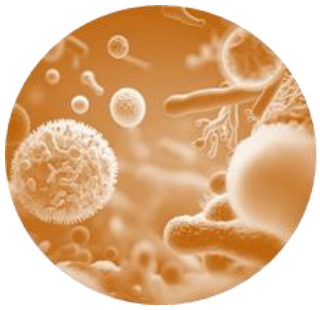
XX TAXON

XX Meeting of the Taxonomy, Phylogeny, and Biodiversity Group




PROGRAMME & ABSTRACT BOOK

September 26-28th, 2024

Salamanca, Spain

Taxonomía, Filogenia y Diversidad
SOCIEDAD ESPAÑOLA DE MICROBIOLOGÍA

VNiVERSiDAD D SALAMANCA

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Welcome Message

We warmly welcome you to XX TAXON, the XX Edition of the Taxonomy, Phylogeny, and Biodiversity Group Meeting. This year, our meeting will be held in the emblematic setting of the Hospedería Fonseca, located in the heart of Salamanca, between September 26 and 28.

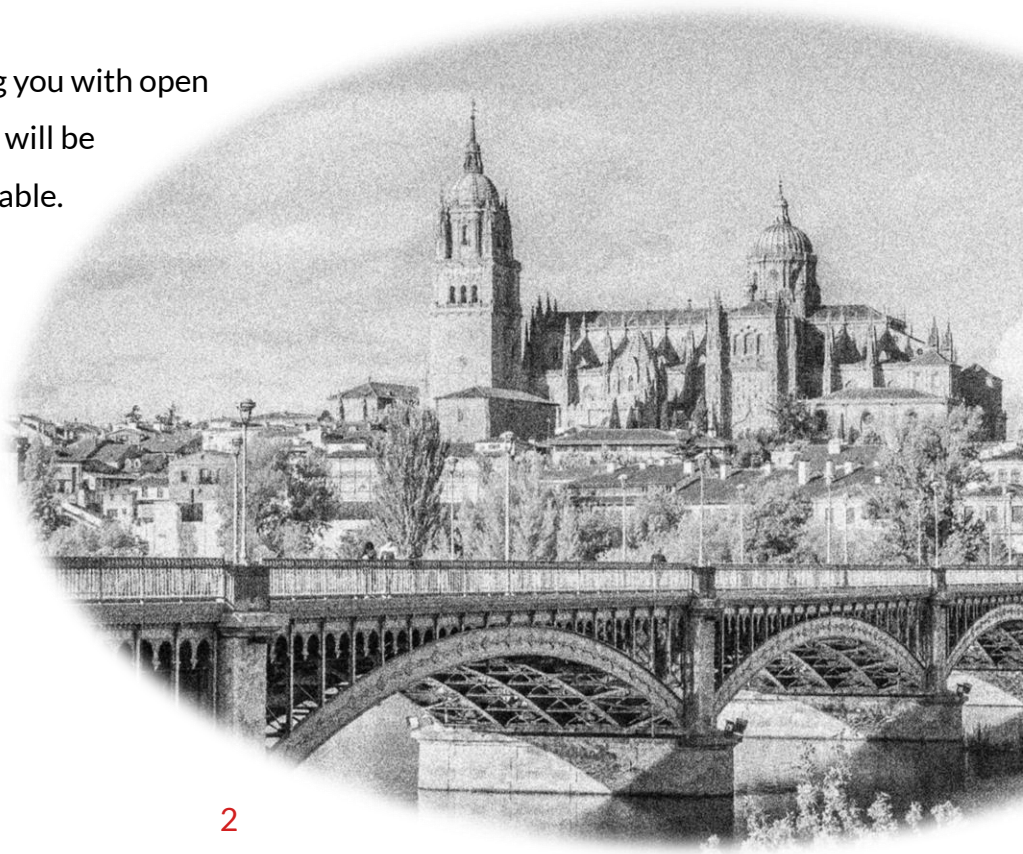
Salamanca, a UNESCO World Heritage city, is not only famous for its prestigious university, one of the oldest in Europe, but also for its rich history and vibrant culture. This academic and cultural environment provides the perfect backdrop for our meeting, promoting a space for enriching exchange and debate.

The Hospedería Fonseca, which will be our venue, is part of the historical heritage of the University of Salamanca. This place not only stands out for its architectural beauty but also for its historical significance and its role in the academic field. It offers modern and comfortable facilities in a building full of history, providing a unique atmosphere for our congress.

We hope that the program prepared for the Meeting will be of great interest to everyone, and that it provides a dynamic forum for the presentation of the latest research in the field of taxonomy and diversity.

We look forward to welcoming you with open arms, hoping that this meeting will be as productive as it is unforgettable.

Organizing Committee



Committees

Scientific Committee

The scientific committee is composed of the following members of the Spanish Society of Microbiology:

- **Jesús López Romalde.** University of Santiago
- **David Ruiz Arahal.** University of Valencia
- **Cristina Sánchez Porro.** University of Seville
- **Margarita Aguilera Gómez.** University of Granada
- **Martha E. Trujillo.** University of Salamanca
- **Margarita Gomila.** University of the Balearic Islands
- **Ana Isabel Vela Alonso.** Complutense University of Madrid

Organizing Committee

The local organizing committee is composed of the following members of the Spanish Society of Microbiology and the University of Salamanca:

- **Martha E. Trujillo**
- **Maite Ortúzar**
- **Raúl Riesco**
- **Andrés Alonso**
- **Víctor Formariz**
- **Jhon Alexander Suescún-Sepúlveda**
- **Ariana Reina-Hidalgo**

Programme

Thursday, September 26

8:30 – 10:00	Registration – Hospedería Fonseca Hall
10:00 – 10:40	Welcome – *Martha E. Trujillo* Vice-chancellor USAL – *José Miguel Roco* President of FEMS – *Antonio Ventosa* President of *Grupo de Taxonomía, Filogenia y Biodiversidad – Jesús L. Romalde*
10:40 – 11:20 **Plenary Talk**	A novel ultra-small bacterium affiliated with the cosmopolitan clade of host-associated and free-living verrucomicrobia *Svetlana Dedysh – Winogradsky Institute of Microbiology*
11:20 – 11:30	Novogene – *Metehan Cifdaloz*
11:30 – 12:30	**Coffee break – Posters, Networking**
12:30 – 13:50	**Session 1: Environmental Microbiology** **Chairs: Svetlana Dedysh and Antonio Ventosa**
12:30 – 12:50	The impact of scots pine genotype to needle mycobiome *Carel E. Carvajal-Arias – Estonian University of life Science*
12:50 – 13:10	Interactions of actinomycetes and Physical Weathering in the deterioration of Egyptian Stone Monument *Hesham Abdulla – Suez Canal University*
13:10 – 13:30	Optimization and validation of a qPCR method for the detection of *Pseudomonas* in environmental samples *Marc Crespo – University of the Balearic Islands*
13:30 – 13:50	Deciphering the metabolic potential of *Ensifer* spp. in bioremediation *María del Carmen Montero-Calasanz – Andalusian Institute of Agriculture and Fisheries Research and Training*
14:00 – 16:00	**Lunch – Fonseca restaurant**

16:00 – 17:30	**Session 2: Applied Systematics** **Chairs: David R. Arahal and Margarita Aguilera**
16:00 – 16:30 **Invited Talk**	Revisiting genome data minimal standards for its use in taxonomy *Raúl Riesco – University of Salamanca*
16:30 – 16:50	*Bacteroidota* is a key phylum for healthy human microbiota and especially susceptible to chemical xenobiotic exposure *Margarita Aguilera – University of Granada*
16:50 – 17:10	Actinomycetes mediated degradation of persistent organic pollutants in a biofilter for Industrial Effluent Treatment *Sahar El-Shatoury – Suez Canal University*
17:10 – 17:30	Impact of bisphenols and parabens on taxa composition of intestinal microbiota in relation to childhood obesity *Jorge Muñoz-Payá – University of Granada*

17:30 –18:30	***Taxonomía, Filogenia y Biodiversidad* Group Meeting**
19:00 – 21:00	**Welcome Reception – Lucía de Medrano**
21:15	**Cultural tour: Ieronimus**

Friday, September 27

9:00 – 11:00	**Session 3: Microbial Diversity** **Chairs: Jesús L. Romalde and Cristina Sánchez-Porro**
9:00 – 9:30 **Invited Talk**	What is a species and a strain? The data has shown us the answers! *Konstantinos T. Konstantinidis – Georgia Institute of Technology*
9:30 – 9:50	Unveiling the Hidden Diversity of the *Stenotrophomonas* Genus Through Phylogenomic Analysis *José Laço – University of the Balearic Islands*
9:50 – 10:10	Bridging Viruses and Prokaryotic Host through Miniature Inverted-repeat Transposable Elements (MITEs) *Ana-Belén Martín-Cuadrado – University of Alicante*
10:10 – 10:30	Genomic exploration of the culturable bacteriome of *Lupinus angustifolius* *Jhon A. Suescún-Sepúlveda – University of Salamanca*
10:30 – 11:00 **Invited talk**	To name or not to name? Species based on single strains *Stephanus N. Venter – University of Pretoria*
11:00 – 12:00	**Coffee break – Posters, Networking**

12:00 – 14:00	**Session 4: Taxogenomics** **Chairs: Raúl Riesco and Margarita Gomila**
12:00 – 12:30 **Invited talk**	Homonymy amid biological codes: analysis of replacement of names among prokaryotes *David R. Arahal – University of Valencia*
12:30 – 12:50	Genomic characterization of seven multiresistant *Enterococcus* spp. isolated from wastewater samples in Galicia, Spain *Jesús L. Romalde – University of Santiago de Compostela*
12:50 – 13:10	Network analysis-aided genus demarcation within the families *Halomonadaceae* and *Oceanospirillaceae* *Rafael R. de la Haba – University of Sevilla*

13:10 – 13:30	Take-home messages from the Genome Taxonomy Database *Maria Chuvochina – The University of Queensland*
13:30 – 14:00 **Invited Talk**	Two codes of prokaryotic nomenclature: how do they become one? *William B. Whitman – University of Georgia*
14:00 – 14:15	**Group Photo**
14:15 – 16:00	**Lunch – Fonseca restaurant**

16:00 – 18:00	**Session 5: Molecular Ecology** **Chairs: Stephanus N. Venter and Maite Ortúzar**
16:00 – 16:20	Metagenomes from deep-sea hydrothermal vents: Sources of novel genomic representation in the Tree of Life *Anne-Louise Reysenbach – Portland State University*
16:20 – 16:40	Understanding the interactions between lupin and its root-associated bacteria *Maite Ortúzar – University of Salamanca*
16:40 – 17:00	Shifts in the composition and dynamics of the almond endosphere in the context of *Xylella fastidiosa* infection through culture-independent methods *Ibai Cano – University of the Balearic Islands*
17:00 – 17:20	Land-use legacies on soil microbial communities' response to wildfires *Andrés Alonso – University of Salamanca*
17:20 – 18:00 **PhD Thesis Award Talk**	Bacterial whole genome sequencing for establishment of reference sequences, comparative genomics, biomarker discovery and characterization of novel taxa *Francisco Salvà-Serra – University of the Balearic Islands*
18:00 – 18:30	**Final Discussion – Martha E. Trujillo**
18:30 – 20:00	**Cultural tour: City of Salamanca**
21:00	**Dinner – Palacio de Figueroa**

Saturday, September 28

Visit to La Alberca (https://laalberca.com/)	
9:30	Departure (Hospedría Fonseca)
11:00 – 13:30	La Alberca – Free Time to Look Around
13:30	Meet at Village Entrance – Walk to Hotel “Abadía de Los Templarios”
14:15	Lunch
16:30	Travel back to Salamanca

Abstracts

PLENARY TALK

A novel ultra-small bacterium affiliated with the cosmopolitan clade of host-associated and free-living verrucomicrobia

Svetlana Dedysh[1], Olga Danilova[1], Varvara Salova[1], Daniil Naumoff[1], Igor Oshkin[1]

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The bacterial phylum *Verrucomicrobiota* accommodates free-living and symbiotic microorganisms, which inhabit a wide range of environments and specialize in degradation of various polysaccharides. Members of this phylum are difficult to culture in the laboratory. Therefore, much of the currently available knowledge of these bacteria originated from cultivation-independent studies. A genus-level clade of *Candidatus* Didemnitutus-affiliated bacteria, which belongs to the family *Opitutaceae* of the order *Opitutales*, is one of these enigmatic as-yet-uncultivated groups. This clade is composed of two dozen metagenomes assembled from aquatic and soil habitats all over the world and named after a verrucomicrobial symbiont of the tunicate *Lissoclinum* sp. Here, we describe the cultivated member of this clade, strain Vm1, which was isolated from a laboratory bioreactor with *Methylococcus*-dominated methane-oxidizing consortium. Strain Vm1 was represented by ultra-small, motile cocci with a mean diameter of 0.4 µm that grew in oxic and microoxic conditions at temperatures between 20 and 42 °C. The range of growth substrates included several sugars and organic acids, as well as some heteropolysaccharides. Multiple transfers of these verrucomicrobia in a mineral medium in a co-culture with *Methylococcus capsulatus* suggested their ability to utilize metabolites excreted by the methanotroph. The genome of strain Vm1 was 4.8 Mb in size and contained about 4300 protein-encoding genes, including a wide repertoire of CAZyme-encoding genes. According to the result of phylogenomic analysis, this bacterium belongs to a clade defined by the symbiotic *Candidatus* Didemnitutus mandelae and the free-living verrucomicrobium from oilsands tailings pond, *Oleiharenicola alkalitolerans*. Analysis of physiology and glycolytic potential of this bacterium may offer an inside in the reasons behind wide distribution of strain Vm1-like verrucomicrobia in the environment.

SESSION 1: Environmental Microbiology

The Impact of Scots Pine Genotype to Needle Mycobiome

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This research investigates the mycobiome on pine needles of *Pinus sylvestris* clones in seed orchards, focusing on the influence of pine genotype and location of orchard. We collected needles from 13 *P. sylvestris* clones across 9 Estonian seed orchards, extracted DNA, and amplified the ITS1-5.8S-ITS2 regions. Sequencing was done using the PacBio platform, and sequences were processed with Pipecraft v0.1.4 to obtain Operational Taxonomic Units (OTUs) at 98% similarity. The fungal community composition was determined using Bray-Curtis matrix, NMDS, and PCoA. ANOSIM and PERMANOVA tests were used to assess variation and differences in the mycobiome. We obtained 516,837 high-quality ITS sequences, representing 918 OTUs, with Ascomycota and Basidiomycota as dominant phyla, con un 99%, Mucoromycota and Chytridiomycota, was minimal, each representing only 0.1% of the OTUs. Our analysis revealed significant diversity in fungal lifestyles, with phytopathogenic fungi being dominant. NMDS showed a central cluster grouping and similarity trend of abundances between most locations and clones. However, some clones showed dissimilarities in the mycobiome structure across different location of seed orchards. PCoA visualization indicated that both the orchard location and the pine genotype may influence the mycobiome composition. ANOSIM revealed variation in the mycobiome composition, with the pine genotype influence being less pronounced than the location. PERMANOVA analyses confirmed that both factors location and the clone have a significant effect the mycobiome composition. Two clones and two locations showed statistically differences in the mycobiome of pine needles. In conclusion, both the location and the genotype of the pine influence the composition of the fungal mycobiome and the richness of the fungi. This also suggests that pine clones can maintain specific fungal richness in different locations, evidencing that genotype may have a greater effect on mycobiome composition.

Interactions of Actinomycetes and Physical Weathering in the deterioration of Egyptian Stone Monument

Hesham Abdulla

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This study investigates the role of actinomycetes in the biodeterioration of Egyptian stone monuments, focusing on their metabolic activities and interactions with physical weathering agents. Stone monuments deteriorate due to physical, chemical, and biological factors, with actinomycetes being significant contributors. We aim to elucidate the mechanisms of actinomycetes-induced decay. To assess this, actinomycetes isolated from Tell Basta tomb stones were examined in artificial weathering chambers. We have simulated natural conditions to accelerate the biodeterioration process of intact stones for one month in artificial weathering chambers for 100 cycles of 0.5 M NaCl / Na_2SO_4 salt treatments, at various intervals. The cabinet's temperature and relative humidity (RH) were set to daily cycles: 11 hours at 35°C and 70% RH, 2 hours at room temperature (for treatments and measurements), and 11 hours at 8°C and 40% RH, reflecting the climate of the Tell Basta area. The study involved three actinomycetes species, *Nocardia brasilinsis*, *Streptomyces sp.3*, and *Streptomyces sp.5*; which we previously isolated from the deteriorated stone monuments. Results showed that actinomycetes significantly contributed to stone decay, particularly under conditions involving Na_2SO_4. The inoculated stones exhibited considerable weight loss, increased porosity, reduced surface hardness, and decreased sound velocity. Notably, *Streptomyces* species were the most aggressive, causing up to 20% more weight loss compared to uninoculated stones. Biofilm production was observed through light and scanning electron microscopy. The examined strains played a crucial role in the degradation process by enhancing the effects of salt crystallization and exerting mechanical stress through hyphal penetration. The study demonstrates that actinomycetes, through their metabolic activities and biofilm formation, significantly enhance the physical weathering of stones, leading to accelerated deterioration of monuments. These results highlight the need for sustainable stone conservation practices to reduce microbial impact, aligning with SDG 11 “Sustainable Cities and Communities” and SDG 15 “Life on Land”.

Optimization and validation of a qPCR method for the detection of *Pseudomonas* in environmental samples

Marc Crespo[1], Magdalena Mulet[1], Elena García-Valdés[1,2] and Margarita Gomila[1]

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The taxonomy of *Pseudomonas* has historically been a challenge due to its complexity. Recent studies based on phylogenomic and comparative analyses have revealed that many species within this genus do not share a common evolutionary history, resulting in the separation of several clades and the description of new genera. In the field of detection, quantitative PCR (qPCR) has been established as a fundamental technique that allows for the specific and quantitative detection of species in environmental samples. For the detection of *Pseudomonas*, specific primers targeting genes such as *rpoD* and *gyrB* have been designed, demonstrating high discriminatory capacity. Despite these advances, significant challenges remain in designing primers that can encompass the entire diversity of *Pseudomonas*. In response to this, some researchers have explored the design of specific primers targeting the 16S rRNA gene or the *oprI* gene as potential solutions (Bergmark *et al*., 2012; De Vos *et al*., 1993).

The aim of this study was to optimize and validate the various qPCR methods described in the literature for the detection of *Pseudomonas* and to evaluate their applicability in environmental samples. Protocols were developed using Taqman probes, optimizing different annealing temperatures with the specific primers, as well as the number of cycles. Methodological sensitivity and specificity were evaluated by amplifying different species representative of the various clades described within the *Pseudomonas* genus and other closely related genera, which were until recently considered members of this genus. The protocol was validated in environmental samples from different origins.

The results showed that a pair of specific primers for the 16S rRNA gene were highly effective in detecting *Pseudomonas* and other closely related genera. An optimized method was developed that successfully validated all species within these clades and demonstrated its effectiveness in a variety of environmental samples.

References

Bergmark L, Poulsen PH, Al-Soud WA, Norman A, Hansen LH, Sørensen SJ. 2012. Assessment of the specificity of *Burkholderia* and *Pseudomonas* qPCR assays for detection of these genera in soil using 454 pyrosequencing. FEMS Microbiol Lett. 333(1):77-84.

De Vos D, Lim JR A, De Vos P. Sarniguet A. Kersters K, Cornelis P. 1993. Detection of the outer membrane lipoprotein I and its gene in fluorescent and non-fluorescent pseudomonads: implications for taxonomy and diagnosis. J Gen Microbiol 139(9):2215-23.

Funding

This work has been made supported by the project PID2020-119449RB-I00 funded by the Ministry of Science and Innovation MCIN/AEI, 10.13039/501100011033.

Deciphering the metabolic potential of *Ensifer* spp. in bioremediation

Maria del Carmen Montero-Calasanz[1], Jose Maria Barcia-Piedras[1], Maria Teresa Cubo[2], Maria Rosario Espuny[2], Maria Camacho[1]

[1]*Andalusian Institute of Agriculture and Fisheries Research and Training - IFAPA Las Torres, Spain,*
[2]*Departamento de Microbiología, Facultad de Biología, Universidad de Sevilla, Seville, Spain.*
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More than 6.24% of the European agricultural area already has toxicity problems related to agricultural practices. Phytomanagement of contaminated soils was presented as a feasible solution and in line with the principles of circular economy. In this sense, it has been shown that *Medicago* spp. (*Leguminosae*, *Fabaceae*) are perfect candidates for phytomanagement, thanks to their high phytoextractive potential, their growth rate, their greater adaptability to stressed environments, their ability to grow in soils contaminated by heavy metals, and, of course, their ability to establish a symbiotic relationship for nitrogen fixation with nitrogen-fixing bacteria. However, heavy metal contamination can seriously affect the alfalfa-rhizobium symbiosis. Therefore, proper selection of well-adapted symbionts is required to maximise the benefits of the use of alfalfa in the remediation of contaminated sites.

The aim of this work was to perform a comparative genomic study of 65 genomes of *Ensifer* sp. to elucidate the metabolic mechanisms that underline their potential stress tolerance. Our results revealed significant genomic differences between members of the genus with respect to the biosynthesis of nodulation factors and protein secretion systems, indicating host-specific nodulation, and provided insights into strong environmental adaptations. Heavy metal resistance genes (HMRs) and complete clusters related to resistance to arsenic, zinc, cadmium, cobalt, chromium, mercury and copper, as well as genes involved in detoxification, were frequently found.

Funding

This work was supported by TED2021-130122B-I00/ AEI/10.13039/501100011033/ Unión Europea NextGenerationEU/PRTR. MdCMC is grateful for funding received from the Ramón y Cajal Research Grant (RYC2019-028468-I) from MCIN.

SESSION 2: Applied Systematics

Revisiting genome data minimal standards for its use in taxonomy

Raúl Riesco[1], Martha E. Trujillo[1]

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The field of microbial taxonomy is dynamic, aiming to provide a stable classification system for prokaryotes. The advent of molecular techniques, particularly DNA sequencing and genomics, has revolutionized our understanding of prokaryotic diversity. Over the past two decades, genome sequencing advances have transitioned taxonomy from DNA–DNA hybridization and 16S rRNA sequencing to a complete in-silico genome-based framework. As technology and databases rapidly expand, maintaining updated standards is crucial. This work revises the 2018 guidelines by Chun et al. for applying genome sequencing data in microbial taxonomy, updating minimal standards and recommendations to reflect technological progress.

Using whole genome sequence data is now standard in delineating new species, utilizing overall genomic relatedness indices (OGRIs) like ANI and dDDH. These indices have limitations and cannot compete with genome-based phylogenetic reconstructions in capturing evolutionary relationships. Additionally, genomes provide useful information about the ecological niche of the bacterium and can include significant markers from shared ecological and metabolic properties that differentiate species within a genus or family. It is recommended to infer ecological properties from the genome for describing prokaryotic species. For genus delineation, a balanced approach combining phylogenomic methods and protein-based OGRIs is recommended. This study evaluated two commonly used protein-based OGRIs, average amino acid identity (AAI) and the percentage of conserved proteins (POCP). While AAI correlates with POCP, the distribution and deviation of POCP values make AAI the preferred OGRI to complement phylogenetic studies. A balanced approach combining multiple analyses and metrics is recommended for a robust microbial taxonomic framework.

Overall, genome-based phylogenies offer deeper resolutions than simple OGRIs and should be central to taxonomic descriptions. This work updates the minimal standards for using genome sequence data in prokaryotic taxonomy, emphasizing the need for comprehensive, high-quality genome data and a balanced approach to classification and identification.

References

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466. doi: 10.1099/ijsem.0.002516.

Riesco R, Trujillo ME. Update on the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2024;74:1–12. doi: 10.1099/ijsem.0.006300.

Bacteroidota is a key phylum for healthy human microbiota and especially susceptible to chemical xenobiotic exposure

Gracia Luque[1,2], Jorge Muñoz-Payá[1,2], Pilar Ortiz[1,2], Alfonso Torres-Sánchez[1,2], Victoria Romero[1,2], Ana López-Moreno[1,2], Alicia Ruiz-Rodríguez[1,2], Mercedes Monteoliva-Sánchez[1], and Margarita Aguilera[1,2]

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The human gut microbiota determines the host's health status based on its composition, resilience, balance, and diversity. Similarly, individual microbiota may be more or less sensitive to environmental stresses, such as xenobiotic exposure, plasticizers, and pollutants, leading to the loss or inhibition of certain taxa or the overgrowth of resilient species. Importantly, the effects of bisphenols on humans are under debate (López-Moreno et al. 2024), as they seem not to be completely metabolized, enabling their transfer to tissues or organs. This systemic accumulation can result in toxic effects on the liver, kidneys, gut and endocrine systems. At the intestinal level, dietary xenobiotics could influence taxa balance of the gut microbiota, which is considered as any other organ for its involvement in metabolism, digestion, acquisition of nutrients, and production of essential metabolites or bioactive lipids (Torres-Sánchez et. al 2023). The triggered microbial dysbiosis could include inflammation, oxidative stress, intestinal and metabolic problems, which increase the risk of developing chronic and non-communicable diseases (Ozdemir et al. 2024).

Gut microbiota *ex vivo* exposure to bisphenol A solubilized in polar solvent exerted a strong effect on the composition and reorganization of microbial communities, with a differential impact on obese comparing to normal-weight individuals. *Bacteroidota* taxa showed remarkable susceptibility to the xenobiotic across all groups, as evidenced by several metric variations (abundance, alpha, and beta diversity). Moreover, culturing data and 16S rDNA sequencing comparisons showed that the genera *Bacteroides*, *Alistipes* and *Prevotella* were significantly modified, revealing imbalanced patterns that may further lead to metabolic, functional and pathophysiological outcomes. The validation of several ratios based on taxa affected by plastic xenobiotics were similar to those described for microbiota dysbiosis found in children with obesity.

References

López-Moreno A, Aguilera M, Ruiz-Rodríguez A. (2024) Reply to Prueitt and Goodman, "Real-world bisphenol A exposure not linked to microbiota dynamics in childhood obesity". mSystems. 3:e0071424.

Torres-Sánchez A, Ruiz-Rodríguez A, … and Aguilera M. (2023) Key Stratification of Microbiota Taxa and Metabolites in the Host Metabolic Health-Disease Balance. Int J Mol Sci. 24;24(5):4519.

Ozdemir C, Kucuksezer UC, … and Akdis CA.(2024). Lifestyle Changes and Industrialization in the Development of Allergic Diseases. Curr Allergy Asthma Rep, 24(7), 331-345.

Actinomycetes Mediated Degradation of Persistent Organic Pollutants in a Biofilter for Industrial Effluent Treatment

Sahar El-Shatoury[1], Magdi Bahgat[2], and Julian Mitchell[3]

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Actinomycetes' extensive enzymatic capabilities make them essential for the biodegradation of complex organic molecules. They are particularly effective in degrading persistent environmental pollutants, contributing significantly to the breakdown and recycling of resistant polymers such as organochlorines. This study aimed to examine the degradation abilities of 102 actinomycete strains that were isolated from a wetland biofilter system for industrial effluent treatment in 10th Ramadan City, Egypt. The focus was on their capacity to metabolize 1,1,1-Trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) and other polycyclic aromatic hydrocarbons (PAHs). A thorough examination showed that 78.5% of the isolated actinomycetes possess the ability to break down catechol, suggesting their potential in the degradation of aromatic compounds. Detailed investigations have shown that actinomycetes from the genera *Nocardioides*, *Streptomyces*, and *Nocardia* have displayed great proficiency in breaking down acenaphthene, fluorene, naphthalene, potassium phthalate, and pentachlorophenol, and a particularly high effectiveness for DDT. Gas chromatography/mass spectrometry (GC/MS) analysis identified multiple DDT metabolites, including benzene dicarboxylic acid esters and 1-chloro-2,2-bis (p-chlorophenyl) ethylene (DDMU), suggesting active metabolic pathways with ultimate mineralization in in some strains. Batch culture experiments have verified that strains such as *Nocardioides fulvus* and *Nocardiopsis dassonvillei* are capable of breaking down DDT, even in co-existence of simple carbon sources like glucose. In addition, experiments conducted on small-scale gravel systems highlighted the enhanced degradation rates in the presence of native biofilm microorganisms, indicating the potential for future bioremediation applications. Pulsed field gel electrophoresis (PFGE) investigation indicated the presence of large linear plasmids in the two strains, potentially encoding degradation genes. This study highlights the important contribution of actinomycetes in breaking down persistent organic pollutants and presents promising strategies for the bioremediation of chlorinated hydrocarbon-contaminated environments.

Impact of bisphenols and parabens on taxa composition of intestinal microbiota in relation to childhood obesity

Jorge Muñoz-Payá[1,2], Margarita Aguilera[1,2], Gracia Luque[1,2], Pilar Ortiz[1,2], Alfonso Torres-Sánchez[1,2], Alicia Ruiz-Rodríguez[1,2], and Ana López-Moreno[1,2]

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Xenobiotics, such as bisphenols and parabens are dietary and environmental plasticizers and preservatives to which humans are exposed throughout life. They pose and trigger obesogenic effects that could be evaluated through exposome and microbiome data analysis/investigation (Lopez-Moreno *et al.*, 2024). The objective of this study was to evaluate the effect of some dietary xenobiotics on the gut microbiota of children with normal weight and obesity. Fecal samples were exposed to bisphenol A (BPA), bisphenol S (BPS), methyl paraben (MeP) and ethyl paraben (EtP) for 10 days and, microbial composition was studied by sequencing the 16S rRNA gene and processing the data with R. The alpha and beta diversity of the microbial community did not vary significantly in response to the xenobiotics. However, the changes produced by BPS in the microbiota of normal weight children made it more similar, in structure and diversity, to that of children with obesity. In line with other research, the gut microbiota of obese children underwent more changes in response to xenobiotics than that of normal-weight children. Besides the relative abundance of 30 taxa varied significantly compared to the experimental control, among which the increase of the following phyla stood out, *Fusobacteriariota*, *Acidobacteriota* and *Euryarchaeota* (Turnbaugh *et al.*, 2006); families, *Prevotellaceae*, *Fusobacteriaceae* and *Gemellaceae*; and genera, *Proteus* and *Clostridium*. Finally, the correlation analysis allowed us to identify 25 taxa as potential biomarkers of exposure to xenobiotics and obesity. Specifically, nine of them correlated positively with obesity parameters. The species *Barnesiella intestinihominis* and *Clostridium saudiense* are postulated as potential biomarkers of BPS exposure for their possible participation in the metabolism of this xenobiotic and their positive correlation with the obesity phenotype. Future studies will aim for validating these species as biomarkers of exposure to xenobiotics, as well as their potential use in mitigation and personalized treatments.

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SESSION 3: Microbial Diversity

What is a species and a strain? The data has shown us the answers!

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Large scale surveys of natural microbial communities (metagenomics) or isolate genomes have revealed species clusters around 95% Average Nucleotide Identity (ANI) of shared genes. That is, members of the same species tend to show >95% ANI among themselves and <85% to members of other species with a clear scarcity (gap) of genome pairs showing between 85-95% ANI. We have recently reported a similar ANI gap within species, around 99.5% ANI, revealing that discrete, intraspecies units may also exist. We suggested referring to these units as genomovars (Rodriguez-R, mBio 2024), and to employ a higher ANI value (99.9%) and level of shared genes (>99% of total genes) to define strains (Viver, Nat. Comms. 2024). To further understand and model these patterns of diversity, however, the underlying genetic and/or ecological mechanisms that maintain discrete units at the species and intraspecies levels need to be elucidated. By analyzing closely related isolate genomes from the same or related samples we show that high ecological cohesiveness among the genomes, coupled to functionally and spatially (across the genome) unrestricted homologous recombination, likely underly these ANI units. Therefore, our results represent a departure compared to previous models of microbial speciation that attributed speciation to either recombination or ecological cohesiveness but not their combined effect.

Unveiling the Hidden Diversity of the *Stenotrophomonas* Genus Through Phylogenomic Analysis

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The genus *Stenotrophomonas* currently comprises 20 validly published species, with *S. maltophilia* being the most studied and well-known species within this genus. The intrinsic antibiotic resistance and adaptability of these bacteria to hospital environments present a potential danger for future healthcare settings.

In this study, we performed whole-genome sequencing (WGS) on 22 *Stenotrophomonas* isolates retrieved from hospital sink drains. All these isolates were first identified as *S. maltophilia* through MALDI-TOF MS and 16S rRNA gene sequencing. Initial phylogenomic analysis, incorporating both our isolates and genomes from the NCBI database, including the 20 type strain genomes, suggested misidentification in some cases. Consequently, a comprehensive analysis using Average Nucleotide Identity (ANIb), Genome-to-Genome Distance Calculation (GGDC), and core genome analysis was conducted on a total of 133 genomes, comprising our 22 isolates and 111 retrieved from the NCBI database. *Xantomonas maliensis* and *Pseudoxanthomonas dokdonensis* (previously *Stenotrophomonas dokdonensis*) were used as outgroups. This analysis identified 48 distinct phylogenomic species (excluding outgroups) within the genus, with 36 confidently belonging to *Stenotrophomonas*. Further studies are required to correctly affiliate the remaining genomes.

Additionally, our findings indicate that *S. maltophilia* is not the sole pathogen within this genus, as novel species show similar virulence and antibiotic resistance traits. The presence of novel species isolated from clinical samples supports this hypothesis. The investigation into the intrinsic chromosomally encoded L1 and L2 beta-lactamases revealed its potential as a key marker for accurate species identification within *Stenotrophomonas*. Interestingly, the L1 gene was identified in 91 out of the 133 genomes, corresponding to the 36 defined groups as this genus, with the L2 gene being more prevalent, present in 128 genomes.

This study underscores the hidden diversity and the pathogenic potential within the genus, emphasizing the need for thorough genomic characterization to improve identification and understand the implications for public health.

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Bridging Viruses and Prokaryotic Host through Miniature Inverted-repeat Transposable Elements (MITEs)

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Transposable elements (TEs) have a pivotal role in the evolution of genomes across all life domains. “Miniature Inverted-repeat Transposable-Elements”, MITEs, are considered non-coding sequences of small size (50-800 bp) that include an internal DNA sequence flanked by terminal inverted repeats. They are non-autonomous TEs mainly located in intergenic regions, relying on external transposases for mobilization.

Using the program MITEtracker, the boundaries of MITEs’ mobilome were explored across 183232 prokaryotic genomes, revealing that a surprisingly vast untapped diversity of MITEs are present in bacterial and archaeal genomes. Also, but in less degree, in viral sequences. MITEs were identified in 56.5% of genomes, totaling over 1.4 million cMITEs (cellular). Cluster analysis revealed that a significant 97.4% of cMITEs were conserved within genera boundaries, with up to 23% being species-specific.

This genus-specificity was evaluated as a tool to link microbial host to their viruses. A total of 51655 cMITEs had counterparts in viral sequences, termed vMITE (viral), resulting in the identification of 2798 viral sequences with vMITEs. Among these, 1501 sequences were positively assigned to a previously known host (41.8% were isolated virus, and 12.3% were assigned through CRISPR data), while 379 new host-virus associations were predicted. Deeper analysis in Neisseria and Bacteroidetes groups allowed the association of 242 and 530 new additional viral sequences, respectively.

Given the abundance of non-culturable virus sequences accumulated in databases lacking affiliations with their microbial targets, MITEs are proposed as a novel approach to establishing valid virus-host relationships.

Genomic exploration of the culturable bacteriome of *Lupinus angustifolius*

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Recent research has identified alternative plant-based sources to address protein deficiencies and promote sustainable agriculture. Leguminous plants, known for their high protein content, have emerged as a promising option for reclaiming poor and marginal lands due to their nitrogen-fixing ability and positive impact on soil quality. The genus *Lupinus*, comprising nearly 600 species, includes three agriculturally significant ones: *L. albus*, *L. angustifolius*, and *L. luteus*. Focusing on *L. angustifolius*, isolating and identifying its culturable bacteriome is crucial for potential applications such as biofertilizer and biopesticide production, as well as enhancing plant productivity. Through culture-dependent methods, our laboratory isolated 722 bacterial isolates belonging to 87 different genera, covering 75% of the genera uncovered by culture-independent methods. Using 16S rRNA sequencing and MALDI-TOF-based screening, representative strains were selected for whole genome sequencing. The main objective was to identify the genetic potential involved in plant-microorganism and microorganism-microorganism interactions. These interactions play crucial roles in determining overall plant health, crop yield, and agricultural productivity. Using the genome sequence, we identified several potential new species in the families *Acetobacteraceae* (1), *Burkholderiaceae* (1), *Caryophanaceae* (2), *Flavobacteriaceae* (1), *Intrasporangiaceae* (1), *Jonesiaceae* (1), *Kineosporiaceae* (1), *Lysobacteraceae* (2), *Methylobacteriaceae* (2), *Microbacteriaceae* (5), *Micrococcaceae* (5), *Mycobacteriaceae* (1), *Neisseriaceae* (1), *Nitrobacteraceae* (1), *Nocardioidaceae* (2), *Oxalobacteraceae* (1), *Propionibacteriaceae* (1), *Sphingomonadaceae* (1). *In silico* characterization using COG, SEED, and CAZy databases, followed by *in vitro* confirmation, provided insights into functional profiles. In addition, using the PLaBAse platform, bacterial plant growth-promoting traits (PGPTs) were identified in these isolates. This comprehensive genomic analysis contributes to understanding potential biological mechanisms and bacterial interactions crucial for plant health and sustainability. In addition, the identification of 30 new species indicates the high bacterial diversity associated with this plant.

To name or not to name? Species based on single strains

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The naming and description of bacterial species based on single strains are often discouraged. Apart from poor circumscription and inability to capture the species' phenotypic variability, this practice places considerable strain on the already strained systems for reviewing and publishing of such papers and for maintaining the associated type strains. Therefore, an alternative approach is needed for dealing with such species as they are invaluable in our efforts to formally capturing the biodiversity and crucial for exploring the biology and evolution of prokaryotic species. Whole genome sequence information offers a feasible solution to the problem. Genome data linked to type strains has revolutionized our approach to species identification, with atypical cultures routinely being identified based on analyses and comparisons of taxonomically informative gene sequences. We are fast moving from identification of species based on distinct phenotypic characteristics linked to the original species description to a situation where whole genome sequence information is used for species diagnosis. In other words, it is possible to validly name a species based only on one strain and its associated genome with little practical impact on end-users. Additionally, the availability of whole genome sequences would negate the need for providing the large range of phenotypic and chemotaxonomic properties, the value of which are often limited. We need a new, streamlined format for naming single strain species and/or for integrating these taxonomic units into papers addressing topics in prokaryotic diversity, evolution or ecology. The availability of stable names would also enable researchers to link them to new information, thereby adding to the body of knowledge regarding the species and the environment it inhabits. Adopting such a cumulative approach will allow for the development of relevant species descriptions through the continuous effort of the wider community, resulting in a better synergy between microbial taxonomy and other branches of microbiology, especially those involving ecology and evolution.

SESSION 4: Taxogenomics

Homonymy amid biological codes: analysis of replacement names among prokaryotes

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In the past two years there has been an unprecedented number of replacement name actions published in the official journal of the ICSP, the International Journal of Systematic and Evolutionary Microbiology (IJSEM). The reason for these publications is the situation of illegitimacy in which the names replaced are found when an older synonym, in accordance with the botanical and zoological codes of nomenclature (Ride *et al*., 1999; Turland *et al*. 2018), is brought up to light. Or in other words, it is a consequent application of Principle 2 and Rule 51b(4) of the International Code of Nomenclature of Prokaryotes (ICNP) (Oren *et al*., 2023). Namely, Principle 2 declares that the nomenclature of prokaryotes is not independent of botanical and zoological nomenclature (from January 2001 and not retroactive). So, one of the tasks of the nomenclature reviewers of IJSEM, aside from checking the etymology of new names, is to check that the proposed name is not in use for other living organisms. However, the recent publications about name replacement shows that there may be loose ends in this process. The question is, how important it is to communicate and repair those cross-code synonymies particularly when the zoological or botanical name disputing priority has not been used for decades? This talk analyses the situation from various perspectives: a) the ruling in the current and past revisions of the ICNP; b) cases over time; c) taxa affected (and taxa not affected); and d) convenience for end users. It also analyses how cross-code synonyms are handled in a universal repository: the Taxonomy Browser maintained at the NCBI/Genbank (Schoch *et al*., 2020). Keeping a pragmatic point of view, the last part of the talk makes considerations to modify the ICNP to prevent replacements of little general utility from proliferating.

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Genomic characterization of seven multiresistant *Enterococcus* spp. isolated from wastewater samples in Galicia, Spain

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Members of *Enterococcus* genus are Gram-positive cocci that colonize the human gastrointestinal tract and, upon being excreted in feces, represent a considerable portion of the bacterial pathogens found in wastewater. In this study, we have performed the genomic characterization of seven *Enterococcus* spp. strains isolated from wastewater in Galicia (NW Spain) in October-November 2022. Wastewater samples were serially diluted, and 100 mL of appropriate dilutions were filtered through 47 mm diameter 0.45 µm pore size nitrocellulose sterile membrane filters. Filters were placed on m-Enterococcus Agar plates supplemented separately with ciprofloxacin (1µg mL-1), trimethoprim (100 µg mL-1) and sulfamethoxazole (7 µg mL-1). Plates were incubated for 24 - 48 h at 37 °C. A total of 7 isolates (4 isolates resistant to ciprofloxacin, 2 isolates resistant to sulfamethoxazole and 1 isolate resistant to trimethoprim) were selected for genomic DNA extraction. Sequencing of 16S rRNA gene indicated that six isolates belonged to *E. faecium* and one to *E. faecalis*. All genomes were sequenced and assembled, and DNA size (bp) and GC (%) contents in their genomes were compiled. In the annotated result, *E. faecium* strains showed a nucleotide identity (ANIb) of 98-99% with the reference sequence of the strain SRR24 (GCF_009734005.1), whereas *E. faecium* strain yielded an ANIb of 98.5% with the reference sequence of strain T5 (GCF_000393015.1). The genome assemblies were screened for the presence of antimicrobial resistance (AMR) genes. The most prevalent AMR genes in all the *E. faecium* genomes were related to resistance to aminoglycosides (*aac(6')-Ii*), fluorquinolones (*efmA*), macrolides (*msrC*), and glycopeptides (vanY gene in vanB cluster). Moreover, *dfrG* gene, which is related with resistance to trimethoprim, was found in 5 *E. faecium* isolates. AMR genes detected in the *E. faecalis* genome were *erm*B (erythromycin), *tet*L and *tet*M (tetracyclines), *efr*A (fluorquinolones), *dfr*E (trimethoprim), and *lsa*A (lincosamides, pleuromutilins and streptogramin A).

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Network analysis-aided genus demarcation within the families *Halomonadaceae* and *Oceanospirillaceae*

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Widespread microbial genome sequencing has entailed a turning point in the taxonomy of prokaryotes, allowing the inference of genome-based phylogenies and the estimation of various metrics and criteria for species circumscription. However, there is currently no consensus on genus definition based on phylogenomic data and overall genome relatedness indexes, with different cut-offs used depending on the microbial group of interest, leading to some degree of subjectivity.

Earlier studies carried out within the family *Halomonadaceae* based on single-copy core-genome analysis and the identification of signature genes characteristic of clades have allowed the taxonomic rearrangement of the representatives of this group, with the creation of six new genera and the reclassification of several species (de la Haba et al., 2023). The aim of the present work is to evaluate the convenience and consistency of the proposed reorganization by including species not previously considered because their genomes were not available, as well as to expand this study to include the genera of the sister family *Oceanospirillaceae*, some of them often regarded as belonging to the *Halomonadaceae*. To this end, we will employ a novel method for genus demarcation that combines normalized phylogenomic tree clustering and validation of relationships between taxa by network analysis of maximum-likelihood distances and genome relatedness indexes (ANI, AAI, and alignment fraction) (Val-Calvo and Vázquez-Boland, 2023). In summary, we intend to be able to establish a non-subjective delimitation of the genera of these two families.

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Take-home messages from the Genome Taxonomy Database

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The Genome Taxonomy Database (GTDB) was developed to address the needs of microbiologists to classify rapidly accumulating genome sequences from both cultured and uncultured microorganisms. To our knowledge, GTDB is the only database that provides a phylogenetically consistent and rank-normalised taxonomy for prokaryotes. It has become an essential taxonomic resource for microbiologists worldwide, attracting approximately 42,000 users annually. Although GTDB serves a multitude of purposes to its global users, we still observe common misunderstandings around interpretations of the GTDB taxonomy or usage of its companion tool, GTDB-Tk, that allows users to classify their own genomes against the GTDB. Here, I would like to address those misunderstandings, specifically regarding taxonomic and nomenclatural changes in GTDB, using examples from GTDB user requests received via the Forum and contact form. I will also present an overview of the GTDB website features which can be useful for taxonomists in their daily jobs.

Two codes of prokaryotic nomenclature: how do they become one?

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Codes of nomenclature enable biologists to create unambiguous and well defined names and are crucial for efficient communication about organisms. The Code of Nomenclature of Prokaryotes Described from Sequence Data (informally the SeqCode) is a new code of nomenclature in which genome sequences are the nomenclatural types for the names of prokaryotic species [Hedlund et al. 2022, Whitman et al. 2022]. While generally similar to the International Code of Nomenclature of Prokaryotes (ICNP) in structure and rules of priority, it does not require deposition of type strains in international culture collections. Thus, it allows for the formation of permanent names for uncultured prokaryotes whose complete or nearly complete genome sequences have been obtained from environmental DNA as metagenome-assembled genomes [MAGs] or single-cell amplified genomes [SAGs]. Fastidious prokaryotes that cannot be deposited in culture collections due to their growth requirements, resistance to isolation, or poor preservation as well as those that cannot be deposited in culture collections for legal reasons but whose genome sequences are known can also be named in this system. The start date of the SeqCode was January 1, 2022, and the online Registry (https://seqco.de/) was created to ensure valid publication of names. The SeqCode recognizes all names validly published under the ICNP prior to 2022. After that date, names validly published under the SeqCode compete with ICNP names for priority. To avoid creation of synonyms, ie. different names for the same taxon, the ICNP should be amended to recognize names formed under the SeqCode. This change in the ICNP will ensure that species and other taxa will have only one name, either from the SeqCode or ICNP, and enable efficient communication between scientists from diverse disciplines.

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SESSION 5: Molecular Ecology

Metagenomes from deep-sea hydrothermal vents: Sources of novel genomic representation in the Tree of Life

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High temperature deep-sea vents support a rich phylogenetic and physiological diversity of thermophilic *Bacteria* and *Archaea*. The structure of the microbial communities associated with the high temperature hydrothermal deposits ("chimneys") is tightly coupled to the hydrothermal geochemistry and geographical location. From metagenomic comparisons across several global hydrothermal sites, we identified several hundred high-quality metagenome assembled genomes (MAGs) that represent a rich abundance of novel families, genera and species in the Archaea and Bacteria. We used these new genomes to re-evaluate the taxonomy of the *Aeropyrum–Thermodiscus–Caldisphaera* clades within the Thermoproteota. At least nine genus-level clades were identified with two or more MAGs. In accordance with SeqCode requirements and recommendations, we proposed names for three novel genera, namely *Tiamatella incendiivivens*, *Hestiella acidicharens* and *Calypsonella navitae*. A fourth genus was also identified related to *Thermodiscus maritimus*, for which no available sequenced genome exists. We proposed the novel species *Thermodiscus eudorianus* to describe our high-quality *Thermodiscus* MAG. All three novel genera and *T. eudorianus* are likely anaerobic heterotrophs, capable of fermenting protein-rich carbon sources, while some *Tiamatella*, *Calypsonella* and *T. eudorianus* may also reduce polysulfides, thiosulfate, sulfur and/or selenite, and the likely acidophile, *Hestiella*, may reduce nitrate and/or perchlorate. Based on phylogenomic evidence, we also proposed the family *Acidilobaceae* be amended to include *Caldisphaera*, *Aeropyrum*, *Thermodiscus* and *Stetteria* and the new genera we described. This study illustrates the value of using high quality MAGs for developing robust taxonomies for understudied lineages of Bacteria and Archaea and where very little genomic or isolate representation exists.

Understanding the interactions between lupin and its root-associated bacteria

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Plant-associated microbial communities are influenced by several factors such as host genotype/species, soil type, plant compartment and climatic season, among others. Understanding how a plant's microbiome is assembled and its functions using specific synthetic communities can reveal very important information about each community member and their functions. *Lupinus angustifolius* is a native plant of Europe, well adapted to the climatic conditions of many countries. It also thrives in poor soils due to its capacity to fix nitrogen.

This study aimed to investigate the bacterial microbiota isolated from *L. angustifolius*. 722 bacteria were isolated from various plant compartments using different isolation media, targeting the most abundant groups identified through 16S rRNA metagenomic profiling. After screening and removing pathogenic strains, we selected 12 strains for further analysis which included *Bradyrhizobium*, *Streptomyces*, *Pseudomonas*, *Variovorax*, *Micromonospora* among others. We sequenced the genomes of these strains and conducted *in silico* analysis. Subsequently, we designed and inoculated several bacterial synthetic communities (SynCom) under various cultivation conditions to examine the association between these bacteria and the host plant. Additionally, we performed RNA-seq analysis on the plant to understand the microorganisms' effects on the plant.

A comparative genomic analysis of the selected consortium was carried out, confirming that all the selected strains had genes with functions related to plant association and growth promotion. Plants grown in a natural soil and in a gnotobiotic system were harvested after 8 weeks. All SynComs improved lupin growth when compared to the un-inoculated plants. Gene ontology enrichment analyses revealed that those functions that were enriched by inoculating the different SynComs were clearly related to plant-microbe interaction functions. The same was observed for the enriched metabolic pathways when KEGG analysis was performed.

Shifts in the composition and dynamics of the almond endosphere in the context of *Xylella fastidiosa* infection through culture-independent methods

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Xylella fastidiosa is currently one of the most relevant phytopathogens. Its infection poses a threat to crops worldwide. Since its arrival in the Balearic Islands, *X. fastidiosa* has caused a noticeable decline in the production and number of the main woody crop in Mallorca, the almond tree. So far, no effective treatment is known for *X. fastidiosa* in affected plants. Thus, recent studies have focused on thoroughly characterizing the endosphere of affected crops. However, there is still a significant gap in knowledge regarding the almond tree endosphere. Studying this not only analyses its dynamics and diversity and the factors that govern it, but it is also the ideal environment to identify potential biocontrol agents against *X. fastidiosa*.

In this study, the endosphere of the almond tree is analyzed using a culture-independent approach. Leaf vein samples were collected in the four seasons of the year from two plots with different bioclimatic variables over two consecutive years. From the obtained DNA, the V5-V7 region (799F-1193R) was amplified to determine the microbial community. For community analysis, data were processed with QIIME2 and DADA2 to obtain amplicon sequence variants (ASVs). The endophytic community of the almond tree was characterized by integrating alpha and beta diversity analyses, as well as co-occurrence and functional prediction analyses. The results showed that seasonality is a key factor in the dynamics and composition of the endophytic community of the almond tree. Additionally, some taxa with potential antagonistic action against the phytopathogen *X. fastidiosa* were identified, making them interesting for *in vitro* and *in planta* studies.

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Land-use legacies on soil microbial communities' response to wildfires

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Soil microorganisms are key players in the processes building ecosystems: they rule nutrient cycles, provide soil structure, and maintain interactions with plant communities. One principal threat to Mediterranean ecosystems is the increasing frequency and severity of wildfires. Comprehending the effects of wildfires on microbiota and their regeneration mechanisms is a required step towards improving the ecological resilience of communities to such impacts. In 2022, over 600 km^2 of the region of Zamora (*Castilla y León*, Spain) burned in the most devastating wildfire in Spanish history. Our team sampled soil from burned and unburned areas, with a parallel design covering different land uses (*forest*, *reforestation*, *grassland*, and *crop*), altitudes (*top*, *slope*, and *plain*) and depths (*top-* and *subsoil*), three months and one year after the fire. Through metagenomic techniques we have observed changes in bacterial and fungal communities after the impact of fire and across the mosaic of land uses. Results indicate a strong land-use legacies in community structure, with clear clustering patterns of β-diversity among each land use and differences of α-diversity in unburned soils. After the fire, differences between land uses mostly disappear and we find an α-diversity decline in burned soils and between years. In bacterial communities, *Pseudomonadota*, *Acidobacteriota* and *Actinomycetota* are the most abundant phyla in burnt soils with apparent differences between land uses and depth. The genera *Pseudolabrys*, *Edaphobacter*, *Segetibacter* and *Ramlibacter* significantly increased their abundances after the fire. Within fungi, *Ascomycota* were remarkably abundant in burned soils, with the genera *Umbelopsis*, *Leohumicola*, *Penicillium*, and *Rasamsonia* being the most prominent. *Rhizomicrobium*, *Conexibacter* and *Solibacter*, in burned subsoil, and *Adhaeribacter*, *Ocallatibacter* and *Segetibacter*, in burned topsoil, were the bacterial genera with more positive correlations of abundance. The sole negative correlation in burned subsoil was between *Sphingomonas* and *Glutinomyces*. This structure and composition of post-fire communities provides a path for restoration initiatives based on potentially resilient taxa and consortia.

PhD Thesis Award

Bacterial whole-genome sequencing for establishment of reference sequences, comparative genomics, biomarker discovery and characterization of novel taxa

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Bacteria are the most widely distributed organisms, playing crucial roles in almost every environment. Understanding their biology is essential for the well-being of the planet and humanity and has been greatly facilitated by high-throughput DNA sequencing. This thesis (Salvà Serra, 2023) employed whole-genome sequencing methodologies to establish bacterial reference genome sequences within three taxa (Papers I–IV, VI, VIII): *Stutzerimonas balearica*, a marine bacterium with capacities for degrading aromatic compounds; the genus *Streptococcus*, which includes commensals and major human pathogens; and the family *Enterobacteriaceae*, a diverse group which members are found in numerous environments and some cause human diseases. The utilized methodologies showcase the evolution of high-throughput DNA sequencing, including accurate determination of complete genome sequences using long-read sequencing. These advancements have led to vast amounts of publicly available genome sequence data, which is essential for downstream studies, yet not everything is positive. This thesis warns about the presence of "false" type strain genome sequences and the importance of quality controls (Paper V). In Paper VI, genome sequences elucidated the genomic diversity of S. balearica and its potential for biodegrading aromatic compounds. A strategy was developed for detecting additional *S.*

balearica strains and determining its habitats, based on 16S rRNA gene signatures and sequence similarities. In Paper VII, hundreds of genome sequences allowed the determination of a biomarker gene specific for the human pathogen *Streptococcus pneumoniae* and enabled a PCR assay for distinguishing it from closely related species, enhancing accurate identification. In Paper VIII, genome sequencing confirmed that a previously unidentifiable clinical isolate of the family *Enterobacteriaceae*, represents a novel genus and species (*Scandinavium goeteborgense*) and allowed accurate determination of its taxonomic position. This thesis highlights how the latest DNA sequencing advancements have pushed the limits of microbiology, enabling the establishment of solid grounds for down-stream research and applications and the high-resolution exploration of bacterial genomic insights.

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Posters

P-1

Climate- induced variations in soil microbiota and plant interactions: Implications amidst changing climate patterns

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Climate fluctuations significantly determine soil microbial communities and plant associations, influencing vital ecosystem functions. Variations in temperature, precipitation, and humidity profoundly impact microbial diversity, altering nutrient cycling and plant interactions. Such changes in microbial composition affect plant health, growth, and resilience. As climate change intensifies, these disruptions could escalate, potentially compromising soil fertility, plant productivity, and overall ecosystem stability. Understanding these dynamics provides crucial insights into anticipating and mitigating the repercussions of climate change on soil microbial communities and plant interactions, thus safeguarding ecosystem health and sustainability.

In this work, we collected 11 soil samples from three climatic regions in Spain (oceanic, continental, and Mediterranean) spanning across 5 bioclimatic zones. Subsequently, two *Lupinus* species, *L. angustifolius* and *L. luteus*, were grown on these soils under greenhouse conditions. Independent cultivation techniques were employed to profile bacterial and fungal amplicons. Additionally, a comprehensive physical-chemical analysis revealed distinct soil properties among the samples. Cultivating both plant species in these soils allowed confirmation of microbial associations with their root systems, and to determine whether there were differences in these associations according to climatic regions. Furthermore, common taxa between the two plants were identified. These results will be used to design synthetic microbial communities to enhance plant resilience in environments experiencing shifts due to changing climatic conditions. This research sheds light on potential strategies for fostering plant growth in regions susceptible to the impacts of climate change through the development of tailored microbial communities.

P-2

Study of the diversity and abundance of endophytic bacteria in *Xylella fastidiosa* infected and non-infected wild plants by culture-dependent methods

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Xylella fastidiosa is a quarantine phytopathogen of great economic and ecological importance due to its high infection capacity in both major agricultural crops and wild species. Its transmission and spread have generated severe repercussions in agriculture and ecosystem conservation, particularly in Mediterranean regions such as the Balearic Islands.

In this study, the diversity and abundance of endophytic microorganisms were investigated using culture-dependent methods in wild plants, *Cistus albidus* and *Rosmarinus officinalis*, both infected and non-infected by *X. fastidiosa*. Seasonal sampling of the same six plants from different localities on the island of Mallorca was carried out in autumn of 2023 and in winter and spring of 2024. The plants were previously diagnosed by qPCR to determine the presence of *X. fastidiosa*.

For the isolation of endophytic microorganisms, R2A agar and Cetrimide agar media were used and incubated at room temperature, while BCYE agar was incubated at 28 °C for 15 days. A total of 656 isolates were obtained: 388 from *C. albidus* (155 in November, 112 in February, 121 in May) and 268 from *R. officinalis* (82 in November, 103 in February, 83 in May). The different isolates obtained were characterized and identified by MALDI-TOF MS and a selection of them were further analyzed by amplification and sequencing of the 16S rRNA gene.

The results obtained so far show a diverse community among the different plants, including within the same species, throughout the study, varying between the different samplings. These differences could be associated with environmental variations. Isolates belonging to taxonomic groups considered potential antagonists against phytopathogens, such as *X. fastidiosa*, have been obtained. Notable species include *Bacillus* sp., *Methylobacterium mesophilicum*,

Paenibacillus sp., *Pantoea agglomerans*, and *Pseudomonas* sp. In future studies, the potential of these as treatments against *X. fastidiosa* could be more thoroughly evaluated. Some of the isolates identified through 16S rRNA gene sequencing could be considered potential new species, necessitating additional taxonomic studies for their proper classification. Preliminary results obtained so far do not show variations in the microbiota associated with infection by *X. fastidiosa*.

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P-3

Characterization of the culturable mycobiome of *Lupinus angustifolius*

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Lupinus angustifolius is a legume of great interest that can be used as a protein source for animals and humans. Microorganisms play a crucial role in the growth and development of plants and are essential for sustainable agriculture systems. Among the microbiota associated to plants, fungi play a pivotal role in the host's adaptation to environmental changes, providing stability and resilience. Many plant-associated fungi also promote plant growth.

The aim of this work was to isolate the fungal community associated with *L. angustifolius*. After analyzing the metagenomic data from previous studies (Ortúzar et al., 2024), fungal strains were isolated from different plant compartments that included the rhizosphere, roots, and nodules. Plant samples were collected in two locations, Cabrerizos and Salamanca. In addition, plants were also grown under greenhouse conditions. For isolation, we designed and applied several protocols to cover the diversity registered by metagenomics. The fungal isolates were then identified by sequencing the ITS region and, the results from the fungal diversity of the different growing conditions were compared.

All the strains were identified as *Ascomycota*, *Basidiomycota*, and *Mucoromycota*. On the other hand, *Penicillium*, *Fusarium*, and *Aspergillus* were the genera with more strains identified. The mycobial communities and their proportions in *L. angustifolius* vary according to the compartment from which they have been isolated, with the greatest diversity found in the rhizosphere. Furthermore, the fungal composition and diversity was specific for each sampling location (Cabrerizos or Salamanca) and the growing conditions (field or greenhouse), with a lower diversity of genera in the greenhouse. It should be pointed out that this is the first time that endophytic fungi have been isolated from nodules of the plant *L.angustifolius*.

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P-4

New isolates of the genus *Spiribacter*: a genomic-based taxonomic reassessment

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The genus *Spiribacter* belongs to the phylum *Pseudomonadota*, family *Ectothiorhodospiraceae*, order *Chromatiales*, within the class *Gammaproteobacteria*. Currently, the genus *Spiribacter* includes validly published six species names. Initially, isolating *Spiribacter* species in laboratory settings proved difficult due to insufficient knowledge of their growth and cultivation requirements. Advances in understanding their ecological niche and metabolic pathways have enabled the successful isolation and identification of additional *Spiribacter* species from diverse locations. These halophilic bacteria are widely distributed in hypersaline environments worldwide, with a particular abundance in environments of intermediate salinity. They are characterized by rather small genomes ranging typically from 1.7 to 2.2 Mb, with a single ribosomal operon, and simplified in terms of metabolic versatility, except for the species *S. halobius*, which harbor a genome of 4.2 Mb. Enriched media with sodium pyruvate as carbon source facilitated the isolation of twelve new strains closely related to the genus *Spiribacter* from hypersaline environments in Spain. Genome sequencing and subsequent analysis of these new strains, along with previously described ones, provided insights into their genomic characteristics and phylogenomic relationships, supporting the proposal of three novel species within *Spiribacter*, designated as *Spiribacter pallidus* sp. nov., *Spiribacter insolitus* sp. nov., and *Spiribacter halophilus* sp. nov. Moreover, the classification of *Spiribacter halobius* and "*Spiribacter salilacus*" within the genus *Spiribacter* presents significant incongruences. Genome mining analysis combined with physiological differences highlight the need for a thorough re-evaluation of their taxonomic positions.

Streamlined genomes of species of *Spiribacter* facilitate survival in hypersaline environments by minimizing non-essential genes and optimizing resource utilization. Key genes involved in osmoprotectant mechanisms, such those for the metabolism of *myo*-inositol, hydroxyproline,

and L-proline, were identified, along with numerous transporters that ensure efficient nutrient acquisition and osmotic balance. Notably, these new species, together with other *Spiribacter* strains, exhibit a broad metabolic diversity in utilizing inorganic sulfur compounds, including thiosulfate and tetrathionate, for energy production and adaptation to hypersaline environments.

P-5

Pseudomonas elenavaldesiana sp. nov., a new endophytic *Pseudomonas* species isolated from *Prunus dulcis*

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The genus *Pseudomonas* is known for its dual interaction with plants, acting both as a pathogen and biocontrol agent. A set of endophytic bacteria of almond trees was characterized in a study conducted in Mallorca between 2021-2022. Of the total endophytic isolates, 182 strains were presumptively characterized as *Pseudomonas*, and of these, 49 strains formed a coherent cluster closely related to *Pseudomonas graminis*. These strains were isolated from different almond varieties in two plots located in different localities.

Eight of these isolates were further characterized using a polyphasic approach. The bacteria, identified as Gram-negative non-fluorescent, catalase-positive, and oxidase-negative, were evaluated using phenotypic (API 20NE, Biolog GEN III, and MALDI-TOF) and genotypic methods, analyzing the housekeeping genes *rpoD* and 16S rRNA. The similarity percentages obtained, 95-96% for the *rpoD* gene, were below the threshold necessary to discriminate species within the genus *Pseudomonas*. Additionally, a phylogenomic approach was performed through a taxonomic study based on the phylogeny of conserved genes (autoMLST), core-genome phylogeny, and genomic indices (ANI and GGDC) to clarify the taxonomy of the new isolates. In the phylogenetic analysis were included the genomes of all species type strains taxonomically closely related, as well as the genome of *P. graminis* UASW1507, described by Crovadore and colleagues (2016), a strain within the species with potential as biological control agent and biofertilizer.

The isolates belong to the *Pseudomonas lutea* subgroup defined by Mulet et al. 2010, although they do not correspond to any of the previously described species. Results suggested that these

isolates represent a new species within the genus, for which the name *Pseudomonas elenavaldesiana* sp. nov. is proposed, being P1B_15Nov21^{T} the type strain.

Additional studies on these strains are necessary to understand the role that these endophytes play in the plant and their potential in the fight against pathogens.

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P-6

Diversity of Bradyrhizobium strains nodulating cowpea (Vigna unguiculata L.) in Northern Peru agricultural fields

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Cowpea (*Vigna unguiculata* L.) production has continuously increased over the last 20 years in Peru where about 30000 ha of the coastal and jungle areas are planted, representing an excellent alternative for both small farmers and export market. In some sites, particularly those located at the desert coastal strip, soils are poor in organic matter and N content and farmers apply inorganic N-fertilizers to maintain production. However, since cowpea establishes nitrogen fixing symbiosis with rhizobia, the use of rhizobial fertilizers is a reliable agronomic practice to partially or completely replace chemical N fertilizers, which lead to the nitrate contamination of soil and waters. Nevertheles, designing suitable biofertilizers for cowpea requires knowledge of the species and symbiovars of rhizobia nodulating this legume in the different countries where it is grown, and to date few studies have focused on the identification of cowpea-nodulating rhizobia in Peru. In this work we isolated several slow-growing strains from effective nodules of cowpea plants growing in two agricultural soils from the provinces of Illimo and Juanjui, in Northern Peru. The isolated strains, which displayed different RAPD patterns, were identified at species level through the analysis of the *rec*A and *gln*II housekeeping genes, which showed that they belong to the genus *Bradyrhizobium*. Most of isolated strains were closely related to *Bradyrhizobium yuanmingense*, one strain was related to *Bradyrhizobium pachyrhizi* and the remaining strains formed independent lineages which constitute putative new species within the genus *Bradyrhizobium*. The identification at

symbiovar level was performed through the *nod*C gene analysis which showed that the strains nodulating cowpea analysed in this study belong to the symbiovar tropiciagri, to the recently described symbiovar americaense and to new symbiovar of the genus *Bradyrhizobium*.

P-7

Dyadobacter helix sp. nov. and *Dyadobacter linearis* sp. nov, from drinking water

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The study is aimed to better characterize and complete the classification of two bacterial strains, AB1^T and AB67^T, isolated from drinking water systems and affiliated to the genus *Dyadobacter* by partial 16S rRNA gene sequence comparison. Hence, we report here the phenotypic, genomic and phylogenetic characterization performed on these strains. Both strains grow on R2A agar forming mucous, bright yellow colonies, developing at 26 °C in 48 h. They produce flexirubin and are oxidase and catalase positive, mesophilic and non-halophilic. The cells of strain AB1^T are curved rods mainly associated in pairs, forming nearly closed rings or resembling the shape of the number three, to long spirals resembling a corkscrew. Its draft genome has an estimated size of 7.23 Mbp (G+C content 45.4%). Strain AB67^T appeared on wet mounts as straight rods, mostly in pairs, sometimes forming long filaments (up to 20 µm). Its draft genome is shorter, estimated size of 6.45 Mbp (G+C content is 46.1%). Overall genome relatedness indexes clearly define them as separate organisms, so based on all the data collected we propose the species *Dyadobacter helix* sp. nov. with type strain AB1^T (= CECT 9275^T = LMG 32341^T) and *Dyadobacter linearis* sp. nov. with type strain AB67^T (= CECT 9623^T = LMG 32342^T).

P-8

Ecological role of the phylum *Acidobacteriota* associated with the plant *Lupinus angustifolius*

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The phylum *Acidobacteriota* has been reported as one of the most abundant and widespread phyla in various habitats using culture independent methods, such as metagenomics (Kalam *et al.*, 2020). However, even if the phylum is known to be abundant in soils, sediments, and hot springs, knowledge about the biology of these microorganisms in these ecosystems remains limited, due to the difficulty of isolation associated with their oligotrophic nature and slow growth rates. For this reason, a combination of bacterial enrichment, selective molecular detection and highly specific media must be used for its isolation and characterization. In 2020 and 2021, metagenomic profiling of soils associated with *Lupinus angustifolious* in Salamanca and Cabrerizos (Spain) detected that the phylum *Acidobacteriota* was the dominating taxon between winter and summer in bulk soils (mean 19.4%), while its abundance decreased in the rhizosphere (6.4%) and nearly disappeared in the endosphere (0.2%) (Ortuzar *et al.*, 2024). Subsequent culture dependent profiling managed to recover 75% of the genera observed in the metabarcoding profiling, but the phylum *Acidobacteriota* remained elusive. In this work, we aimed to specifically isolate members of the phylum *Acidobacteriota* from bulk and rhizosphere soils to understand the functions and ecological importance of these bacteria in this specific environment. High-yield cultures were conducted in 96-well plates using SE/HD (Huber *et al.*,2016) and SSE/HD liquid media (DSMZ media 1426), with serial dilutions of the soil extract, incubated at room temperature and in darkness for 12 weeks. During incubation, the presence of *Acidobacteriota* in each well was monitored and detected using PCR every 15 days with specific primers (31F – 1492R) targeting the 16S rRNA coding sequence. Upon positive detection, cultures were transferred to SSE/HD agar plates for selective isolation and subsequent molecular and phenotypic characterization.

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P-9

Diversity and interaction among the halophilic prokaryotic community in hypersaline environments

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Hypersaline environments are extreme habitats with a limited prokaryotic diversity, mainly restricted to halophilic or halotolerant archaeal and bacterial taxa adapted to high saline conditions. This study attempts to analyze the taxonomic and functional diversity of the prokaryotes that inhabit ponds of a solar saltern located at the Atlantic coast, in Isla Cristina (Huelva, Southwest Spain), and the influence of salinity on the diversity and metabolic potential of these prokaryotic communities, as well as the relationships among the individuals within that environment. Brine samples were obtained from different saltern ponds, with a salinity range between 19.5% and 39% (w/v). Total prokaryotic DNA was sequenced using the Illumina shotgun metagenomic strategy and the raw sequence data were analyzed using supercomputing services following the MetaWRAP and SqueezeMeta protocols. The most abundant phyla at moderate salinities (19.5-22% w/v) were *Methanobacteriota*, *Pseudomonadota*, and *Bacteroidota*, followed by *Balneolota*, *Actinomycetota*, and *Uroviricota* in smaller proportions, while at high salinities (36-39% w/v) the most abundant phylum was *Methanobacteriota* (formerly "*Euryarchaeota*"), followed by *Bacteroidota*. The most abundant genera at intermediate salinities were *Halorubrum* and the bacterial genus *Spiribacter*, while the haloarchaeal genera *Halorubrum*, *Halonotius* and *Haloquadratum* were the main representatives at high salinities. A total of 65 Metagenomic Assembled Genomes (MAGs) were reconstructed from the metagenomic datasets and different functions and pathways were identified in them, allowing to find key taxa in the prokaryotic community able to synthesize and supply essential compounds, such as biotin, β-carotene, and bacterioruberin, to other dwellers in this habitat lacking the required enzymatic machinery to produce them. This work shed light on the ecology of aquatic hypersaline environments, such as Isla Cristina saltern, and on the dynamics and factors affecting the microbial populations under such extreme conditions (García-Roldán et al., submitted).

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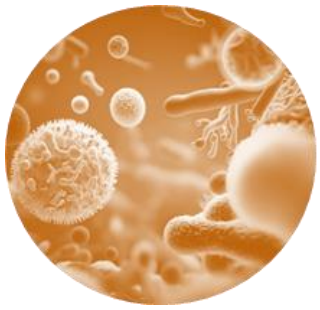


PROGRAMME & ABSTRACT BOOK

September 26-28th, 2024

Salamanca, Spain

Taxonomía, Filogenia y Diversidad
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