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# sciforum-111227: Use of medicinal plants for treatment and prophylaxis of canine parasitic infections: a literature review

Patrícia Santos<sup>1,\*</sup>, Mariana Sousa<sup>2</sup> and Agostinho Cruz<sup>3</sup>

<sup>1</sup> Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal

<sup>2</sup> Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 4200-072, Porto, Portugal LAQVIREQUIMTE, Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Po

<sup>3</sup> LAQVIREQUIMTE, Escola Superior de Saúde, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

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## Introduction

Dogs have been perceived as providing companionship to all members of the family, yet they can act as a vector for the transmission of parasitic agents to humans, particularly those who own or care for them. Traditional medicine seem to demonstrate effects in the treatment of diseases in animals, however, it is necessary to prove their effectiveness using more recent scientific evidence.

## Objective

The objective is to identify medicinal plants commonly used in the treatment and prophylaxis of parasitic infections in dogs.

## Methods

A literature review was conducted by gathering data from the MEDLINE database, using the following search equations: “(“Plants”[Mesh] OR (“Plants/parasitology”[Mesh]) AND (“Phytotherapy/veterinary”[Mesh]))”; “Medicinal Plants Treatment for Infested Dogs”; “Parasitic Infections in Dogs Treatment with Plants”. The selection criteria established ensured that only articles published between 2000 and 2023, which referenced the utilisation of medicinal plants in the management and prevention of parasitic infections in canines, were included. Furthermore, review articles and articles that referenced pharmacological treatments or treatments for other comorbidities were excluded.

## Discussion and Conclusion

A total of 26 articles were selected, resulting in the identification of 112 species from 16 countries that can be used to treat or prevent infections and parasitic infestations. The most commonly used medicinal plants were: *Azadirachta indica*, *Juniperus communis* L., *Melissa officinalis*, *Clibadium surinamense* L., *Olea europaea* L., *Juglans regia* L., *Allium sativum* L., *Coriandrum sativum* L., *Artemisia cina* Berg ex Poljakov, *Calendula officinalis* L., *Mentha piperita* L., *Cocos nucifera* L., *Ricinus communis* L., *Andrographis paniculata* (Burm.f.) Wall. ex Nees and *Juglans nigra* L. The species in question contain a variety of compounds that may confer medicinal properties; however, the anthelmintic and anti-parasitic activity may be attributed to the presence of phenolic compounds, which are known to possess a range of beneficial properties, including anti-parasitic and anthelmintic effects.



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## **Session 4. Emerging Infectious Diseases**



# sciforum-115415: “Influence of Non-Treatment of Partners on the Rate of Congenital Syphilis: A Comparative Analysis between Demographic Regions of Brazil (2014–2024)”

**Maria Fernanda Valentim De Paula \***, Pedro Henrique Paesi Dutra, Gabriela Gerevini Dal Alba and Camilly Vitória Cansan Loss

Academic of Medicine, School of Medicine Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, 90050-170, Brazil

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## Introduction

Congenital syphilis continues to be a serious public health problem in Brazil, with significant impacts on neonatal morbidity and mortality. One of the main factors associated with the high rate of this condition is the lack of adequate treatment for pregnant women's partners, which facilitates the vertical transmission of the infection.

## Methods

Secondary data were collected from the Department of Informatics of the Unified Health System (DATASUS) regarding congenital syphilis cases in Brazil from 2014 to 2024. The analysis focused on comparing rates of congenital syphilis across demographic regions, considering the influence of untreated partners on these rates.

## Results

Between 2014 and 2024, 250,310 cases of congenital syphilis were reported in Brazil. Of these, 135,693 cases (54.2%) were from untreated partners, 46,096 (18.4%) were from treated partners, and 68,521 (27.3%) were classified as ignored/blank. The southeast region has the highest rate with 108,874 (43.5%) cases and untreated partners represent 57,762 (53%) cases in this region. This is followed by the northeast region with 72,113 (28.8%) total cases and 39,608 (54.9%) cases of untreated parents. The highest rate of untreated parents occurs in the northern region with 12,599 (58.9%) cases.

## Conclusions

The data indicate that congenital syphilis remains a critical public health issue in Brazil, with a significant proportion of cases linked to untreated partners. The southeast and northeast regions exhibit the highest absolute numbers, while the northern region shows the highest percentage of cases from untreated partners. These findings highlight the urgent need for targeted interventions, including comprehensive prenatal screening and effective partner treatment strategies. Strengthening public health policies and awareness campaigns is essential to reducing vertical transmission rates and mitigating neonatal morbidity and mortality associated with congenital syphilis.



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# sciforum-115739: Design and Pilot Scale Production of an Alternative Non-Live Attenuated BTV Vaccine in Yeast

**Ikram Joubair<sup>1,\*</sup>, Jesus Zueco<sup>2</sup>, Ismail MOUKADIRI<sup>1</sup>, sergi Maicas<sup>3</sup> and Salim Bounou<sup>1</sup>**

<sup>1</sup> Biomedical & Biotechnology School of Engineering, Euro-Mediterranean University of Fez, Eco-Campus UEMF, Route de Meknès (RN6, Rond-Point Bensouda), 30070 Fez, Morocco

<sup>2</sup> Department of Microbiology and Ecology. Faculty of Pharmacy and Food Science. Universitat de València. Burjassot. Spain

<sup>3</sup> Department of Microbiology and Ecology. Faculty of Biology. Universitat de València. Burjassot. Spain

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Bluetongue (BT) is an infectious disease of domestic and wild ruminants caused by Bluetongue virus (BTV), an arbovirus of the *Orbivirus* genus within *Sedoreoviridae* family. It's primarily transmitted by biting midges of the *Culicoides* genus. BTV poses a significant threat to the livestock industry, particularly sheep. To date, a total of 36 serotypes of BTV have been characterized worldwide, causing periodic outbreaks, occurring most frequently in the Mediterranean basin. The control of BT disease is based on vaccination and several vaccines have been developed. Live attenuated vaccines and inactivated vaccines are widely used to prevent BT. However, despite their demonstrated efficacy, those vaccines have several limitations, including safety concerns and incomplete cross-protection among BTV serotypes. Recombinant subunit BT vaccines based on BTV structural proteins or virus-like particle vaccines (VLPs) may solve some of these limitations. In this study, we aim to design and develop vaccines that constitute an alternative to conventional attenuated vaccines. For this, we intend to express the VP2 and VP5 BTV antigens in *Saccharomyces cerevisiae*, a GRAS microorganism, by fusing the antigens to the secretion and cell wall retention signals of the Pir4 yeast cell wall protein. The VP2 and VP5 BTV structural proteins will be expressed in *S. cerevisiae*, targeting them to the yeast cell surface, or to the culture medium. The possibility of obtaining virus-like particles (VLPs), mimicking the virus, will also be studied.

Given that the research is currently underway, cloning, gene confirmation, sequencing and transformation of the constructs into the yeast expression system have been successfully completed. The confirmation of VP2, VP5, VLPs expression and assembly is currently under investigation, using immunofluorescence and Western blot analysis. The assessment of antigenicity and evaluation of the potential immunogenicity will be performed to validate the activity of the vaccine candidate in animals.



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# sciforum-115431: Epidemiological Profile of Gestational Syphilis Cases in Brazil Between 2020 and 2023: Insights for Public Health Improvement

Yasmin Marques Loureiro \*, Maria Fernanda Valentim de Paula, Enrico Lázaro Guidugli and Lucas Conzatti Rodrigues

Academic of Medicine, School of Medicine, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, 90050-170, Brazil

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**Introduction:** Gestational syphilis remains a significant public health issue due to its severe maternal and neonatal complications. Understanding its epidemiological profile in Brazil is essential for guiding public health interventions and reducing its impact on vulnerable populations. **Methods:** A cross-sectional, descriptive, retrospective, and quantitative study was conducted on cases of gestational syphilis across Brazil's five regions between January 2020 and December 2023. Data were collected through the Department of Informatics of the Brazilian Unified Health System (DATASUS), as well as from the 2022 census conducted by the Brazilian Institute of Geography and Statistics (IBGE). The analysis included variables such as age groups, patient race, sex, and regional distribution. **Results:** Between 2020 and 2023, 246,265 cases of gestational syphilis were reported in Brazil. The Northern region of the country presented the highest incidence rate, with approximately 157 cases per 100,000 inhabitants. The most affected age group was women aged 20 to 39 years, accounting for approximately 76% of the cases, followed by adolescents aged 15 to 19 years (21%). Furthermore, brown women (pardas) comprised about 53% of the reported cases, while white women represented 28%. **Conclusions:** The elevated number of gestational syphilis cases in Brazil from 2020 to 2023 underscores persistent regional disparities in healthcare access. The disproportionately high rates in the North may be linked to limited healthcare infrastructure and socioeconomic vulnerabilities. These findings highlight the urgent need for targeted public health interventions to address the unique challenges faced by this region.



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# sciforum-110902: Exploring methodologies from isolation to excystation for *Giardia lamblia*: a systematic review

Susie Sequeira <sup>1,\*</sup>, Mariana Sousa <sup>2</sup> and Agostinho Cruz <sup>2</sup>

<sup>1</sup> ESS, Polytechnic of Porto, rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

<sup>2</sup> REQUIMTE/LAQV, ESS, Polytechnic of Porto, rua Dr. António Bernardino de Almeida, 4200- 072 Porto, Portugal

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**Introduction:** *Giardia lamblia* is a flagellated protozoan and the etiological agent of giardiasis, a leading cause of epidemic and sporadic diarrhoea globally. The clinical significance and public health impact of giardiasis underscores the need for robust methodologies to investigate and manage this pathogen. This study systematically reviews the main methodologies described in the literature for studying the life cycle of *G. lamblia*, focusing on isolation, purification, axenization, excystation, and encystation processes. **Methods:** A systematic literature review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement. The search was performed in the MEDLINE, ScienceDirect, and Web of Science databases using the following search equation: "(“axenization” OR “isolation” OR “excystation” OR “encystation” OR “purification”) AND (“method”) AND (“giardia”)”. **Results:** A total of 39 studies were included in the review, revealing 56 methods for isolation and purification, 7 methods for excystation, and 3 methods for axenization and encystation. Isolation and purification methods exhibited significant variability, often involving two phases: an initial separation using simple techniques such as filtration and centrifugation, followed by a purification phase using a density gradient for faecal samples and immunomagnetic separation for water samples or nucleic acid extraction. The effectiveness of these methods differed depending on the source and sample type, highlighting the need for standardized protocols to ensure consistent and reliable results. **Conclusion:** Methods for the isolation and purification of *G. lamblia* exhibit notable variability and lack uniformity compared with the more consistent methods that are used for other life cycle stages. These findings underscore the urgent need for the development of standardized methodologies to enhance the reproducibility and reliability of research outcomes in this field.



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# sciforum-115036: Gestational toxoplasmosis: An analysis of cases over the last 5 years in Brazil according to clinical evolution

Isabela Karina Vilas Boas \*, Andihel Dionísio Ramos, Gabriela Gerevini Dal Alba and Maria Fernanda Valentim de Paula

Academic of Medicine, School of Medicine, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, 90050-170, Brazil

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## Introduction

Gestational toxoplasmosis (GT) results from *Toxoplasma gondii* infection during pregnancy, potentially causing severe fetal complications. Studying GT in Brazil is crucial to understanding clinical outcomes and gaps in epidemiological records.

## Methods

A descriptive analysis of GT cases recorded in DATASUS from 2019 to 2024 was conducted. Data were extracted from the notification module in SINAN. Cases were classified into four outcomes: blank, cure, death due to GT, and death from other causes.

## Results

The data showed 58,912 reported cases. Of these, 36,182 (61.4%) recovered, while 22,654 (38.4%) had unknown outcomes. Deaths totaled 76, with 39 attributed to GT and 37 to other causes.

Annual analysis revealed the highest notifications in 2022 (12,447 cases, 24.6%), followed by 2021 (11,093 cases, 22%) and 2023 (10,849 cases, 21.5%). The lowest numbers were in 2019 (8,436 cases, 14.3%) and 2024 (6,961 cases, 13.8%). Comparing pre-pandemic (2019) and post-pandemic years (2020-2024), the average number of reported cases remained stable, suggesting little impact from the pandemic.

## Conclusions

GT remains a significant concern in Brazil, with 58,912 cases between 2019 and 2024. Most cases (61.4%) recovered, but 38.4% had unknown outcomes, reflecting gaps in reporting. The 76 deaths, including 39 from GT, highlight its severity. The 2022 peak may reflect improved surveillance or outbreaks, while the 2024 decline suggests control or underreporting. The stable case average pre- and post pandemic suggests minimal pandemic impact. However, limitations include unexamined confounders and high missing data rates, which may affect accuracy. Strengthening reporting, monitoring pregnant women, and investing in prevention are key to reducing GT's impact.



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# sciforum-114897: HHV-6 in Liver Damage: Innocent Presence or Active Player in Alcohol-Induced Injury?

**Anda Upane<sup>1,\*</sup>, Simons Svirskis<sup>2</sup> and Sandra Skuja<sup>2</sup>**

<sup>1</sup> Joint Laboratory of Electron Microscopy, Institute of Anatomy and Anthropology, Rīga Stradiņš University, Riga, Latvia

<sup>2</sup> Institute of Microbiology and Virology, Rīga Stradiņš University, Latvia

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Human herpesvirus 6 (HHV-6), a widely distributed member of the Herpesviridae family, establishes lifelong latency in monocytes and macrophages. The persistence of HHV-6 under immunosuppressive conditions, such as chronic alcohol consumption, may potentiate liver inflammation and exacerbate tissue damage by synergising with the hepatotoxic effects of ethanol. This interaction could raise significant concerns in the context of infectious diseases, as it poses a heightened risk to individuals with a history of heavy alcohol use, particularly those with underlying comorbidities or compromised immune function. The transcription factor NF- $\kappa$ B, essential for transactivating target genes involved in immune and inflammatory responses and CD163, expressed on anti-inflammatory macrophages, provides valuable insights into tissue damage during the presence of HHV-6 infection.

Fifty-four liver tissue specimens were collected from the following groups: control (n=11), young alcohol users (n=15), and chronic alcohol users (n=28). Specimens were immunohistochemically stained with anti-CD163, anti-NF- $\kappa$ B, and anti-HHV-6 antibodies and analysed via light microscopy. HHV-6- and CD163-positive cells were counted quantitatively, while both the intensity and distribution of NF- $\kappa$ B expression were analysed semi-quantitatively. Statistical analysis was performed using SPSS 28.0.

HHV-6-positive liver lobules were identified in 48.75% of the controls, 63.89% of young alcohol users, and 72.04% of chronic alcohol users. The mean CD163-positive cell count in the lobular area increased significantly in young alcohol users (mean=135  $\pm$  43 SEM) and chronic alcohol users (mean=226  $\pm$  38 SEM) compared to the controls (mean=59  $\pm$  14 SEM). NF- $\kappa$ B expression intensity in the lobular area was significantly higher in young alcohol users (p0.005), and both intensity and distribution were notably increased in chronic alcoholics (p0.001, p=0.02) compared to the controls.

Chronic alcohol consumption increases liver inflammation and damage, potentially exacerbated by HHV-6 persistence. Further studies are needed to confirm these interactions and explore the mechanisms driving the synergistic effects of HHV-6 and ethanol on liver tissue damage.



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# sciforum-115422: Impact of the 2024 Floods on the Increase in Leptospirosis Cases in the City of Canoas, Southern Brazil: A Comparison with 2023

Letícia Souza<sup>1\*</sup>, Lucas Conzatti Rodrigues<sup>2</sup>, Enrico Lázaro Guidugli<sup>2</sup> and Gabriela Pereira Macelaro<sup>2</sup>

<sup>1</sup> Universidade Federal do Rio Grande do Sul

<sup>2</sup> Universidade Federal de Ciências da Saúde de Porto Alegre

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## Introduction

Leptospirosis, a zoonosis caused by *Leptospira* bacteria, is transmitted through contact with infected animals' urine, often in flooded, unsanitary environments. In 2024, Rio Grande do Sul experienced severe floods due to heavy rains, inundating cities like Canoas and creating ideal conditions for leptospirosis spread through contaminated water exposure.

## Methods

Data on confirmed leptospirosis cases in Canoas for 2023 and 2024 were sourced from the Department of Informatics of the Unified Health System (DATASUS). Monthly notifications were analyzed using time-series analysis to detect trends and descriptive statistics to highlight peak incidence periods. Historical data from 2020–2022 were reviewed to place recent findings in a multi-year context.

## Results

In 2023, 14 cases were recorded, while 27 cases were reported in the first half of 2024. From March to June, aligned with the 2024 flooding, cases increased from 7 in 2023 to 27 in 2024, with May 2024 representing 52% of the year's total. Historical data (2020–2022) show an annual average of 12 to 15 cases, suggesting the 2024 surge exceeds typical fluctuations, particularly during flood-affected months. This indicates a significant deviation linked to environmental conditions.

## Conclusions

The 2024 floods in Rio Grande do Sul likely contributed to the notable rise in leptospirosis cases in Canoas, as floodwaters heightened infection risk. Compared to historical averages, this increase appears anomalous, reinforcing the influence of extreme weather on disease incidence. These findings emphasize the importance of tailored public health responses in flood-prone regions, such as improved sanitation and early warning systems, to mitigate future outbreaks and protect vulnerable populations.



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# sciforum-115387: Increase in Dengue Cases in Rio Grande do Sul in 2024 and Its Relation to Floods

Lucas Konzatti Rodrigues\*, Gabriela Pereira Macelaro, Guilherme Ferreira Cruz and Andihel Dionísio Ramos

Academic of Medicine, School of Medicine, Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), Porto Alegre, 90050-170, Brazil

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**Introduction:** Dengue is a public health challenge in Brazil, characterized by its mosquito-borne nature and potential for severe clinical outcomes. The distribution and dynamics of this disease can be influenced by various factors, including environmental, social, and economic changes. During periods of heavy rainfall and subsequent flooding, like those experienced in Rio Grande do Sul in May of 2024, there is often an increase in reports of Dengue cases. Understanding the epidemiological trends of Dengue is essential for guiding prevention and control efforts. **Methods:** A cross-sectional, descriptive, retrospective, and quantitative study was conducted on cases of dengue in the Brazilian state of Rio Grande do Sul during 2023 and 2024. The data were obtained from the Department of Informatics of the Unified Health System (DATASUS). **Results:** In May and June 2024, following the flooding in Rio Grande do Sul, a total of 57,899 dengue cases were reported (47,733 in May and 10,166 in June). In comparison, during the same period in 2023, 18,195 cases were recorded (13,494 in May and 4,701 in June), representing a 218% increase in total cases between the two years. This surge was particularly significant in May, which saw more than triple the number of cases compared to the previous year. **Conclusion:** The significant increase in Dengue cases in Rio Grande do Sul in 2024, particularly following the severe flooding in May, underscores the influence of environmental factors on disease transmission. The 218% surge in cases compared to 2023 highlights the vulnerability of affected regions to vector proliferation during and after extreme weather events. These findings emphasize the urgent need for integrated public health strategies, including enhanced surveillance, community education, and rapid response measures, to mitigate the impact of environmental changes on Dengue outbreaks.



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# sciforum-115647: ULTimate Y2H: A Powerful Platform to decipher Host–Pathogen Protein Interactions and Biological Pathways

Susanne Reichinnek<sup>1,\*</sup>, Petra Tafelmeyer<sup>2</sup> and Jean-Christophe Rain<sup>1</sup>

<sup>1</sup> Hybrigenics Services

<sup>2</sup> Hybrigenics Corporation, Boston MA

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The understanding of the molecular mechanisms by which pathogens like viruses, bacteria, fungi, and parasites operate, particularly in relation to their host interactions, is essential to effectively develop therapeutic lead candidates. Protein–protein interactions serve as fundamental components of cellular protein complexes and pathways and play a crucial role in determining protein functionality. The yeast two-hybrid (Y2H) screening method remains a valuable tool for analyzing the interactomes of various model organisms and pathogens, as well as for investigating host–pathogen interactions.

In order to achieve reproducible and comprehensive Y2H outcomes, we developed a patented mating protocol that allows 83 million interactions to be tested, on average, per screen. Additionally, we used a domain-based strategy to create highly intricate, random-primed cDNA/genomics libraries with several million independent clones in yeast derived from several bacteria and viruses, such as *Caulobacter crescentus*, *Escherichia coli*, and *Streptococcus pneumoniae*, as well as human tissues, rodents, flies, and plants. The use of protein domain libraries allows for the isolation of multiple independent fragments for each interactant, facilitating the immediate identification of minimally interacting domains and the calculation of confidence scores.

We used this methodology to investigate biological pathways in *Staphylococcus aureus*. A total of 26 essential proteins from *S. aureus* were screened against our highly complex genomic library of the same organism. Among the identified proteins are critical genes associated with cell wall biosynthesis, cell division, and antibiotic resistance to allow for an understanding of the underlying molecular pathways and the development of therapeutic targeting and antimicrobial resistance research. The resulting protein interaction map identified 167 CDSs that are connected by 191 protein–protein interactions (PPIs), and, on average, every bait protein has 9.6 PPIs and can therefore serve as a foundational resource for identifying potential new targets for antimicrobial drug development and future treatments.



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# sciforum-110891: Assessment of COVID-19 Antibody Response Using Magnetic Bead-Coupled S-Protein in Flow Cytometry

**Roland Hetenyi**<sup>1,2,3\*</sup>, **Daniel Hanna**<sup>2,3,4</sup>, **Péter Szabó**<sup>2,3,4</sup>, **Zoltán Kopasz**<sup>4</sup>, **József Szentpéteri**<sup>5</sup>, **Krisztián Bányai**<sup>6</sup> and **Edina Szabó-Meleg**<sup>3,7</sup>

<sup>1</sup> University of Pécs, Pécs, H-7624, Hungary

<sup>2</sup> RoLink Biotechnology Kft., Pécs, Hungary

<sup>3</sup> Doctoral School of Pharmaceutical Sciences, Medical School, University of Pécs, Pécs, H-7624, Hungary

<sup>4</sup> National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs, H-7624, Hungary

<sup>5</sup> Institute of Transdisciplinary Discoveries, Medical School, University of Pécs, Pécs, H-7624, Hungary

<sup>6</sup> Szentágothai Research Centre, University of Pécs, Pécs, H-7624, Hungary

<sup>7</sup> Department of Biophysics, Medical School, University of Pécs, Pécs, H-7624, Hungary

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## Introduction

The COVID-19 pandemic has emphasized the need for precise and reliable methods to assess immune responses, improve viral surveillance, and evaluate vaccine efficacy. This study employs flow cytometry in conjunction with magnetic beads functionalized with SARS-CoV-2 S-proteins to quantify virus-specific antibodies. Compared to ELISA, this method offers superior specificity, higher sensitivity, reduced background interference, and more precise quantitative assessment.

## Methods

Magnetic beads coated with the SARS-CoV-2 S-protein were incubated with human serum samples to capture virus-specific antibodies. These bead-antibody complexes were subsequently analyzed using flow cytometry. Antibody-bound beads were differentiated from unbound beads based on fluorescence intensity detected by flow cytometry. Bound antibodies were labeled with fluorescently conjugated secondary antibodies specific for human immunoglobulins, resulting in increased fluorescence intensity compared to unbound beads, enabling precise quantification and differentiation. The results obtained were validated against the commercially available anti-SARS-CoV-2 QuantiVac IgG ELISA assay.

## Results

Flow cytometry demonstrated remarkable sensitivity and specificity in detecting SARS-CoV-2 antibodies at serum dilutions of 1:100 and 1:400. The results closely aligned with commercial ELISAs, confirming the accuracy and reliability of this alternative diagnostic method.

## Conclusion

Flow cytometry utilizing magnetic bead-coupled S-protein presents a robust and precise tool for evaluating COVID-19 antibody responses. This technique enhances existing serological diagnostic platforms by significantly reducing background interference and enabling precise antibody quantification and provides valuable insights into immune response assessments in both clinical and research settings.

## Acknowledgment

This research was conducted in collaboration with the National Laboratory of Virology and the Flow Cytometry Core Facility at the University of Pécs. Funding was provided by the National Research, Development, and Innovation Office of Hungary through grants 2020-2.1.1-ED-2020-00100 and RRF-2.3.1-21-2022-00010.



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# sciforum-115426: Demographic analysis of Zika virus cases in Brazil from 2020 to 2024

Enrico Lázaro Guidugli<sup>1,\*</sup>, Leticia De Paula e Souza<sup>2</sup>, Theodora Garcia Furtado<sup>1</sup> and Pedro Henrique Paesi Dutra<sup>1</sup>

<sup>1</sup> Academic of Medicine, School of Medicine, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, 90050-170, Brazil

<sup>2</sup> Academic of Medicine, School of Medicine, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, 90035-003, Brazil

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**Introduction:** The Zika virus is a flavivirus transmitted through the bite of the *Aedes aegypti* mosquito, which led to an epidemic in Brazil in 2016. In the majority of adult cases, infection is asymptomatic. However, in pregnant women, Zika virus infection can result in fetal abnormalities such as microcephaly. This study intends to analyze Zika virus cases in Brazil during the period from 2020 to 2024. **Methods:** A cross-sectional, descriptive, retrospective, and quantitative study on the number of Zika Virus cases in Brazil from 2020 to 2024 is described. Data from the Notification of Diseases Information System available in the DATASUS database were utilized. Among the analyzed variables, age group, education level, and sex were particularly highlighted. **Results:** In the analyzed period, 133,202 cases were recorded, with women being the most affected, accounting for 62% of the cases. Regarding age, the highest number of registered cases (52,423) was in the 20-39 years age group, followed by the 40-59 age group, computing 31,412 cases. Furthermore, illiterate people recorded the lowest incidence of Zika (1,077), as the highest incidence was recorded in the group that completed high school (22,030). **Conclusions:** The data from 2020 to 2024 demonstrate that Zika virus infection in Brazil predominantly affected women, particularly those in their reproductive and working-age groups (20-39 years), highlighting the continued vulnerability of this population and their possible children. The higher incidence observed among individuals with higher educational levels, particularly those who completed high school, suggests that exposure and reporting factors may play a role in case distribution. These findings emphasize the importance of tailored public health interventions, with a focus on reproductive-age women, as well as the need for enhanced education and outreach strategies to address at-risk populations effectively.



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# sciforum-115656: Detection and Antifungal Susceptibility of Fungal Pathogens Associated with Otomycosis in a Tertiary Health Center in Kano, Nigeria.

**Khadija Muhammad Kawu <sup>1,\*</sup>, Muhammad Ibrahim Getso <sup>1</sup>, Taysir Ramadan <sup>1</sup>, Alhassan Abdullahi Sharif <sup>1</sup>, Kanishka Hrishi Das <sup>2</sup>, Al-muktar Yahuza Adamu <sup>1</sup> and Mansur Aliyu <sup>1</sup>**

<sup>1</sup> Department of Medical Microbiology and Parasitology, College of Health Sciences, Bayero University Kano, Kano-Nigeria.

<sup>2</sup> Department of Allied Health Science, Chitkara School of Health Sciences, Chitkara University, Rajpura, Punjab, India.

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## Introduction

Otitis externa is estimated to account for 5-20% of all ear-related visits to Ear, Nose, and Throat (ENT) clinics, of which 10-25% were attributed to otomycosis. Our study aimed to identify the fungal pathogens associated with otomycosis and to evaluate their antifungal susceptibility in a low-resource-setting community.

## Methods

The study was conducted in the Department of Medical Microbiology and Parasitology, Bayero University Kano, Nigeria, from January to June 2024. A total of 300 patients with a clinical diagnosis of otitis externa were recruited from the otorhinolaryngology clinic of Aminu Kano Teaching Hospital. Two swab samples were collected from each patient for mycological examinations. The first swab was evaluated for microscopy using KOH mount and Gram Staining. The second swab was subjected to fungal culture on SDA. Isolates were identified using other mycological examinations where necessary, including the sequencing of the fungal rDNA. In vitro AFST against Amphotericin B, Fluconazole, Itraconazole, Voriconazole, Ketoconazole, and Nystatin was performed using the disk diffusion method according to the CLSI guidelines.

## Results

Out of 300 patients, 117 subjects (39.0%) were mycologically positive. Females (69.0%) and the age group  $\leq 10$  years (44.3%) were the most affected. Cases were predominantly unilateral in 285 (95%) patients involving the right ear (71.7%). Ear itching (95.7%) and pain (89.0%) were the most common presentations. The markedly observed risk factors were frequent ear cleaning (88.0%) and ear manipulation with a foreign object (76.3%). *Aspergillus* species and *Candida* species were present in 90 (77.0%) and 27 (23.0%) cases, respectively. Isolates showed high sensitivity to Voriconazole and Nystatin but poor response to Ketoconazole and Fluconazole.

## Conclusion

Otomycosis accounts for 39% of otitis externa seen in our center, mostly presenting with pruritus and otalgia. Awareness campaigns to the general population on predisposing factors might significantly reduce the incidence of otomycosis.



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# sciforum-110652: Development of a Novel Multicomponent Vaccine to Address the Burden of Otitis Media in High-Risk Populations

**Ayesha Zahid**<sup>1,2,\*</sup>, Jennifer C. Wilson<sup>3</sup>, I Darren Grice<sup>3,4</sup> and Ian R. Peak<sup>3,4</sup>

<sup>1</sup> Institute for Biomedicine and Glycomics, Griffith University, Australia

<sup>2</sup> School of Pharmacy and Medical Sciences, Griffith University, Australia

<sup>3</sup> School of Pharmacy and Medical Science, Griffith University, Gold Coast, QLD, Australia

<sup>4</sup> Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, QLD, Australia.

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## Background

Middle ear infection (otitis media) remains the most common childhood disease diagnosed among Australian Indigenous children. Despite extensive antibiotic usage, it persists as a significant health concern, characterized by substantial morbidity and economic burden. *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, and *Moraxella catarrhalis* are the most common culprits responsible for acute otitis media. Currently, pneumococcal and influenza vaccines are utilized as a preventive measure against otitis media. However, their efficacy is limited to prevent carriage and/or illness induced by non-vaccine serotypes. Otitis media stemming from non-vaccine serotype pneumococci, non-typeable *H. influenzae*, and *M. catarrhalis* continue to pose significant healthcare challenges.

## Methods and Findings

Given the expansive heterogeneity of pathogens involved in otitis media, a multicomponent vaccine is essential to confer adequate protection. This study is focused on devising a novel multicomponent vaccine targeting two major etiological agents, *H. influenzae* and *M. catarrhalis*. Our vaccine formulation features novel protein antigens derived from *M. catarrhalis*, non-typeable *H. influenzae*, and genetically modified outer-membrane vesicles (OMVs) from *M. catarrhalis*. We successfully expressed and purified recombinant protein antigens from NTHi and *M. catarrhalis* using *E. coli* expression system and immobilized metal affinity chromatography. We also developed knockout mutants of *M. catarrhalis* lacking targeted immunodominant strain-variable proteins. These mutants are currently being used to purify OMVs. Preclinical investigations will encompass a rigorous evaluation of vaccine immunogenicity and cross-reactivity against heterologous strains in mice.

## Conclusion

By combining novel protein antigens with genetically modified outer-membrane vesicles, this study aims to overcome the limitations of current vaccine approaches. Forthcoming preclinical evaluations will provide critical insights into its immunogenicity and cross-reactivity, paving the way for future advancements in otitis media prevention.



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# sciforum-111209: First report of potential pathogenic fungus *Papiliotrema aurea* in *Glossiphonia complanata*: Implications for leech-mediated fungal transmission

Michał Kułakowski <sup>1,\*</sup>, Maciej Grobelski <sup>2</sup> and Karolina Górzyska <sup>1</sup>

<sup>1</sup> Department of Systematic and Environmental Botany, Faculty of Biology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland

<sup>2</sup> Department of Animal Morphology, Faculty of Biology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland

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Leeches (*Hirudinea*), a diverse group of annelids, are primarily classified as either parasites or predators, depending on their feeding methods and life strategies. These groups differ in habitat and dispersal. This diversity plays a significant role in the functioning of aquatic ecosystems, especially in the context of epidemiological threats posed by the transmission of pathogenic fungi by leeches. Due to fungi's ability to adapt rapidly to new environments and given their dissemination through various vectors, including leeches, these diseases can spread quickly. This study examined the mycobiome of the parasitic leech *Glossiphonia complanata*, which has a wide range of hosts, making it a potentially effective vector for the spread of fungal diseases.

Leeches were collected from five water reservoirs located on the Morasko Campus of Adam Mickiewicz University. Anesthetized leeches were surface-sterilized with 75% ethanol, dissected into fragments, and placed on PDA medium. DNA was extracted from the cultivated fungal colonies, followed by PCR amplification of the ITS region sequences. The PCR products were sequenced, and additional morphological analyses of the fungi were conducted.

In addition to widely recognized environmental fungi such as *Cladosporium* spp., the fungal species *Papiliotrema aurea* was detected and identified within the bodies of the leeches. This species, previously isolated from the Chinese mitten crab (*Eriocheir sinensis*), exhibits a notable capacity for colonization in various organisms. Such findings imply that *P. aurea* not only possesses the ability to thrive in specific microenvironments but also raises concerns regarding its potential pathogenicity.

In summary, the ecological diversity of leeches and their ability to interact with other organisms, including pathogenic fungi, highlight their importance in aquatic ecosystems. Research on the mycobiome of leeches provides valuable insights into potential vectors of fungal diseases. Understanding these mechanisms can contribute to a better management of epidemiological risks and the protection of aquatic ecosystems from pathogen invasions.



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# sciforum-102625: Fungal Pathogenesis in Bovine (*Bos Taurus indicus*) Mastitis: Therapeutic Protocol With Ethno Veterinary Formulation

Amruthavarshini S N \* and Senthilkumar Rajagopal

Department of Biotechnology School of Applied Sciences, REVA University

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Bovine are susceptible to various infections caused by a variety of pathogens. Due to their low immunity or poor hygiene conditions, they succumb to diseases, contributing to an important set of problems. These include animal welfare, productivity losses, uncertain food security, and loss of income and health. Fungal diseases with respect to their correlation to infection have not been explored.

In this study, we aimed to understand the effects of fungi in bovine by extracting and analyzing blood and milk samples from infected mastitis, as well as evaluating the rate of growth, general topography, reverse pigmentation, and microscopic examination for mycelial studies and slide culture. Mycelial studies will be performed to further identify these fungi. The fungal culture slide will be prepared by the tease mount technique using methylene blue stain. The slides will then be analyzed to characterize the fungi. Identification will be performed according to the guidelines recommended by Larone.

The research methodology used will benefit the farmers by reducing the cost on herbal medicine and the treatment of fungal pathogenesis by lowering the price of treatment and medication. Comparatively, in regular treatment checks, it takes 5-7 days for treatment and 7 days for recovery, whereas ethno veterinary formulations take 5 days and recovery takes 3 days.

This study shows that when bovine are infected with diseases such as mastitis, they can also succumb to opportunistic pathogens that are commensal. Due to their weakened immune system, during an infection, these fungal pathogens can take advantage and cause pathogenesis in bovine. This is the first study on the opportunistic pathogenesis of fungal pathogens during an infection in bovine. Further studies have to be carried out in order to study the mechanisms and roles involved in pathogenesis.



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# sciforum-115162: Fungi and wildlife: the case of avian aspergillosis

Andreia Manuela Vieira Garces \* and Isabel Pires

<sup>1</sup> Animal and Veterinary Research Centre (CECAV), Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), University of Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal

<sup>2</sup> Universidade de Trás os Montes e Alto Douro

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Avian aspergillosis is a fungal disease caused predominantly by *Aspergillus fumigatus*, affecting the respiratory system of birds. It occurs in both wild and domesticated avian species, often leading to significant health and conservation concerns. The disease typically arises from the inhalation of fungal spores, which proliferate in damp or poorly ventilated environments, such as contaminated feed, litter, or nesting material. Clinically, aspergillosis manifests in acute or chronic forms. Acute cases are common in young or immunocompromised birds, presenting with respiratory distress, lethargy, and sudden death. Chronic aspergillosis, frequently seen in adult birds, leads to granuloma formation in the air sacs and lungs, causing progressive respiratory issues, anorexia, and weight loss. Diagnosis relies on clinical signs, imaging, fungal cultures, or histopathology. This study aims to describe the epidemiology and the macroscopic, histological, and microbiological lesions from wild birds during postmortem exams. Samples were collected for histology and Sabouraud agar. The necropsy and histological examination established the aspergillosis diagnosis in 10 animals examined over 7 years. The main species affected was *Buteo buteo*. Macroscopic alterations in the animals with aspergillosis were varied. All animals presented thickened air sacs with abundant caseous and necrotic debris and greyish-green fungal colonies and nodules in the air sacs and lungs. One animal presented dissemination to the bone and muscle. The main lesions observed were on the lungs and air sacs as expected. This work shows that aspergillosis is an important disease that causes mortality in wild birds, particularly during the recovery process of rehabilitation of these animals in the recovery centres. This work shows that the part of mycological identification is still often overlooked during the necropsy, and the diagnosis is based only on the macroscopic diagnosis. Avian aspergillosis remains a critical issue in veterinary medicine and wildlife conservation, requiring continued research for improved diagnostic and therapeutic approaches.



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# sciforum-115156: Harnessing the Intrinsic Disorder in Human Metapneumovirus for Therapeutic Development

Deepak Chaurasiya

Biomedical Informatics Lab, Indian Institute of Information Technology, Allahabad, 211002, India

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Human metapneumovirus (hMPV) is a significant respiratory pathogen affecting populations worldwide, particularly young children, the elderly, and immunocompromised individuals. Despite its clinical importance, the intrinsic disorder status of hMPV proteins and their roles in the pathogenic mechanism remain largely unexplored. Intrinsically disordered proteins (IDPs) and disordered protein regions (IDPRs) are members of the "dark proteome," characterized by the absence of a stable three-dimensional structure but with critical biological functions, such as host-virus interactions and immune evasion. In this study, we evaluated the prevalence and functionality of IDPs/IDPRs in the hMPV proteome using computational tools, including PONDR®, IUPred2A, DISOPRED, and ESpritz, validated with NMR, X-ray crystallography, and DisProt data. Our findings revealed significant levels of intrinsic disorder in key hMPV proteins, such as the fusion (F), nucleocapsid (N), and glycoprotein (G), which are implicated in viral replication, host entry, and immune modulation. Proteins SPQ8B9Q8 and SPQ6WB94 showed 100% intrinsic disorder, highlighting their potential roles in immune evasion, while structural proteins like SPQ6WB99 displayed minimal disorder. The hMPV proteome exhibited a mean disorder percentage of 19.19%, with nearly half of the proteins containing disordered regions exceeding 30 amino acids. These findings emphasize the critical role of intrinsic disorder in the hMPV life cycle and identify IDPs/IDPRs as promising targets for disorder-based drug design.



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# sciforum-115793: Impact of the COVID-19 Pandemic on Visceral Leishmaniasis Reporting in Brazil: A Retrospective Analysis of Epidemiological Trends

Gabriela Pereira Macelaro\*, Yasmin Marques Loureiro, Guilherme Ferreira Cruz and Isabela Karina Vilas Boas

UFCSPA - Universidade Federal de Ciências da Saúde

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**Introduction:** Visceral Leishmaniasis represents a significant public health challenge in Brazil, characterized by its zoonotic nature and potential for severe clinical outcomes. The distribution and dynamics of this disease can be influenced by various factors, including environmental, social, and economic changes. Understanding its epidemiological trends is essential for guiding effective prevention and control efforts. **Methods:** A cross-sectional, descriptive, retrospective, and quantitative study was conducted on confirmed cases of Visceral Leishmaniasis in the five regions of Brazil during the pre-pandemic period (2016 to 2019) and the pandemic period (2020 to 2023). The data were obtained from the Department of Informatics of the Unified Health System (DATASUS), specifically from the "Notifiable Diseases and Conditions" section. **Results:** During the pre-pandemic period (2016–2019), a total of 14,589 cases of Visceral Leishmaniasis were reported in Brazil, with a 46% overall reduction during the pandemic period (2020–2023). The decrease in the number of cases varied across the country's regions, with the North region showing the largest decline of 56%. The Northeast experienced a 44% reduction, followed by the Southeast with a 52% decrease. In the South, the reduction was 16.9%, and in the Central West, it was 6.1%. **Conclusions:** Based on data analysis, it is possible to infer that the COVID-19 pandemic had an impact on the reporting of Visceral Leishmaniasis cases in Brazil. The sharp decline in notifications during the pandemic, particularly in the Northern region, suggests the possibility of an overburdened healthcare system, reduced demand for diagnosis and treatment due to social isolation, and a decrease in control programs and epidemiological surveillance. Therefore, it can be proposed that the reduction in Visceral Leishmaniasis cases is associated with lower case detection and reporting.



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# sciforum-114956: Molecular analyses and Sindbis virus pathogenesis in human neuroblastoma cell line

Kornélia Bodó<sup>1,\*</sup>, Viktória Nyári<sup>1</sup>, Zoltán Kopasz<sup>1</sup>, Péter Engelmann<sup>2</sup>, Krisztina Leiner<sup>1</sup>, Mónika Madai<sup>1</sup>, Brigitta Zana<sup>1</sup>, Zita Potzné-Árvai<sup>1</sup>, Gábor Kemenesi<sup>1,3</sup> and Anett Kuczmog<sup>1,3</sup>

<sup>1</sup> National Laboratory of Virology, Szentágotthai Research Center, University of Pécs, H-7624 Pécs, Hungary

<sup>2</sup> Department of Immunology and Biotechnology, Medical School, Clinical Center, University of Pécs, H-7624 Pécs, Hungary

<sup>3</sup> Institute of Biology, Faculty of Sciences, Department of Microbiology and Molecular Biology

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Alphaviruses cause mosquito-borne erythrogenic diseases. Sindbis virus (SINV) circulates in an enzootic cycle between its vectors and birds. In humans, SINV triggers musculoskeletal syndromes characterised by fever, rash, acute and chronic polyarthritis, etc. Few studies examining the virus's impact on the nervous/immune systems are available. SINV often causes cerebral infection, so understanding the brain's response is crucial. The SH-SY5Y cell line, from neuroblastoma, is used in many neurobiological and virological studies. This study aimed to understand viral infection in vitro and examine immune markers using SINV infection in SH-SY5Y cells.

Due to the lack of adequate knowledge of SINV, optimization was necessary for all experimental designs. After the virus stock preparation, the median tissue culture infectious dose was used to determine the multiplicity of infection (MOI). Taqman-probe-based RT-qPCR was designed for the SINV nucleic acid, and immunofluorescence-based ds-RNA antibody staining was used to reinforce the virus replication. Apoptotic markers were also investigated by means of immunofluorescence. To monitor specific immune gene expressions (*pattern recognition receptors (PRRs)*, *regulator gene*, *cytokines*) in a time-dependent manner (3/6/12/16/24/30 hr), RT-qPCR was also performed.

SINV infected the cells, and after 24 hr, a cytopathogenic effect and virus replication could be observed. SINV induced the caspase-dependent apoptotic pathway. Most of the investigated genes, including *PRRs*, (*TLR-3/7*, *RIG1/MDA5*), a regulator (*b-catenin*), and inflammatory (*IL-1b*, *IL-6*, *TNFa*), and antiviral (*IL-10*, *IFNβ*) genes, exhibited a consistent induction up to 12/16 hr; by the end of the 30 hr, their expression mainly (e.g., *RIG1/MDA5*, *IFNβ*) decreased compared with those of our controls. We can conclude that in vitro, SINV can be transmitted even in small quantities, and an inflammatory environment can be developed, which later leads to cell death.

These preliminary results allow us to gain insights into SINV-SH-SY5Y interactions, which are better understood by examining them in much finer molecular detail.



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# sciforum-111309: Rapid Benchtop Purification of SARS-CoV-2 for Immunological Studies: Minimizing Cytotoxic Effects on Monocytes

**Daniel Hanna**<sup>1,2,3,\*</sup>, **Roland Hetenyi**<sup>1,2,3</sup>, **Peter Szabo**<sup>3</sup>, **Zoltan Kopasz**<sup>3</sup>, **Anett Kuczmog**<sup>3</sup>, **Krisztian Banyai**<sup>2,4</sup> and **Edina Szabo-meleg**<sup>2,5</sup>

<sup>1</sup> RoLink Biotechnology Kft., Pécs, Hungary

<sup>2</sup> Pharmacological and Pharmaceutical Sciences, Medical School, University of Pecs, Hungary

<sup>3</sup> National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Hungary

<sup>4</sup> Szentágothai Research Centre, University of Pécs, Hungary

<sup>5</sup> Department of Biophysics, Medical School, University of Pecs, Hungary

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## Introduction

Understanding COVID-19 pathogenesis and immune responses relies on robust in vitro systems that utilize purified SARS-CoV-2 virions. This study focused on developing a rapid, benchtop purification method for SARS-CoV-2, designed to eliminate non-viral substances and cytopathic agents, enabling immunological experiments.

## Methods

The SARS-CoV-2 delta variant was propagated in Vero E6 cells within a BSL-4 laboratory. Complete virus inactivation was achieved using UV irradiation and was confirmed via standard virological assays. The purification procedure involved filtration through a 0.22 µm membrane, followed by anion exchange chromatography, with desalting performed as an optional step. PBMCs were isolated from healthy donor blood using SepMate™ tubes with slight modifications to the manufacturer's protocol. These PBMCs were incubated with either purified or unpurified virus, and cells and immunological interactions were analyzed through flow cytometry.

## Results

The purification method successfully removed 99% of non-viral proteins, leaving primarily viral proteins. RT-PCR and rapid antigen tests demonstrated an approximately 70% recovery of viral RNA. Flow cytometry confirmed that the unpurified virus caused significant, almost total, CD14+ monocyte destruction. In contrast, monocytes incubated with purified virus maintained viability, indicating reduced cytotoxic effects.

## Discussion

This streamlined purification process provides a reliable, rapid tool for preparing SARS-CoV-2 for immunological studies. While anion exchange chromatography was highly effective, observed discrepancies between PCR and rapid antigen test results after desalting merit further exploration. Future studies with active viruses will help determine the infectivity of purified preparations.

## Acknowledgment

This research was conducted in collaboration with the National Laboratory of Virology and the Flow Cytometry Core Facility of the University of Pécs. The project was supported by grants 2020-2.1.1-ED-2020-00100 and RRF-2.3.1-21-2022-00010 from the National Research, Development, and Innovation Office of Hungary.



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# sciforum-115767: Serological and Molecular Prevalence of Tick-Borne Pathogens in Dogs from Southern Italy

Santina Di Bella \*, Vincenza Cannella, Valeria Blanda, Delia Gambino, Valeria Vaglica, Davide Pepe, Giada Spene and Annalisa Guercio

Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri", Ragusa, RG 97100, Italy

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## Introduction

Tick-borne diseases are an increasing concern for both animal and human health, particularly in Mediterranean regions. Ticks act as vectors for pathogens, including those from the genera *Rickettsia*, *Anaplasma*, and *Ehrlichia*, many of which are zoonotic and pose significant risks to human health. This study presents a preliminary analysis of the serological and molecular prevalence of these pathogens in dogs from southern Italy by examining both blood and tick samples.

## Methods

Blood samples and ticks were collected from dogs in Sicily in 2023-2024. Indirect immunofluorescence assays were performed to detect antibodies against *Rickettsia* spp., *Anaplasma phagocytophilum*, and *Ehrlichia canis* in the blood samples. Polymerase chain reaction was carried out on blood and ticks to detect *Rickettsia* spp., *Anaplasma* spp., and *E. canis*. Positive samples were sequenced for species identification.

## Results

A total of 242 blood samples were analyzed, revealing high seropositivity rates for *Rickettsia* spp. (47.1%), *A. phagocytophilum* (32.2%), and *E. canis* (23.3%), suggesting extensive pathogen circulation. *Rickettsia* DNA was not detected in the blood samples, likely due to its transient nature. *Anaplasma* spp. was detected in five blood samples (2%), with sequencing confirming two positives for *A. phagocytophilum*, one for *A. ovis*, and two for *A. platys*. Two samples (1.6%) tested positive for *E. canis*. Of the 100 ticks collected, 32% tested positive for *Rickettsia* spp., including *R. aeschlimannii*, *R. felis*, *R. massiliae*, *R. africae*, *R. conorii*, and *R. conorii* subsp. *israelensis*. One tick (1%) tested positive for *E. canis* and another (1%) for *A. platys*.

## Conclusions

This study confirms the high prevalence of tick-borne pathogens in dogs, identifying zoonotic agents in both blood and tick samples. The co-circulation of pathogens highlights the need for integrated control strategies, including tick surveillance and veterinary prophylaxis. Dogs serve as valuable sentinels for monitoring zoonotic risks, underscoring the importance of further research to elucidate pathogen dynamics.



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# sciforum-112185: Seroprevalence of HEV, Mycobacterium avium, and Brucella suis in the pig population in Southern Italy

Gianmarco Ferrara

Department of Veterinary science, University of Messina, Italy

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The pig is susceptible to numerous zoonoses and represents the main reservoir for some zoonoses. These include Pasmahepevirus balayani (formerly called HEV), Mycobacterium avium paratuberculosis (MAP), and Brucella suis. These infections cause mild symptoms in pigs (the only one to cause truly evident symptoms is Brucella suis, which causes reproductive disorders) but have serious consequences in humans. The purpose of this study was to assess the swine population's exposure to three zoonotic infections (Brucella suis, Mycobacterium avium, and HEV) in the Campania region (Southern Italy). A total of 370 blood samples were collected and tested with commercial indirect ELISAs to evaluate the circulation of these pathogens. Prevalences of 41.4%, 3.5%, and 0% were found for HEV, MAP, and Brucella suis, respectively. The data obtained are in line with what has been found in other European countries or in other industrialized countries undergoing eradication plans for Brucella. The data relating to HEV have also been confirmed in other epidemiological studies in the literature (pigs and deer are the main reservoirs of the infection, and therefore, the presence of anti-HEV antibodies in these species is very frequent). The evaluation of risk factors highlighted how higher seroprevalences were obtained in animals at the end of their production careers. This study highlights the spread of some zoonoses in the pig population and the need for continuous surveillance in reservoir species.



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# sciforum-115808: Surge in Dengue Cases in Rio Grande do Sul in 2024: Epidemiological Trends and Contributing Factors

João Paulo Farezin Fortti \* and Gabriela Pereira Macelaro

UFCSPA - Universidade Federal de Ciências da Saúde de Porto Alegre

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**Introduction:** Dengue is a viral disease transmitted by *Aedes* mosquitoes, endemic in over 100 countries, including Brazil. It represents a significant public health challenge due to its high incidence and the simultaneous circulation of all four virus serotypes, creating a hyperendemic scenario. This situation complicates efforts for prevention and control, making it a persistent concern for healthcare systems. The state of Rio Grande do Sul has seen a notable rise in dengue cases in 2024, highlighting the need to understand its epidemiological trends, impact on healthcare infrastructure, and contributing factors. **Methods:** Data were collected from the Department of Informatics of the Unified Health System (DATASUS) on dengue cases in Rio Grande do Sul from January 2021 to December 2024. The data were analyzed based on the following variables: epidemiological profile, hospitalization rates, and clinical outcomes. **Results:** From 2021 to 2024, 329,185 dengue cases were reported in Rio Grande do Sul, with 212,321 (64%) occurring in 2024, peaking in April (84,513 cases). A 400% increase was observed in 2024 compared to 2023, 200% compared to 2022, and 1800% compared to 2020. The epidemiological profile remained consistent, with 54% of cases in females and 60% in patients aged 20–49. Most patients were of White ethnicity (82%). Regarding outcomes, 73% did not require hospitalization, and 75% recovered, following trends from previous years. **Conclusions:** The significant rise in dengue cases in Rio Grande do Sul in 2024, particularly with a 400% increase compared to 2023, highlights the influence of favorable climate conditions, including increased rainfall and higher temperatures, which promote mosquito proliferation. Additionally, factors such as urban water accumulation and insufficient mosquito control measures contribute to the surge. Despite this, the epidemiological profile and clinical outcomes remained consistent, with most cases not requiring hospitalization and the majority of patients recovering.



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# sciforum-115054: Zika Virus Surveillance in Brazil (2010–2024): Epidemiological Insights

**Gabriela Gerevini Dal Alba**<sup>1,\*</sup>, Yasmin Marques Loureiro<sup>1</sup>, Andihel Dionísio Ramos<sup>1</sup>, Camilly Vitória Cansan Loss<sup>2</sup> and Isabela Karina Vilas Boas<sup>1</sup>

<sup>1</sup> Universidade Federal de Ciências da Saúde de Porto Alegre

<sup>2</sup> Universidade Federal do Rio Grande do Sul

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## Introduction

The Zika virus emerged as a significant public health challenge in Brazil, particularly after its epidemic peak in 2016. Initially identified in Africa, Zika spread to the Americas in the mid-2010s, causing widespread concern due to its association with severe neurological complications, including microcephaly in newborns and Guillain–Barré syndrome in adults.

## Methods

Secondary data were collected from the Department of Informatics of the Unified Health System (DATASUS) regarding Zika virus notifications in Brazil between January 2010 and December 2024, analyzing the number of cases by year of occurrence.

## Results

Between 2010 and 2024, 497,550 cases of Zika were reported in Brazil. The peak occurred in 2016, with 277,072 cases (55.7%). In 2017 and 2018, the numbers dropped to 32,225 (6.5%) and 20,092 (4%), respectively. After an increase in 2019, with 30,800 cases (6.2%), there was another decline in 2020 and 2021, with 20,637 (4.1%) and 19,112 (3.8%) cases. In 2022, notifications rose to 34,786 (7%), followed by 22,778 (4.6%) in 2023 and 35,146 (7.1%) in 2024. The initial period (2010–2014) recorded fewer than 100 cases annually, reflecting the absence of outbreaks.

## Conclusions

The data reveal a significant epidemiological dynamic of the Zika virus in Brazil. After an initial period of low notification numbers (2010–2014), the 2016 outbreak marked the peak of the epidemic, accounting for more than half of the cases recorded over the 14 years investigated. A sharp decline was observed in the following years, until a slight increase in 2019. New fluctuations occurred, with rises in 2022 and 2024. The post-2016 reduction may be associated with population immunity and public health interventions, while the occasional increases suggest seasonal or regional factors. Continuous surveillance is essential to monitor and prevent new outbreaks.



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## **Session 5. Foodborne Pathogens and Food Safety**

# sciforum-110804: *Listeria monocytogenes* isolated from fresh pork meat commercialised in La Plata, Buenos Aires, Argentina

**Raúl Emilio Iza**<sup>1,\*</sup>, Hernan Darío Nievas<sup>1</sup>, Camila Aurnague<sup>1</sup>, Victorio Fabio Nievas<sup>1</sup>, Santiago Urtizberea<sup>2</sup>, Silvana Magalí Arce<sup>2</sup>, Francisco Bucchianeri Faccin<sup>2</sup>, Fabiana Alicia Moredo<sup>1</sup> and Magdalena Costa<sup>3</sup>

<sup>1</sup> Laboratorio de Bacteriología y Antimicrobianos, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata 1900, Buenos Aires, Argentina

<sup>2</sup> Student

<sup>3</sup> Instituto de Genética Veterinaria "Ing. Fernando N. Dulout" (UNLP-CONICET), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata 1900, Buenos Aires, Argentina

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*Listeria monocytogenes* is one of the most important bacterial foodborne pathogens. The ability to survive in diverse environmental conditions and the capacity to form biofilms are critical to guaranteeing food safety. No current data are available regarding the detection of this microorganism in fresh pork meat in the region. The aim of this study was to determine the presence of *L. monocytogenes* in fresh pork meat from meat retailers in La Plata, Buenos Aires, Argentina.

The present study is part of a food safety observational, transversal, and descriptive research project. From August to November 2024, a total of 138 pork meat samples were collected from 46 local meat retailers selected randomly in the city of La Plata. In each one of the stores, three samples were pooled to form a unique sample. Each pool of 25 g was cultured in 225 ml of Half Fraser broth for 24 h at 30 °C. After the pre-enrichment step, 0.1 ml of each culture was subcultured in 10 ml of secondary enrichment medium (Fraser) supplemented with 0.1 ml of ferric ammonium citrate for 48 h at 37 °C. Plating out and identification was performed with CHROMagar™ for *Listeria* according to the manufacturer's instructions. Suspected *L. monocytogenes* were selected for biochemical identification according to ISO 11290-1, and those confirmed were subcultured and preserved at −20 °C. Of the 46 retailers sampled, 32.61 % resulted positive for *Listeria monocytogenes*. Although this is the first report of *Listeria monocytogenes* in fresh pork meat in the region, similar values were reported from beef butcher shops.

The isolation of this microorganism, its survival characteristics, and the context of meat retailers that sell ready-to-eat food products might indicate the presence of other foodborne pathogens implicated in cross-contamination and food safety, highlighting the importance of implementing good manufacturing practices.



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# sciforum-105451: Antimicrobial activity of peptides produced by *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus* and *Tetrapisispora phaffii* against foodborne pathogens

Patrícia Branco <sup>1,2\*</sup>, Diogo Mendes <sup>1</sup>, Elisabete Muchagato Maurício <sup>1,3</sup> and <sup>4</sup>

<sup>1</sup> BIORG—Bioengineering and Sustainability Research Group, Faculdade de Engenharia, Universidade Lusófona, Av. Campo Grande 376, 1749-024 Lisbon, Portugal

<sup>2</sup> Linking Landscape, Environment, Agriculture and Food (LEAF), Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>3</sup> CBIOS—Research Center for Biosciences & Health Technologies, Universidade Lusófona, Campo Grande 376, 1749-024 Lisbon, Portugal

<sup>4</sup> Elisa Câmara, Lda, Dermocosmética, Centro Empresarial de Talaíde, n°7 e 8, 2785-723 Lisbon, Portugal

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In the food industry, synthetic preservatives are commonly used to inhibit microbial spoilage, prevent chemical and nutritional changes, and extend shelf life. However, growing consumer demand for healthier, minimally processed foods without synthetic additives has led to a search for natural preservation alternatives, such as antimicrobial peptides from yeasts. Pathogenic microorganisms such as *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp. represent major contamination risks to food products. To address these risks, various bioactive compounds with antimicrobial properties, including antimicrobial peptides, bacteriocins, and mycocins, have been identified as potential natural preservatives. This study aimed to assess the antimicrobial efficacy of peptide fractions from *Saccharomyces cerevisiae*, *Wickerhamomyces anomalus*, and *Tetrapisispora phaffii* against *E. coli* (ATCC 25922), *L. monocytogenes* (ISA 4008), and *Salmonella* spp. (ISA 4008). MIC tests revealed varying levels of activity, with peptides from *S. cerevisiae* demonstrating the strongest effect, achieving an MIC of 250 µg/mL against all three pathogens. Peptides from *W. anomalus* showed MICs of 2300 µg/mL for both *E. coli* and *L. monocytogenes*, and 1150 µg/mL against *Salmonella* spp. In contrast, peptides from *T. phaffii* required 1250 µg/mL to inhibit *E. coli* and *Salmonella* spp., and 2500 µg/mL to inhibit *L. monocytogenes*, indicating differing levels of antimicrobial efficacy. Additionally, a challenge test was conducted to determine the shelf life of the juice. The objective was to assess the antimicrobial efficacy of yeast-derived peptides for preserving watermelon juice at 25°C. The results showed that *S. cerevisiae* peptides achieved a >5-log CFU/mL reduction in *E. coli* within seven days, reducing the pathogen to undetectable levels and suggesting irreversible cellular damage. Peptides from *W. anomalus* also exhibited antimicrobial effects, though less pronounced, with a reduction of 1.1 log CFU/mL. These findings suggest that yeast-derived peptides, particularly from *S. cerevisiae*, could serve as effective natural biopreservatives to enhance the microbial safety of fruit juices.



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## sciforum-115816: Essential Oils for the Inhibition of *Listeria monocytogenes* in food applications

**Ana Olívia Serra Jorge**<sup>1,\*</sup>, M. Carpena<sup>2</sup>, S. Seyyedi-Mansour<sup>2</sup>, P. Barciela<sup>2</sup>, A. Perez-Vazquez<sup>2</sup>, Rafael Nogueira-Marques<sup>3</sup>, M. Beatriz P. P. Oliveira<sup>4</sup> and M.A. Prieto<sup>2</sup>

<sup>1</sup> REQUIMTE/LAQV, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

<sup>2</sup> Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Instituto de Agroecoloxía e Alimentación (IAA) – CITEXVI, 36310 Vigo, Spain.

<sup>3</sup> REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal.

<sup>4</sup> LAQV@REQUIMTE, Department of Chemical Sciences, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal.

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# sciforum-115789: Evaluation of Lactose-Sulfite-Phenol Red-Rifampicin (LSPR) Agar for the Co-Enumeration of *Salmonella enterica* and Shiga-toxigenic *Escherichia coli* in sausage batter

Boluwatife Esther Ajayi \* and Thomas Matthew Taylor

Animal science, Texas A&M University

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Accurate enumeration of *Salmonella enterica* and Shiga-toxigenic *Escherichia coli* (STEC) is critical for validating pathogen control in dry fermented sausage production. Lactose-Sulfite-Phenol Red-Rifampicin (LSPR) agar is a selective medium designed to recover and enumerate these pathogens under environmental stress. *S. enterica* produces black colonies on LSPR, while STEC produces cream-colored colonies, enabling visual differentiation. This study evaluates LSPR's performance for recovering *S. enterica* and STEC after lactic acid (pH 3.0) and salt (35.9 g NaCl/100 mL) stress and its suitability for co-enumeration in sausage batter.

Cocktails of seven rifampicin-resistant serotypes of *S. enterica* and STEC were stressed for 0, 30, 60, and 120 seconds, then plated on BHI, LSPR, and modified LSPR (MLSPR). Initial bacterial loads averaged 7.5 log CFU/mL. After 120 seconds of acid stress, STEC declined by 4.2 log CFU/mL, while *S. enterica* declined by 3.1 log CFU/mL ( $p = 0.05$ ). Under salt stress, reductions were 2.8 and 2.1 log CFU/mL, respectively ( $p > 0.05$ ). Two-way ANOVA revealed that exposure time significantly affected bacterial survival ( $p = 0.0027$ ), with no significant differences in recovery between media types ( $p = 0.0776$ ). MLSPR did not enhance recovery over LSPR ( $p = 0.238$ ).

The interaction between media and exposure time was not statistically significant for either acid ( $p = 0.2380$ ) or salt stress ( $p = 0.9031$ ), indicating consistent bacterial reductions across media.

The findings indicate that media type had no statistically significant effect on bacterial counts, as LSPR, BHI, and MLSPR supported similar levels of pathogen recovery showing the suitability of the media for co-enumeration of *Salmonella* and STEC in sausage batter. However, exposure time to stress solution showed a significant impact on bacterial survival, with longer exposure times resulting in greater reductions in STEC and *S. enterica* counts.



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# sciforum-115707: EVALUATION OF THE PRESENCE OF FOOD-BORNE VIRUSES IN DIFFERENT FOOD MATRICES IN SICILY

**Francesca Gucciardi \***, Maria teresa todaro, Antonina princiotta, Virginia Talarico, Alessandra Cannarozzo, Annalisa Guercio and Giuseppa Purpari

Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Via Marinuzzi, Palermo, Italy

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## Introduction

Enteric viruses of human and animal origin are widely present in the environment and are often responsible for foodborne and waterborne epidemics, constituting a major public health risk worldwide. In this study, we assessed the presence of foodborne pathogenic viruses, including Hepatitis A Virus (HAV), Norovirus (NoV), Rotavirus (RoV), and Adenovirus (AdV), in different food matrices and mains water collected during official control monitoring programs from harvesting areas, restaurants, shellfish markets, retail stores, and supermarkets in Southern Italy (Sicily).

## Methods

The survey was conducted over a six-year period (2019-2024) at the Istituto Zooprofilattico Sperimentale della Sicilia. A total of 598 Shellfish, 25 mains water samples, 79 vegetables, 163 fruits, and 5 gastronomic preparations containing fish products and/or raw vegetables were sampled. HAV, NoV, RoV, and AdV were detected by nested PCR and real-time PCR.

## Results

Of the 598 molluscs, 20 were positive for NoV (3.34%), of which 3.17% were positive for NoV GII and 1.33% for NoV GI (0.65%). The positive samples belonged to the species *Mytilus galloprovincialis*, *Chamelea gallina*, *Tapes decussatus*, and *Crassostrea gigas*. One mains water sample was positive for NoV GII (4%). No enteric viruses were detected in the vegetables, fruit, and food preparations tested. All samples were negative for HAV, RoV, and AdV.

## Conclusion

The identification of enteric viruses in shellfish and mains water provides information on the circulation of foodborne pathogens capable of infecting humans in the local area. In recent years, the large-scale production and distribution of food products and the increase in consumption of uncooked or ready-to-eat foods have exposed an increasing number of consumers to health risks. Molecular surveillance can be useful for implementing food safety by extending virological control along the entire food chain and for defining more effective risk communication strategies to consumers.



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# sciforum-114921: Microbiological assessment and acceptability of minced meat from hypermarkets: pathogenic and spoilage microorganisms

**Joana Paiva** <sup>1,2,3,\*</sup> and **Cristina Saraiva** <sup>2,3,4</sup>

<sup>1</sup> University of Trás-os-Montes e Alto Douro

<sup>2</sup> Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), 5001-801 Vila Real, Portugal

<sup>3</sup> Centro de Ciência Animal e Veterinária (CECAV), University of Trás-os-Montes and Alto Douro (UTAD), 5001-801 Vila Real, Portugal

<sup>4</sup> Veterinary and Animal Research Centre, Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes and Alto Douro (UTAD), 5000-801 Vila Real, Portugal

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Minced meat contains spoilage microorganisms, reducing shelf-life and causing economic and environmental impacts. It can also harbour pathogens that pose risks to public health, leading to foodborne illnesses and product recalls. The aim of this study was to evaluate the deteriorative and pathogenic microbiota of minced meat obtained in hypermarkets. Thirty samples of minced meat from bovine (n=15), swine (n=6), and poultry (n=9), produced from hypermarkets in Vila Real, were analysed for the presence of three important foodborne pathogenic bacteria, i.e., *L. monocytogenes*, *S. aureus*, and *E. coli*. In parallel, total aerobic microorganisms at 30°C (mesophiles), Enterobacteriaceae, Lactic Acid Bacteria (LAB), and *Pseudomonas* spp., were also enumerated as hygiene / safety indicator organisms. Microbial concentrations (log<sub>10</sub> cfu/g) of 6,01 ± 0,19 (total mesophiles), 3,79 ± 1,27 (Enterobacteriaceae), 4,14 ± 0,96 (LAB), 1,35 ± 1,31 (*Pseudomonas* spp.), 0,07 ± 0,29 (*L. monocytogenes*); 0,21 ± 0,66 (*S. aureus*), and 0,52 ± 0,81 (*E. coli*) were found. Significant differences in microbial counts were found only for total mesophiles and *E. coli*, with poultry showing higher levels than beef and pork (*p* 0.05). Although poultry meat had the overall highest microbial counts, which is associated with its lower acceptability, followed by beef and pork minced meat, this difference was not statistically significant (*p* > 0.05). Enterobacteriaceae was the microorganism with the lowest acceptability, contributing to a reduction in the general product acceptability (83.3%) compared to the higher rates for other microorganisms. These findings emphasize the role of minced meat in the transmission of pathogenic and deteriorative microorganisms, highlighting the importance of proper handling and thorough cooking to prevent foodborne diseases.



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# sciforum-111320: Natural Extracts as Antimicrobial Agents: A Comparative Study of *Castanea sativa*, *Hibiscus sabdariffa* L., and *Punica granatum* L.

**Joana C.L. Martins**<sup>1, 2, 3, 4, 5,\*</sup>, **Juliana Garcia**<sup>1, 2, 5</sup>, **Rafaela Guimarães**<sup>1, 5</sup>, **Maria José Alves**<sup>1, 5, 6, 7</sup> and **Maria José Saavedra**<sup>2, 3, 4</sup>

<sup>1</sup> AquaValor – Centro de Valorização e Transferência de Tecnologia da Água, Portugal

<sup>2</sup> CITAB – Centre for the Research and Technology of Agro-Environment and Biological Sciences and Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production (Inov4Agro), University of Trás-os-Montes e Alto Douro, 5001-801 Vila Real

<sup>3</sup> CECAV – Veterinary and Animal Research Centre and Associate Laboratory for Animal and Veterinary Science (AL4AnimalS), University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal

<sup>4</sup> AB2Unit – Antimicrobials, Biocides & Biofilms Unit and Veterinary Sciences Department University of Trás-os-Montes e Alto Douro (UTAD), 5001-801 Vila Real, Portugal

<sup>5</sup> LiveWell – Research Centre for Active Living & Wellbeing, Instituto Politécnico de Bragança, 5300-253 Bragança, Portugal

<sup>6</sup> CIMO – Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Bragança, Portugal

<sup>7</sup> Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

Food safety and food security cannot be considered without considering the One Health concept (1). This approach underscores the interdependence of health challenges compromising people, animals, plants, and the environment in which they coexist. Antimicrobial resistance is one of these threats, and the food chain is considered an important reservoir of antibiotic resistance genes (2). The food industry produces wastes and by-products from a variety of sources which have the potential to mitigate environmental impacts and be effective against pathogenic foodborne bacteria (3,4). In addition to by-products, natural sources have demonstrated significant antimicrobial and antioxidant properties, emphasizing the potential of natural extracts derived from the food chain to enhance food safety (5). This study aimed to assess the antimicrobial activity of natural products from northern Portugal, including *Hibiscus sabdariffa* L., *Punica granatum* L., and *Castanea sativa* hedgehog, to understand their potential as bioactive compounds. To prepare the crude extract, we employed a solid-liquid extraction process followed by freeze-drying to obtain the dried extracts. Initially, a screening of the extracts was conducted using the MIC method to evaluate their antimicrobial activity against key pathogens, including *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and more. Subsequently, the best-performing extract was analyzed for its physicochemical properties, including antioxidant capacity and phenolic compound content. *Hibiscus sabdariffa* L. exhibited MIC values of 7.81 mg/mL against both Gram-positive and Gram-negative bacteria, demonstrating bactericidal activity. *Punica granatum* L. showed MIC values ranging from 1.95 mg/mL to 3.91 mg/mL, while the hedgehog extract of *Castanea sativa*, displayed the most effective activity, with MIC values between 0.250 mg/mL and 1.00mg/mL. *Castanea sativa* hedgehog extract was further analyzed for its antioxidant activity and phenolic compound contents. Overall, this study highlights the promising role of these natural extracts as antimicrobial agents against pathogenic bacteria and the necessity for further investigations.



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# sciforum-113578: Strategies for the decontamination of *Acanthamoeba* spp. and *Naegleria fowleri* in different water systems in Bombali and Tonkolili districts (Sierra Leone): lessons from the UK Recovery Handbook for Biological Incidents

**Antonio Peña-Fernández<sup>1,2,\*</sup>, María de los Ángeles Peña<sup>3</sup>, Raoul Emeric Guetiya Wadoum<sup>4</sup> and Umar Anjum<sup>2</sup>**

<sup>1</sup> Department of Surgery, Medical and Social Sciences, Faculty of Medicine and Health Sciences, University of Alcalá, Ctra. Madrid-Barcelona, Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain.

<sup>2</sup> Leicester School of Allied Health Sciences, De Montfort University, Leicester, LE1 9BH, UK.

<sup>3</sup> Departamento de Ciencias Biomédicas, Universidad de Alcalá, Ctra. Madrid-Barcelona Km, 33.6, 28871 Alcalá de Henares, Madrid, Spain

<sup>4</sup> Department of Public Health, Microbiology and immunology, Ernest Bai Koroma University of Science and Technology, Makeni, Sierra Leone.

The US Environmental Protection Agency's Contaminant Candidate List includes *Acanthamoeba* spp. and *Naegleria fowleri* due to their ability to withstand harsh environmental conditions and their function as reservoirs for various important human pathogens. These human-pathogenic free-living amoebas (FLAs) pose significant challenges for their elimination from water systems because of their resistance to commonly used disinfectants, including those employed in treating drinking water. Our research group has previously isolated and detected cysts/amoebas of *Acanthamoeba* spp. in water from various open water systems across Bombali and Tonkolili Districts in Sierra Leone (SL), emphasising a potential public health risk to the Sierra Leonean population. We have used the emerging guidelines to decontaminate different environments, including open and drinking water systems, available in the UK Recovery Handbook for Biological Incidents (UKRHBI) to identify applicable recovery options (ROs) to mitigate the presence of FLAs in water systems in SL. The UKRHBI provides customised ROs for decontaminating/managing environments affected by any biological hazard, which are appropriately selected according to the physiological characteristics of the biological hazard (in this case, FLAs) and the characteristics of the site, following an accessible methodology. The following ROs might be applicable to the characteristics of the water systems and areas monitored: restricting water use, encouraging bottled water for drinking, removing contamination sources, modifying water treatment systems (e.g., integrating ozone-based treatments), and sludge treatment (use of ultrasonic disintegration treatment and topsoil stabilisation treatment). Emerging technologies, such as photolysis of sodium chloride and sodium hypochlorite, have demonstrated efficacy in inactivating *Acanthamoeba castellanii* and have the potential to be adapted to target other chlorine-resistant pathogens. The adoption of these interventions by the relevant authorities in SL could lead to the effective protection of vulnerable populations, including children, the elderly, immunocompromised individuals, and Ebola survivors.



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## **Session 6. Microbiome and Soil Science**

# sciforum-111303: The microbial and physicochemical interactions of soils in the degradation of buried polyethylene pipes in Colombia

**Maria Andrea Reyes Reyes<sup>1,2,\*</sup>, Brayan Danilo Vergel<sup>1</sup>, Jenny Andrea Oviedo<sup>2</sup>, Clara Inés Sánchez<sup>1</sup> and Jorge Hernando Panqueva<sup>2</sup>**

<sup>1</sup> Escuela de Microbiología, Universidad Industrial de Santander (UIS), Bucaramanga, 680002, Colombia

<sup>2</sup> Corrosión, Corporación para la Investigación de la Corrosión (CIC), Piedecuesta, 681011, Colombia

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The widespread use of polyethylene (PE) in industries such as agriculture and construction poses environmental challenges due to its recalcitrance in natural ecosystems. This study focuses on the microbial and physicochemical interactions of soils from Colombia, in the degradation of buried polyethylene pipes, combining molecular, microbiological, and physicochemical approaches to understand soil–microbe–plastic dynamics. Soil samples were collected from sites with buried polyethylene pipes exhibiting degradation signs. Microbial communities were characterized using 16S rRNA and ITS sequencing, with Nanopore technology, to identify bacterial and fungal taxa. Additionally, microbiological analysis was performed by taking swabs from the surface of degraded pipes to directly observe microbial colonization. Fungal isolates were cultured on Potato Dextrose Agar (PDA) to correlate traditional isolation techniques with molecular findings. Soil physicochemical properties, including pH, moisture, redox potential, particle size distribution, and others, were analyzed. Polyethylene degradation was assessed through surface analysis by scanning electron microscopy (SEM). Bacterial phylum Proteobacteria and genera such as *Pseudomonas*, along with fungal genera such as *Aspergillus* and *Fusarium*, were identified as key players in soils with higher degradation rates. Microbiological analysis of pipe surfaces confirmed microbial colonization, consistent with soil sequencing data. Fungal isolates grown on PDA matched molecular findings, validating the integration of traditional and molecular approaches. SEM imaging revealed significant surface erosion and microbial colonization on the polyethylene pipes. Physicochemical analyses showed that soils with neutral pH and higher moisture content supported more active and diverse microbial communities. This study demonstrates the critical role of microbial activity and soil physicochemical properties in polyethylene degradation. The integration of microbiological, molecular, and physicochemical methods offers a comprehensive framework to advance sustainable strategies for managing plastic pollution in soil environments.



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# sciforum-115555: Analysis of Soil Biological Activity in the Surroundings of Sfântu Gheorghe in Covasna

Éva Abod<sup>1,\*</sup>, László Veres<sup>2</sup> and Levente Csiszér<sup>1</sup>

<sup>1</sup> Department of Life Sciences, Faculty of Life Sciences and Sports, Sfântu Gheorghe, Sapiientia Hungarian University of Transylvania, 520036 Sfântu Gheorghe, Romania

<sup>2</sup> Master's student between 2019-2021, Sapiientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences - Târgu-Mureş, Department of Horticulture, 540485 Târgu-Mureş, Romania

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Covasna County in Romania is regarded as an agricultural region due to the exceptionally high proportion of arable land. One of the most significant indicators of agricultural productivity is soil fertility, which is influenced by numerous factors. Among these, the most important is the biological condition of the soil.

In our research, we analyzed the biological activity of soils in the surroundings of Sfântu Gheorghe. Soil samples were collected from five different soil types (preluposol, faeoziom, cambic faeoziom, aluviosol and gleysol) in autumn 2018 and spring 2019. For each field, 20 samples were collected, homogenized and the biological activity was measured in three replications using the fluorescein diacetate (FDA) hydrolysis method.

The faeoziom soil type showed enzymatic activity of  $3.9631 \pm 0.1818 \mu\text{g/g}\cdot\text{h}$  in autumn and  $2.9788 \pm 0.3277 \mu\text{g/g}\cdot\text{h}$  in spring; the cambic faeoziom showed  $4.0072 \pm 0.1145 \mu\text{g/g}\cdot\text{h}$  in autumn and  $3.0548 \pm 0.1621 \mu\text{g/g}\cdot\text{h}$  in spring; the gleysol type showed  $3.2869 \pm 0.1817 \mu\text{g/g}\cdot\text{h}$  in autumn and  $2.9988 \pm 0.4679 \mu\text{g/g}\cdot\text{h}$  in spring; the aluviosol soil type showed  $2.4186 \pm 0.0616 \mu\text{g/g}\cdot\text{h}$  in autumn and  $1.3421 \pm 0.3779 \mu\text{g/g}\cdot\text{h}$  in spring and the preluposol type recorded the highest values, with  $5.4517 \pm 0.2609 \mu\text{g/g}\cdot\text{h}$  in autumn and  $5.3677 \pm 0.1548 \mu\text{g/g}\cdot\text{h}$  in spring. For the faeoziom, cambic faeoziom, and aluviosol soil types, an approximately one-unit decrease was observed in spring due to the lower temperatures during the spring sampling period. For the gleysol soil type, the decrease was much smaller, and the differences between the values were not statistically significant. We attribute this to the application of manure by farmers after the autumn sampling, as manure contains a considerable number of microorganisms. For the preluposol soil type, enzymatic activity also decreased slightly, which can be explained by the fact that this area is a pastureland with continuous vegetation cover and no soil disturbance, providing a stable habitat for microorganisms, therefore a stable microbial activity.



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# sciforum-111511: Comparative evaluation of 16S rRNA and housekeeping gene-specific primer pairs for rhizobia and agrobacteria community metagenomics

Romain Kouakou Fossou

Laboratoire de Microbiologie, Biotechnologie et Bio-Informatique ; Unité Mixte de Recherche et d'Innovation en Sciences Agronomiques et des Procédés de Transformation ; Institut National Polytechnique Félix HOUPHOUËT-BOIGNY ; Yamoussoukro ; BP 1093 ; Côte

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Rhizobia are scattered across 18 genera, in which several taxa are known to have conserved genetic structure across rRNA operons. Recent metagenomic studies have revealed that the 16S rRNA gene and corresponding variable regions may have insufficient taxonomic resolution for the accurate identification of rhizobia and related plant-associated bacteria such as agrobacteria. Therefore, some housekeeping genes, including *gyrB* and *rpoB*, are used as alternative markers to 16S to analyze rhizobia communities. However, the extent to which the targeted genes and their corresponding primers could be suitable in metagenomic studies of all the genera of rhizobia remains elusive. This work evaluates *in silico* the taxonomic resolution of partial regions of two housekeeping and 16S rRNA genes to differentiate between rhizobia and agrobacteria communities. For the evaluation, nine V-regions (V1 to V9) spanning the entire 16S rRNA gene and commonly targeted in bacterial metagenomic-based studies were selected. The full-length 16S rRNA gene sequences retrieved from genome data archived in GenBank were first aligned for 19 rhizobia and agrobacteria genera, and the alignment was adjusted to the total number of positions that correspond to those of each V-region. The number of positions in each edited dataset was used to calculate pairwise similarity distances, which were used to identify uniquely distinguishable taxa for a given V-region, mainly at 97% (OTU) and 100% (ASV) cut-offs. The same uniquely distinguishable taxa approach was applied to *rpoB* (two regions) and *gyrB* (one region) at ~98% and 100% thresholds to allow for a comparative evaluation. This study suggested V5-V7 as the best 16S rDNA v-region for differentiating all genera at a 100% threshold. However, the *rpoB* and *gyrB* markers outcompeted the 16S rDNA in terms of taxonomic resolution regardless of the threshold, possibly replacing the use of 16S rDNA V-regions in the metagenomics of rhizobia and agrobacteria.



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# sciforum-111158: Microbial Diversity in Composting: identification and analysis of Fungus-related and bacterial communities for enhanced organic waste recycling

**Patrícia Branco**<sup>1,2,\*</sup> and **Elisabete Muchagato Maurício**<sup>1,3</sup>

<sup>1</sup> BIORG—Bioengineering and Sustainability Research Group, Faculdade de Engenharia, Universidade Lusófona, Av. Campo Grande 376, 1749-024 Lisbon, Portugal

<sup>2</sup> Linking Landscape, Environment, Agriculture and Food (LEAF), Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>3</sup> CBIOS—Research Center for Biosciences & Health Technologies, Universidade Lusófona, Campo Grande 376, 1749-024 Lisbon, Portugal

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Composting is an ancient and sustainable method of recycling organic waste into humus, a nutrient-rich substance that enhances soil productivity and reduces the environmental impact of waste disposal. Microorganisms, including fungi and bacteria, are essential to this process, as they drive the decomposition of organic matter.

This research aimed to identify fungal and bacterial species involved in composting and optimize their roles in decomposition. Compost samples were collected from Lusófona University's composting system after six months of processing organic waste, primarily fruit peels and vegetable scraps, mixed with dry plant material. Microbiological analyses, including DNA extraction, PCR amplification, and Sanger sequencing, were used to identify the microorganisms present.

The results showed that the most abundant microorganisms were filamentous fungi, with the most predominant species being *Aspergillus heyangensis*, *Aspergillus creber*, *Cladosporium asperulatum*, *Pestalotiopsis lespedezae*, and especially *Penicillium brevicompactum*, which was the most prevalent. These species dominate the decomposition of the complete residue and have potential applications in industry and composting. The bacterial species identified were predominantly represented by the genera *Bacillus* (e.g., *B. halotolerans*, *B. subtilis*, *B. amyloliquefaciens*, *B. mojavensis*), as well as species from *Sphingobacterium* (*S. kitahiroshimense*), *Serratia* (*S. fonticola*), and *Pseudomonas* (*P. fluorescens*). The collective contribution of these microorganisms to the breakdown of organic material during composting is significant.

This study highlights the diversity and significance of fungal and bacterial communities in composting, emphasising their potential to optimise organic waste recycling processes. Understanding the microbiota involved in composting opens avenues for developing biotechnologies, such as microbial inoculants, to enhance process efficiency and sustainability. Future research should focus on optimizing environmental conditions to support beneficial microorganisms, further advancing composting practices and producing higher-quality humus.



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# sciforum-114742: Phage-based biocontrol of nitrification in agricultural soil

**Laura Sala-Comorera**<sup>1,\*</sup>, Pedro Blanco-Picazo<sup>1</sup>, Gloria Vique<sup>1</sup>, Pablo Quirós<sup>2</sup>, María Dolores Ramos-Barbero<sup>1</sup>, Clara Gómez-Gómez<sup>1</sup>, Lorena Rodríguez-Rubio<sup>1</sup>, Tula Yance-Chávez<sup>2</sup>, Ignasi Salaet<sup>2</sup> and Maite Muniesa<sup>1</sup>

<sup>1</sup> Departament de Genètica, Microbiologia i Estadística. Universitat de Barcelona

<sup>2</sup> Departamento de I+D+i de Fertinagro Biotech S.L., Teruel, Spain

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## Introduction

Nitrogen fertilization boosts crop yields but is inefficient due to the rapid activity of nitrifying bacteria, which leads to the loss of useful nitrogen forms. This requires the use of large amounts of fertilizers, leading to environmental pollution from compounds like NO<sub>3</sub> and N<sub>2</sub>O. Although chemical inhibitors can improve fertilization, their impact on the environment and human health remains uncertain. A new strategy involves the use of bacteriophages (phages) that specifically target nitrifying bacteria.

## Methods

Bacteriophages were isolated from sewage samples. The inhibition of nitrifying activity was tested in AOB medium and soil to monitor nitrite production with a colorimetric assay. The increase in bacteriophage particles during infection was assessed with qPCR. Controls without the addition of bacteriophages or bacteria were included for in vitro and soil samples. The persistence of infectious bacteriophages was analyzed in water suspensions and soil under different pH levels (3, 5, 6, 7, 8) and temperatures (4, 15, 22, 37 °C) over 6 months.

## Results

We isolated a bacteriophage (FNF-1) capable of infecting four species of *Nitrosomonas*. FNF-1 inhibited bacterial growth, resulting in a reduction in nitrite formation in phage-treated samples, both in vitro and in the soil. Meanwhile, FNF-1 increased by 2-log<sub>10</sub> units within 48 hours. Infectious FNF-1 remained stable in water suspensions for 6 months at 4, 15, 22, and 37 °C and at pHs 6, 7, and 8 but was inactivated when exposed to pHs 3 and 5. In soils, FNF-1 infectivity showed a 2-log<sub>10</sub> reduction over 6 months.

## Conclusions

This study presents a novel approach using bacteriophages to eliminate nitrifying bacteria. Bacteriophages as biocontrol agents offer an effective and environmentally friendly alternative to inhibit nitrification while overcoming the limitations of chemical products. While their application shows potential for improving agricultural fertilization, further research is required to scale up and optimize a product.



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# sciforum-110346: Preliminary data on biodiversity effects of replacing plastic row covers with legume and non-leguminous covers in organic artichoke cropping

**Savanah Senn**<sup>1,\*</sup>, **Brianna Zimmerman**<sup>1</sup>, **Arianna Bozzolo**<sup>2</sup>, **Ray A. Enke**<sup>3</sup>, **Maxim Recke**<sup>1</sup>, **Jordan Schoonover**<sup>1</sup>, **Julia Perederiy**<sup>4</sup>, **Daila Melendez**<sup>1,5</sup> and **Les Vion**<sup>1</sup>

<sup>1</sup> Los Angeles Pierce College Department of Agriculture Sciences, Plant Science program, Woodland Hills, CA, 91371, USA

<sup>2</sup> Rodale Organic Institute, California Organic Center, Camarillo, CA, 93010, USA

<sup>3</sup> James Madison University, Biology Department, Harrisonburg, VA, 22807, USA

<sup>4</sup> OmBiome, Torrance, CA, 90502, USA

<sup>5</sup> Oregon State University, Department of Horticulture, Corvallis, OR, 97331, USA

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The purpose of this study was to investigate how replacing polyethylene row covers with other types of cover influences soil properties, microbial diversity, the composition of soil communities, and carbon metabolism in artichoke cropping. We carried out five treatments in four replicates, including plastic film, buckwheat, kurapia, crimson clover, and white clover. The samples were collected after rainfall events totalling 2 in. of precipitation during February 2024.

In the cross-sectional data, results were comparable between treatments with regards to physical and chemical properties, but there were significant differences in soil moisture and pH, with plastic having the lowest moisture and lowest pH. Higher CFUs in plastic at a  $10^{-4}$  dilution were noted.

For the composition and abundance of bacteria, the results indicated the following differences between white clover vs. plastic: higher *Adhaeribacter* sp. and *Phycococcus* sp. abundance in white clover treatments; higher *Rhodobacter* sp. and *Sphingobium* sp. abundance in plastic treatments. The following differences were observed between the legume treatment vs. the control: *Phycococcus* sp. and *Stenotrophomonas* sp. abundance was higher in the legume treatment. Results with low fold change values 1.33 were omitted.

The Chi-square test was negative for differences in proportions of carbon sources that could be metabolized in Biolog Ecoplate trials by each group ( $p=0.16$ ). There was a gap in the mosaic plot between the proportion of carbon sources metabolized by white clover communities and other treatments. For the artichokes, the GLM logit model indicated a difference between white clover treatments when compared with other treatments ( $p=7.76 \times 10^{-5}$ ). The carbon sources that differed significantly in the microbes' ability to metabolize them were delta-glucosaminic acid, L-alpha-glycerol phosphate, glucose-1-phosphate, i-erythritol, itaconic acid, phenylalanine, serine, and threonine.

Further studies should include microbial biomass analyses and nutrient analyses, and consider the effects of the treatments on yield. Longitudinal analysis of soil physical and chemical properties will confirm the effects of the treatments on physical and chemical properties of the soil.



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## **Session 7. Microbe-Plant Interactions**

# sciforum-114860: Impact of Co-Inoculating *Bradyrhizobium japonicum* with *Bacillus subtilis* or *Priestia megaterium* on Nitrate Inhibition of Symbiotic Nitrogen Fixation in Soybean

Steven Orito \*, Norikuni Ohtake and Takuji Miyamoto

Faculty of Agriculture, Niigata University, Niigata 950-2181, Japan

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Cultivated soybean is a vital source of protein and oil. Soybean obtains nitrogen (N) primarily from two sources: symbiotic nitrogen fixation (SNF) facilitated by microbes like *Bradyrhizobium japonicum*, and mineral nitrogen from soil or nitrate fertilizers. While nitrate fertilizers enhance plant growth, high nitrate levels inhibit nodule formation and SNF activity, reducing the proportion of nitrogen derived from SNF.

This study investigated the potential of nitrate-reducing bacteria (*Bacillus subtilis* and *Priestia megaterium*) to mitigate the nitrate-induced inhibition of SNF when co-inoculated with *B. japonicum* USDA 110 or USDA 6<sup>T</sup> strains. Nitrogenase activity, nodulation, root elongation, and plant biomass were evaluated under hydroponic conditions at 1 mM and 10 mM nitrate levels, with nitrate assimilation analyzed using the <sup>15</sup>N stable isotope. The growth responses of *B. japonicum* to varying nitrate concentrations were also examined. The growth of *B. japonicum* peaked at 7 mM nitrate but declined at 10 mM, suggesting inhibitory effects at higher concentrations.

Nitrogenase activity significantly decreased under high-nitrate conditions. However, co-inoculation with *B. subtilis* partially alleviated this inhibition, enhancing nitrogenase activity by 15.23% (USDA 110) and 72.62% (USDA 6<sup>T</sup>) at 10 mM nitrate. Conversely, *P. megaterium* co-inoculation further reduced nitrogenase activity in some treatments. Nodulation was similarly inhibited by high nitrate levels but improved with the co-inoculation of *B. subtilis* under both nitrate conditions, while the effects of *P. megaterium* were strain-dependent. Root length and plant biomass responded positively to co-inoculation with both bacteria, although nitrate-induced reductions were observed in nodule and root N concentrations.

This study highlights the potential of *B. subtilis* to counteract the nitrate-induced inhibition of SNF and improve soybean growth under nitrogen-rich conditions. The findings provide insights into microbial interactions that could inform sustainable agricultural practices aimed at enhancing legume productivity in nitrogen-rich soils such as those found in intensively managed agricultural systems.



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# sciforum-112715: PLFA Analysis of Soil Microbial Communities: Key Insights from Organic Artichoke Cropping Systems

**Savanah Senn<sup>1,\*</sup>, Arianna Bozzolo<sup>2</sup> and Brianna Zimmerman<sup>1</sup>**

<sup>1</sup> Los Angeles Pierce College Department of Agriculture Sciences, Plant Science program, Woodland Hills, CA 91371

<sup>2</sup> Rodale Organic Institute, California Organic Center, Camarillo, CA 93010

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The effects of replacing polyethylene row covers in artichoke production with legume and non-legume cover crops were investigated at Rodale Organic Institute in Camarillo, CA. The treatments included plastic film mulching (PFM) and cover crops: Buckwheat, Kurapia, Crimson Clover, and White Clover with four replicates. While various studies have established the benefits of PFM versus bare soil and cover cropping versus bare soil in agriculture, few studies have directly compared plastic with legume and non-legume cover crops.

The phospholipid fatty acid (PLFA) test on soil provides a generalized community profile. Microbial biomass is measured; bacteria, fungi, and protozoans can be differentiated, along with subgroups such as Gram-positive and Gram-negative bacteria, saprophytes, and mycorrhizae. Functional Diversity Analysis is provided. The PLFA analysis was performed by Trace Genomics during year 2 post production. Kruskal-Wallis tests were performed for the response variables to determine if cover cropping treatments were comparable to the control. A positive Kruskal-Wallis Chi-square test was followed up with the Dunn test used for multiple comparisons between groups, using the Holm correction.

There was a trend toward the plastic treatment having the lowest total bacteria biomass, the lowest fungi-bacteria ratio, lower levels of saprophytes, and less total microbial biomass in the plastic treatment. The results showed that the treatment was significant in determining the functional diversity index; plastic tended to have a lower functional diversity index than white clover ( $p=0.015$ ); the Dunn Test was negative ( $p\text{-adj}=0.076$ ). The treatment was significant in determining the mass of Arbuscular mycorrhizae in plots ( $p=0.010$ ). Arbuscular mycorrhizae biomass was different between the plastic and Kurapia treatments, according to the results ( $p\text{-adjusted}=0.023$ ).

The main result of the PLFA analysis showed that Arbuscular mycorrhizae biomass was significantly higher in the Kurapia treatments than the plastic controls. These results, combined with soil fertility analysis, will inform growers on best practices in organic artichoke cropping.



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# sciforum-110662: A reclamation strategy to stabilize degraded riverbanks: prospective benefits of using plant growth-stimulating bacteria associated with the riparian plant *Salvia procurrens*

Gabriela Cristina Sarti<sup>1,2\*</sup>, Josefina Ana Eva Cristóbal-Miguez<sup>3</sup>, Marta Kaplanski<sup>4</sup>, Isabel María Maza<sup>4</sup>, Antonio Paz-González<sup>2</sup>, Ernesto Benito Giardina<sup>4</sup>, Ana Rosa García<sup>3</sup> and Mirta Esther Galelli<sup>5</sup>

<sup>1</sup> Inorganic and Analytical Chemistry Cathedra, Department of Natural Resources and Environment, Faculty of Agronomy, University of Buenos Aires

<sup>2</sup> AQUATERRA Research Group, Interdisciplinary Center for Chemistry and Biology, CICA, As Carballeiras, s/n Campus de Elviña, University of A Coruña, 15008 Coruña, Spain

<sup>3</sup> Inorganic and Analytical Chemistry Cathedra, Department of Natural Resources and Environment, Faculty of Agronomy, University of Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina

<sup>4</sup> School of Floriculture and Gardening "Juan O. Hall". Department of Agricultural Engineering and Land Use, Faculty of Agronomy, University of Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina

<sup>5</sup> Agrofood Area, Department of Applied Biology and Food, Faculty of Agronomy, University of Buenos Aires, Av. San Martín 4453, Buenos Aires C1417DSE, Argentina

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The riparian plant *Salvia procurrens* Benth (commonly named blue creeper) is a native species from South America that is commonly used to remediate degraded lands, and in particular, to stabilize riverbanks due to its environmental adaptation. This study proposes co-inoculating this plant species with two plant growth-promoting microorganisms, *Azospirillum brasilense* SP7 and *Bacillus subtilis* subsp. *spizizenii*, to enhance restoration effects on riversides. Single-node blue creeper cuttings were placed in forestry plug trays to produce rooting using sand as a substrate. After 20 days, the plants were transplanted into 3L pots and inoculated with the bacterial mixture, applying 5 mL of inoculum at the base of each plant's stem. After six months, the plants were harvest and the following parameters were evaluated: plant height, root length, number of leaves and nodes, shoot and root biomass, and above- and belowground heavy metal concentrations. In the bioinoculant, the number of *B. subtilis* spores was quantified, reaching  $1.6 \times 10^5$  CFU mL<sup>-1</sup>. Bacterial inoculation had positive effects, increasing plant height (37%), root length (8%), and the number of leaves (57%) with respect to the control, but it had no effect on the number of nodes. Shoot and root dry weight in the inoculated plants more than doubled compared to the control, increasing by 117% and 167%, respectively. The roots of the inoculated plants showed higher contents of Fe, Cu, Mn, Cd, and Pb than those of the control plant. In particular, root Pb levels were more than 100% higher in the inoculated plants than in the non-inoculated plants. Co-inoculation was proven to have a beneficial effect not only in enhancing the growth of *S. procurrens*, making it ideal as ground cover in revegetation programs for riparian areas, but also for its capacity to accumulate metals.



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# sciforum-115323: Biocontrol of plant pathogenic fungi by endophytic *Trichoderma harzianum* through production of volatile organic compounds

Rajani Pijakala <sup>1,\*</sup>, Rajasekaran Chandrasekaran <sup>2</sup>, Shannon B. Olsson <sup>3</sup>, Ravikanth Gundasalamani <sup>4</sup> and Uma Shaanker Ramanan <sup>5</sup>

<sup>1</sup> Department of Microbiology, BMS College for women, Basavanagudi, Bengaluru, 560019, India

<sup>2</sup> Department of Biotechnology, School of Biosciences and Technology Vellore Institute of Technology, Vellore, 632014, India

<sup>3</sup> National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru, 560097, India

<sup>4</sup> Ashoka Trust for Research in Ecology and the Environment, Royal Enclave Bengaluru, 560064, India

<sup>5</sup> Department of Crop Physiology and School of Ecology and Conservations, GKVK, Bengaluru, 560065, India

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**Introduction:** Endophytic fungi were isolated from medicinal plants on the assumption that these would possess the ability to produce a diverse array of secondary metabolites that might help deter the plant pathogens.

**Methods:** The plant *Pterocarpus santalinus* was collected from the campus of the University of Agricultural Sciences, GKVK, Bengaluru (13.0801° N, 77.5785° E), in the year 2014-15. Endophytic fungi were isolated from the leaf, stem and flower (if available), as described by Shweta et al. (2010). Endophytes were screened against the soil-borne plant pathogens by a dual culture assay and a double-plate assay. Mechanisms were confirmed using SEM and GC-MS. Data were statistically analyzed.

**Result:** In this study, involving four pathogenic fungi and an endophytic fungus, *Trichoderma harzianum* isolated from *Pterocarpus santalinus*, show that besides mycoparasitism, VOCs play a major role in antagonism. In the double-plate assay, the endophytic *T. harzianum* significantly inhibited the mycelial growth of three of the four pathogens (*Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, and *Fusarium oxysporum*), while that of *Macrophomina phaseolina* was not affected. The GC-MS profile of the VOCs of the endophytic *T. harzianum* and their interaction with pathogens showed the presence of different classes of VOCs. The VOC profiles of endophyte-pathogen depended on the interacting pathogen, suggesting a species-specific response. In the presence of *M. phaseolina*, the VOC repertoire was subdued and in some cases not detected. Such dynamism in the chemical warfare between the endophyte and pathogenic fungi is little known and could be potentially interesting for unraveling the molecular mechanisms of VOC induction. Soybean seeds pre-treated with the endophyte *T. harzianum* showed significantly reduced mortality upon infection with *S. rolfsii* and *S. sclerotiorum* compared with those not enriched with the endophytes.

**Conclusion:** Understanding the immediate mechanisms involved in this endophyte-mediated resistance against pathogens could lead to a more effective use of endophytes in disease control in the future.



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# sciforum-114284: Exploring the Biotechnological Potential of Bacterial Endophytes from the Lathyrus Genus: Isolation, Screening, and Characterization for Agricultural Applications

Nihad Kerrou <sup>\*1</sup> and Djellali Belhadi <sup>2</sup>

<sup>1</sup> Microbial Ecology Laboratory, Department of Environmental Sciences, University of Bejaia, Algeria

<sup>2</sup> Microbial Ecology Laboratory, Department of Microbiology, University of Bejaia, Algeria

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Endophytic bacteria live alongside rhizobia in the nodules of legumes and can boost soil fertility by enhancing stress tolerance, producing growth-promoting hormones, and improving nutrient absorption.

The aim of this study was to isolate and characterize endophytic bacterial strains from the nodules of Lathyrus. A total of 301 strains were collected through direct sampling or trapping from soil samples gathered from different regions in Algeria. Twelve potential plant growth-promoting strains were then selected for their high inorganic phosphate-solubilizing ability. These strains were screened for enzymatic activities, including cellulase, protease, esterase, and urease production, which are critical for nutrient mobilization and plant growth promotion. API NE strips were used to obtain a comprehensive biochemical profile of the isolated endophytes.

The results demonstrated that oxidase exhibited the highest level of positive isolates, followed by DNase. Significant enzymatic activities were observed for pectinase and lipase, while moderate levels were detected for esterase and cellulase activities. Urease showed low levels of positive isolates, and none of the isolates tested positive for gelatinase. These findings underscore the metabolic diversity and potential agricultural benefits of the endophytic isolates in enhancing soil fertility, nutrient cycling, and plant health. The API 20 NE results revealed that glucose and arabinose assimilation, along with esculin hydrolysis, had the highest levels of positive isolates.  $\beta$ -galactosidase was also notable. Moderate activity levels were observed for arginine dihydrolase, while lower levels of positive isolates were seen for nitrate reduction, tryptophan deamination, and gelatin hydrolysis.

The promising enzymatic activities and plant growth-promoting traits of these endophytes make them suitable candidates for the development of eco-friendly agricultural inputs aimed at enhancing crop productivity and sustainability.



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# sciforum-109606: Harnessing Antioxidant-Producing Rhizobacteria to Boost Loquat Oxidative Stress Defense Under Adverse Environmental Conditions

**Mian Muhammad Ahmed**<sup>1,2,\*</sup>, **Pan Zhiyong**<sup>3,4</sup>, **Muhammad Asim**<sup>5</sup>, **Guo Xingyu**<sup>2</sup> and **Hamid Syeda Maira**<sup>6,7</sup>

<sup>1</sup> College of life Science and Technology, Tarim University, Alar, Xinjiang, 843301, China

<sup>2</sup> National and Local Joint Engineering Laboratory for High-Efficiency and High-Quality Cultivation and Deep Processing Technology of Characteristic Fruit Trees in Southern Xinjiang, China

<sup>3</sup> College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan, P.R. China

<sup>4</sup> National Key Laboratory for Germplasm Innovation & Utilization of Horticultural Crops, Huazhong Agricultural University, Shizhishan Street, Hongshan District, Wuhan, Hubei, 430070 China.

<sup>5</sup> National Key Laboratory for Germplasm Innovation and Utilization of Horticultural Crops, Huazhong Agricultural University, Wuhan, 430070, Hubei, China

<sup>6</sup> Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, Alar, Xinjiang, China.

<sup>7</sup> College of Life Science and Technology, Tarim University, Alar Xingjiang, China

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Loquat (*Eriobotrya japonica*) is economically important, but abiotic stresses like drought, salinity, and severe temperatures are having an increasing effect on its productivity. Plant cells experience oxidative stress, which leads to the buildup of reactive oxygen species (ROS). The production of antioxidant enzymes by specific bacteria in the loquat rhizosphere can reduce ROS levels and possibly increase the plant's resistance to these stresses. However, little is known about the processes by which these bacteria aid loquat in defending against oxidative stress. The purpose of this work is to identify and describe the bacterial strains linked to loquat that produce antioxidant enzymes and to clarify how they help loquat plants. After separating bacterial strains from the loquat rhizosphere, we examined their antioxidant enzyme activity, paying particular attention to the synthesis of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) under stress-like circumstances. To identify and examine the genes in charge of the synthesis of antioxidant enzymes and the control of stress responses, high-performing strains were chosen for in-depth characterization utilizing enzyme activity tests and molecular methods such as qPCR and genome sequencing. The chosen bacterial strains were administered to loquat seedlings, which were subsequently subjected to simulated oxidative stress, to evaluate their practical effects. To assess the effect on plant health, measurements were made of three important physiological indicators: electrolyte leakage, relative water content, and chlorophyll concentration. Furthermore, the amount of ROS in loquat tissues was measured, and qPCR was used to examine the expression of the plant's own antioxidant genes. This study found that antioxidant-producing bacteria improved loquat's oxidative stress tolerance, lowering ROS levels, increasing chlorophyll, and reducing electrolyte leakage, thus boosting resilience to oxidative damage. These findings pave the way for sustainable microbial inoculants to reduce oxidative stress in loquat farming, enhancing crop stability under tough conditions.



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## sciforum-115027: Plant-microbial interactions in the phytosphere of thyme (*Thymus vulgaris* L.)

Ekaterina Zharkova

Core Shared Research Facility, Industrial Biotechnologies, Federal Research Center, Fundamentals of Biotechnology,  
Russian Academy of Sciences, Moscow, 119071, Russian Federation

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Medicinal plants are one of the groups of economically important species and a valuable natural resource. One of these is thyme (*Thymus vulgaris* L.), the source of essential oil with high antimicrobial activity against microorganisms from various systematic groups. The study of thyme-microbial interactions was carried out in 2014-2024 for the collection of medicinal plants of the V. I. Edelstein Vegetable Experimental Station by cultural, chromatography, and NGS methods. The data obtained indicated the importance of the phytosphere's physiological and biochemical features for microbial community structure and representation depending on the development stage, since these factors significantly affected the plant-microbial interaction in the thyme-soil system. In other words, the features of the studied plant-microbiological interaction depended on which microorganisms inhabited specific parts of the thyme phytosphere and at what stage of plant development this occurred. Moreover, for bacterial communities, the key roles were played by the features of the phenological stage of the plant (54.3%) and the colonized part (44.7%), while for fungi, the interaction of these two factors was the most significant (75.8%). For microbial biotechnologies, it is necessary to obtain cultured identified forms of microorganisms. So, typical representatives of microbial communities were isolated and sequenced. At least nine phytosphere microorganisms can be recommended as protective and growth-regulating agents for thyme cultivation in the Non-Chernozem zone due to the suppression of root rot pathogens that actively develop during thaws and high humidity conditions.



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# sciforum-115708: Proteomic Insights into Bacterial Endophyte Responses to Chickpea Root Exudates Highlight the Role of GacS

Ana Paço<sup>1</sup>, Denise Pereira Torres<sup>1</sup>, Jean Armengaud<sup>2</sup>, Gérard Steinmetz<sup>2</sup> and Clarisse Brígido<sup>3,\*</sup>

<sup>1</sup> MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

<sup>2</sup> Département Médicaments et Technologies pour la Santé (DMTS), Université Paris-Saclay, CEA, INRAE, SPI, Bagnols-sur-Cèze 30200, France

<sup>3</sup> MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Portugal

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Legumes not only establish beneficial symbiosis with rhizobia but are also colonized by other endophytic bacteria, which are believed to play a significant role in plant fitness. However, the molecular mechanisms through which non-rhizobial bacteria respond to host-derived signals remain poorly understood compared to those of the well-studied rhizobia-legume symbiosis. In this study, the responses of two non-rhizobial endophytic bacteria, *Pseudomonas sp.* Q1 and *Kosakonia sp.* MH5, to chickpea root exudates were analyzed using high-throughput proteomics. Proteomic analysis revealed the differential expression of proteins involved in metabolism, cell envelope biosynthesis, stress response, oxidative stress defense, chemotaxis, nitrogen metabolism, type IV secretion systems, and transmembrane transport.

A notable finding was the upregulation of the *gacS* gene (2,754 bp) in the proteome of *Pseudomonas sp.* Q1 following exposure to chickpea root exudates. This gene encodes GacS, a hybrid histidine kinase and a critical component of the Gac two-component signal transduction system. To explore the functional role of GacS, a *gacS* knockout mutant of *Pseudomonas sp.* Q1 was generated. The mutant  $\Delta gacS$  strain showed no significant differences in swarming or swimming motility, biofilm formation, or phosphate solubilization compared to the wild-type strain. However, siderophore production in the mutant was reduced by approximately 8.8%, while the biosynthesis of antimicrobial metabolites remained unaffected. Additionally, under iron deficient conditions, subclover plants inoculated with the mutant  $\Delta gacS$  exhibited significantly lower growth compared to those inoculated with the wild-type strain.

These findings indicate that GacS in *Pseudomonas sp.* Q1 is only partially involved in regulating key plant-beneficial traits, suggesting that other two-component signal transduction systems may compensate for GacS function in this strain. Further studies are needed to fully understand the role of GacS in plant-bacteria interactions. This research enhances our understanding of the molecular dialogue between chickpea and its endophytic bacteria and identifies potential genetic targets to improve plant-microbe interactions.



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## sciforum-115019: The nematicide dodecan-1-ol has a reduced impact of on plant growth-promoting bacteria compared to conventional pesticides

Ricardo Machado<sup>1,\*</sup>, Paula Fareleira<sup>2,3</sup> and Jorge M. S. Faria<sup>2,3</sup>

<sup>1</sup> INIAV, Instituto Nacional de Investigação Agrária e Veterinária, Quinta do Marquês, 2780-159 Oeiras, Portugal

<sup>2</sup> INIAV, I.P., National Institute for Agrarian and Veterinarian Research, Quinta do Marquês, 2780-159 Oeiras, Portugal.

<sup>3</sup> GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Av. da República, 2780-157 Oeiras, Portugal

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Conventional pesticides commonly used in modern agriculture pose significant risks to plant health by disrupting soil microbiota, including beneficial plant growth-promoting bacteria. These non-target effects contribute to long-term soil degradation and ecological imbalances, ultimately undermining plant productivity and sustainability. Consequently, there is an urgent need to identify safer and more sustainable pest management alternatives that support plant health. Volatile phytochemicals, known for their bioactivity against plant pests, offer a promising solution due to their reduced environmental impact and lower toxicity.

This study evaluated the toxicity of dodecan-1-ol, a volatile compound with nematicidal properties, on crop-associated bacterial species of importance for plant growth and soil health: *Bacillus megaterium*, *Pseudomonas azotoformans*, *Paenibacillus zeisoli*, and *Burkholderia phymatum*. Direct-contact bioassays in 96-well microplates were used to monitor bacterial growth spectrophotometrically. For comparison, the conventional pesticides emamectin benzoate and oxamyl were tested under identical conditions.

Dodecan-1-ol inhibited bacterial growth by a maximum of 20%, significantly lower than the 93% and 11% inhibition observed with emamectin benzoate and oxamyl, respectively. These results were obtained at a concentration of 0.02 mg/mL for all compounds. Importantly, at this concentration, dodecan-1-ol achieves its lowest maximal effective concentration (EC<sub>100</sub>) against plant-parasitic nematodes, whereas emamectin benzoate and oxamyl exhibit limited nematicidal efficacy and require substantially higher application rates in agricultural settings.

By combining effective nematicidal activity with minimal impact on plant-beneficial bacteria, this study highlights the potential of dodecan-1-ol to support plant health while addressing pest control needs. These findings emphasize its role as a safer and more sustainable alternative to conventional pesticides, with promising applications in integrated pest and soil management strategies to promote healthy crop growth and long-term agricultural sustainability.



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# sciforum-115784: Treasures of Tunisian Wild Legumes: Diversity of Root Nodule Endophytes

**Roukaya Ben Gaied**<sup>1,2,\*</sup>, **Imed Sbissi**<sup>3</sup>, **Clarisse Brígido**<sup>4</sup> and **Mohamed Tarhouni**<sup>3</sup>

<sup>1</sup> Institute of Arid Lands, Laboratory of Pastoral Ecosystems and Promotion of Spontaneous Plants and Associated Micro-Organisms University of Gabes, Tunisia,

<sup>2</sup> MED – Mediterranean Institute for Agriculture, Environment and Development, Universidade de Évora

<sup>3</sup> Laboratory of Pastoral Ecosystems and Promotion of Spontaneous Plants and Associated Micro-Organisms, Institute of Arid Lands, University of Gabes, Medenine 4119, Tunisia.

<sup>4</sup> MED-Mediterranean Institute for Agriculture, Environment and Development & CHANGE-Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, Évora, 7006-554, Portugal.

Overgrazing and climate change have resulted in the expansion of marginal lands and the deterioration of soil quality, leading to a decline and extinction of numerous Fabaceae species. In this context, particular attention was given to study the symbiotic interactions between native legumes and their associated microbiome, as a sustainable alternative for restoring vegetation cover and reestablishing soil fertility in vulnerable areas. The main objective of this study was to isolate and characterize the root nodules endophytes from 15 wild legumes, originating from diverse Tunisian climatic zones (humid, arid, and Saharan). The taxonomic diversity of the isolates was determined by means of morphological, genomic, and phylogenetic analyses based on molecular screening by BOX-PCR and sequencing of 16S rRNA and *atpD* genes. To further assess the symbiotic characteristics of certain isolates, we proceeded with the amplification of *nodC* and *nodZ* genes. In a collection of 210 strains, phylogenetic analysis revealed the presence of 46 rhizobial strains distributed among 6 genera, namely *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Neorhizobium*, and *Agrobacterium*. Our results showed the predominance of *Sinorhizobium* genus in arid and Saharian zones of southern Tunisia, represented by three different symbiovars: sv. *meliloti*, sv. *Tunisiaense*, and sv. *Vachelliae*. However, no *nodC* genes were detected in *Bradyrhizobium*, which was only present under humid climate suggesting the impact of environmental conditions on the diversity of the rhizomicrobiome and its symbiotic interaction with wild legumes. In addition, various non-rhizobial isolates were identified, including strains from  $\alpha$ -,  $\beta$ -, and Gamma-proteobacteria, Firmicutes, and Actinobacteria. These strains were not only highly tolerant to different abiotic stresses, but also presented different plant growth promoting features (PGP), making them perfect candidates to boost the survival and productivity of food legumes in drylands.



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# sciforum-115129: Unraveling the bioremediation potential of Arbuscular Mycorrhizal Fungi through a bio-inoculation approach

Ranjna kaundal \* and Vipin Parkash

Forest Pathology Section, Forest Protection Division, ICFRE- Forest Research Institute, Dehradun, Uttarakhand-248006, India

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Arbuscular Mycorrhizal Fungi (AMF) even exist in soils polluted by heavy metals and play an important role in improving plant tolerance under stress conditions. A pot culture experiment was carried out to study the effects of AMF inoculation on growth and Cadmium (Cd) uptake through soil enzymatic activities under varying levels of Cd concentrations (3, 6, and 9 mg kg<sup>-1</sup> of soil) in soil by *Albizia lebbeck* (L.) Benth. seedlings. AMF inoculation significantly improved both shoot and root biomass at a Cd concentration of 3 mg.kg<sup>-1</sup>, while a reduction in biomass was observed at 9 mg.kg<sup>-1</sup> concentration of Cd. AMF also enhanced plant growth, with maximum shoot length observed at 3 mgkg<sup>-1</sup> and root length at 9 mgkg<sup>-1</sup>. However, AMF spore count decreased while the root colonization rate significantly increased with Cd concentration, leading to a reduction in Cd levels in the rhizosphere of AMF-inoculated seedlings. Soil enzymatic activities like dehydrogenase, acid phosphatase, alkaline phosphatase, and catalase were significantly reduced in non-inoculated seedlings under Cd stress, whereas AMF inoculation improved these enzymatic activities. These results indicate that AMF can alleviate Cd toxicity by decreasing translocation and enhancing the bioaccumulation factor in *Albizia lebbeck* seedlings. Thus, AMF in combination with *Albizia lebbeck* offers a promising approach for the phyto-stabilization of Cd-contaminated soils. These findings also highlight the potential of the AMF–*Albizia lebbeck* interactions system in the bioremediation of Cd-polluted ecosystems.



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MDPI AG  
Grosspeteranlage 5  
4052 Basel  
Switzerland  
Tel.: +41 61 683 77 34

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