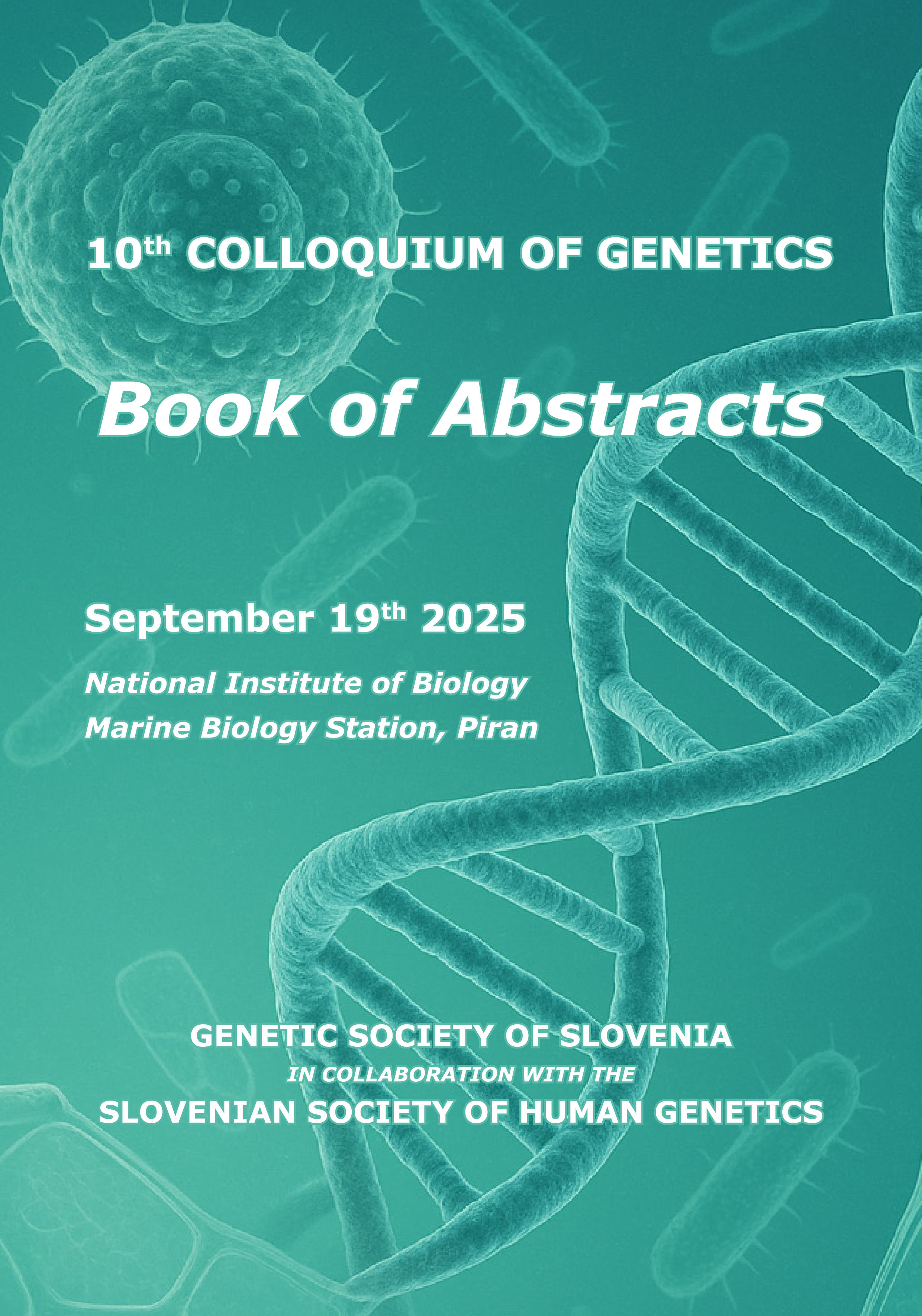

10th COLLOQUIUM OF GENETICS

Book of Abstracts

September 19th 2025

National Institute of Biology
Marine Biology Station, Piran

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SLOVENSKO DRUŠTVO
ZA HUMANO GENETIKO

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PROGRAM

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SLEEPING BEAUTY TRANSPOSON SYSTEM: TOWARDS GENE THERAPY FOR RARE BLOOD DISORDERS

Karen Vanhoorelbeke

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Rare blood disorders such as congenital thrombotic thrombocytopenic purpura (TTP) and type 3 von Willebrand disease (VWD) are caused by severe deficiencies in key hemostatic proteins-ADAMTS13 and von Willebrand factor (VWF), respectively-leading to life-threatening complications. Current therapies rely on frequent infusions of plasma-derived products, which offer only transient relief and impose a significant burden on patients. In this study, we explore the potential of the nonviral Sleeping Beauty (SB) transposon system as a long-term gene therapy platform for these disorders.[1,2]

Using hydrodynamic tail vein injection, we achieved sustained supraphysiological expression of murine ADAMTS13 in *Adamts13−/−*mice, resulting in efficient clearance of prothrombotic ultralarge VWF multimers and protection against TTP episodes for up to 25 weeks.[3] Similarly, in a severe VWD mouse model (*Vwf−/−*), we developed a liver-targeted SB transposon approach capable of delivering full-length murine VWF cDNA (8.4 kb) via a sandwich vector design. This strategy supported stable VWF expression for up to 1.5 years, with vector integration confirmed through liver regeneration studies. However, while long-term VWF expression was achieved, the bleeding phenotype was not fully corrected in all mice, likely due to reduced levels of high-molecular-weight VWF multimers.[4]

Together, these findings underscore the promise of the SB transposon system as a versatile and durable gene therapy platform for rare hematologic diseases. Future efforts will focus on optimizing cell type-specific targeting and multimer composition to enhance therapeutic efficacy.

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GENOMIC STRUCTURE OF THE LIPIZZAN HORSE POPULATION FROM THE LIPICA STUD

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The Lipizzan horse is an indigenous Slovenian breed representing the Baroque horse type, originating from the local Karst horses and imported Andalusian horses at the end of the 16th century to the Lipica stud farm. During the history of the breed, the additional introduction of Neapolitans and Arabs also played an important role. Currently, there are six classic stallion lines (Neapolitano, Favory, Siglavy, Pluto, Maestoso and Conversano) and 18 classic mare family lines established. In this study, we genotyped 232 Lipizzan horses from the Lipica stud farm born between 1992 and 2018, using the ISAG set of microsatellite markers and GGP Equine 70k SNP chip (*Neogen*, UK). The pedigree was checked for pedigree loops, duplicated animals, and conflicts between the stated sex and the sex of the animal's parent. We traced the pedigrees of genotyped horses back to the horses that are considered the founders of the Lipizzan breed. The pedigree we were using in this study comprised 2911 animals, 438 of which were founders. After quality control 70,896 from originally 71,607 SNP markers on the GGP Equine 70k chip remained for the analysis. The principal component analysis based on SNP markers showed a relatively homogeneous genetic structure of the studied population with weak traces of grouping individuals belonging to the same stud lines. We estimated inbreeding based on microsatellites, SNPs and pedigree with the longest ancestral path ranging from 11 to 17. Values for F_{str} ranged from 0.06 to 0.43 (in average 0.16). Values for F_{ped} ranged from nearly 0 to 0.15 (in average 0.04). Runs of homozygosity (ROH) based inbreeding F_{roh} ranged from 0.11 to 0.28 (in average 0.17). ROH islands were identified on chromosomes 14, 5, 11 and 3, revealing possible selection signatures in genomic regions encoding the genes *OAZ3* associated with male fertility, *KIF2B* associated with equine withers height, *NR3C1* associated with stress response and *ARHGAP26* associated with skeletal muscle response to exercise in horses. Special attention was given to the health and trait markers included in the SNP array. Because the Lipizzan horses are mostly grey (as a result of the *STX17* mutation causing age related greying) we searched for the presence of hair colour alleles that might occur in the absence of dominant greying. We identified the presence of allele combinations causing black, bay and brown, as well as a few sorrel or chestnut coat colour.

ADVANCING SPATIAL TRANSCRIPTOMICS IN COMPLEX TISSUES: A XENIUM WORKFLOW FOR SKELETAL MUSCLE

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Spatial transcriptomics (ST) enables gene expression profiling while preserving spatial context of tissues, providing insights into cellular organization, tissue architecture, and interactions that are lost in dissociative methods such as single-cell RNA sequencing or RNAseq. Imaging-based platforms like 10x Genomics Xenium provide subcellular resolution and precise transcript localization, making them particularly suited for complex, highly structured tissues like skeletal muscle.[1]

Skeletal muscle is a heterogeneous tissue of large, multinucleated fibres of different types arranged in a highly ordered, spatially patterned structure. These features make ST analysis particularly challenging, especially in terms of cell segmentation.[2] Most existing tools were developed without using skeletal muscle data and have not been optimised for the Xenium platform. To address these challenges, we are developing a Python-based pipeline tailored to Xenium skeletal muscle data.

To demonstrate functionality, we applied the pipeline to two human skeletal muscle samples—one healthy and one diseased—processed using the Xenium 377-gene panel. Sections were also processed for histological and immunofluorescent staining.

The workflow begins with cell segmentation using Cellpose[3], followed by transcript assignment to individual cells. Quality control, filtering, and normalization are performed with Scanpy[4], a Python library for single-cell sequencing and ST. To explore tissue organization, we apply clustering based on gene expression alone and graph-based methods that also incorporate spatial context.

For cell type annotation, we defined a training set of cells using manual annotation guided by immunofluorescence staining. Distinct clusters were assigned identities based on gene expression differences in the training set. Beyond clustering, spatially variable genes were analysed to reveal location-specific expression. Transcript localization was examined by measuring distances between transcripts, nuclei, and cell centroids at the subcellular level.

Our preliminary results revealed several challenges. Clustering was constrained by the small gene panel in our Xenium samples, making interpretation difficult. Segmentation was particularly challenging in inflamed regions, where histological staining proved essential for improving both segmentation and annotation. Overall, our pipeline demonstrates the feasibility of spatial transcriptomics in skeletal muscle and lays the groundwork for more robust tools tailored to complex tissues.

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DEVELOPMENT OF EXPERIMENTAL MODELS TO ASSESS THE EFFICACY OF ELECTROCHEMOTHERAPY IN OSTEOSARCOMA

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Osteosarcoma (OS) is one of the most common primary bone cancers in humans. Despite advancements in therapy, the standard of care, i.e. surgical resection combined with adjuvant chemotherapy, has remained largely unchanged since 1970. While the 5-year survival rate for non-metastatic OS is approximately 70%, it decreases to 25% in metastatic cases.[1] Electrochemotherapy (ECT) combines electric pulses with chemotherapy to enhance drug uptake. It has already been applied in the treatment of bone metastases. However, the lack of bone-adapted protocols has limited its therapeutic efficacy.[2,3] To explore the feasibility of ECT for OS, we developed predictive in vitro and in vivo models to evaluate treatment efficacy and safety. We established two-dimensional (2D) and three-dimensional (3D) cell models using the murine OS cell line K7M2 and an orthotopic mouse model using luciferase-expressing K7M2 (K7M2luc) cells. The 2D model was used for cytotoxicity and cell membrane permeabilization assays following electroporation and ECT. Electric pulses (8 square waves, 100 µs, 1 Hz) were delivered using parallel stainless-steel electrodes (2.4 cm apart) at 300-1300 V/cm. For 3D modeling, tumor spheroids were generated in a Clinostar® rotary incubator using seeding densities from 1,000 to 10,000 cells/spheroid and cultivated for 7 or 14 days. Immunohistochemical (HE and Ki-67) analyses were performed to characterize necrotic and proliferative regions. To more closely simulate the bone tumor microenvironment, an orthotopic model was created by injecting K7M2luc cells into the proximal tibia of mice using a 29G needle. Tumor progression was monitored weekly via IVIS bioluminescence and x-ray imaging. Mice were euthanized when clinical signs of bone lysis and distress appeared (approximately 6 weeks post-inoculation; Animal Ethics Committee Approval no. U34401-17/2023/1). Together, these models offer a robust platform for evaluating ECT protocols specifically adapted for bone tissue. The aim is to improve therapeutic outcomes for OS by optimizing parameters that enhance cytotoxicity while preserving surrounding healthy structures.

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GOREVERSELOOKUP: PHENOTYPE-TO-GENE DISCOVERY VIA REVERSE GENE ONTOLOGY ANALYSIS

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The Gene Ontology (GO) is a widely used framework for functional interpretation of genes and proteins. It is a structured representation of current biological knowledge, in which standardized biological descriptors, known as GO terms, encapsulate molecular functions, biological processes, and cellular components, and are linked to specific genes based on various degrees of underlying evidence, from experimental to computational.[1] Gene Ontology is most frequently applied in gene enrichment analyses, where the goal is to infer functional or anatomical information from a subset of genes. Before the development of our tool, there was no systematic way to determine which genes are involved in complex phenotypes defined by multiple GO terms—researchers had to manually cross-check and compile lists of genes shared among selected terms.

GOReverseLookup[2], our recently developed tool, addresses this limitation by implementing a phenotype-to-gene workflow that starts from custom, user-defined states of interest, each represented by a set of GO terms, and outputs a list of genes most strongly associated with those states. Each gene is assessed for association strength with interest states versus remaining terms in the GO universe. Our tool is open-source, uses a simple configuration-driven interface, and is built on asynchronous programming principles, enabling rapid and reproducible analyses.

Validation of GOReverseLookup was demonstrated through two case studies designed to test its reliability and versatility. In the first, focused on rheumatoid arthritis, we used a streamlined setup with only a single defined state of interest, showing that even a straightforward input file can yield valid and literature-supported results. In the second, we highlight our tool's capacity to detect genes associated with multiple, biologically related processes. We defined two states of interest, chronic inflammation and tumorigenesis, aiming to discover genes that drive the development of these states. These two analyses underscore the tool's utility for uncovering both well-known, as well as novel contributors to complex phenotypes. Furthermore, when broader comparative insights are required, the integration of orthologous gene data enables cross-species analysis, enhancing the tool's applicability for translational research.

The core contribution of GOReverseLookup lies in its systematic reverse-query process, which replaces manual curation with a transparent approach for discovering evidence-based candidate genes from phenotype definitions. By converting researcher-defined biological states directly into candidate genes, GOReverseLookup streamlines exploratory studies in physiology and functional genomics, enabling both the validation of well-established genes and the discovery of novel candidates relevant to complex phenotypes.

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AI-DRIVEN DESIGN OF MINI-USH2A AND GENE ELECTROTRANSFER FOR MUTATION INDEPENDENT THERAPY OF USHER SYNDROM TYPE 2

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Usher syndrome type 2 (USH2) is the most common form of syndromic retinitis pigmentosa, with mutations in USH2A being the primary cause.[1–3] Current therapeutic approaches are constrained either by mutation-specificity or by the size limitations of viral vectors. Here, we propose a novel, mutation-independent therapeutic strategy that leverages AI-driven protein design to generate a functionally optimized, miniaturized USH2A construct (mini-USH2A). Using advanced molecular dynamics modelling, we systematically reduced the size of USH2A by ~50% while preserving its essential structural and functional domains.[4] In contrast to conventional AAV-based gene therapy, which is restricted by limited payload capacity and immunogenicity, our plan is to employ gene electrotransfer (GET) for retinal plasmid delivery. GET provides targeted and sustained transgene expression while reducing systemic immune responses and bypassing the inefficiencies of multi-vector AAV systems (5,6). We designed three pEFS plasmids: a full-length wild-type USH2A construct (16 kb) and two miniaturized versions (8 kb). Preliminary in vitro experiments demonstrated successful transfection of all three constructs into HEK293T cells under various GET conditions, with expression confirmed by qPCR. Importantly, transfection efficiency was independent of construct size. Beyond USH2A, this pipeline can be extended to other large genes implicated in Usher syndrome, including ADGRV1 and MYO7A, offering broader therapeutic applicability. By integrating computational protein engineering with a non-viral delivery system, our study introduces a paradigm shift in gene therapy for inherited retinal diseases.

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RADIOTHERAPY INDUCES ACTIVATION OF TUMOR ENDOTHELIUM AND FORMATION OF HIGH ENDOTHELIAL VENULES, SUPPORTING ANTI-TUMOR IMMUNE RESPONSE

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Introduction: Radiotherapy (RT) not only affects tumor cells but also the tumor microenvironment, including endothelial cells (ECs), with the vascular response remaining unclear.[1] Tumor-associated high endothelial venules (TA-HEVs) have recently been identified as specialized vascular structures that facilitate lymphocyte entry into tumors; however, whether they can be activated by IR remains unanswered.[2]

Aim: To determine how irradiation (IR)–induced changes in vascular structure and endothelial activation regulate immune-cell homing and extravasation in colon cancer.

Methods: The effects of IR were examined using *in vitro* endothelial assays, a 3D vasculature-on-a-chip model, murine CT26 and MC38 colon carcinoma models exposed to either single-dose 15 Gy or fractionated 5×5 Gy RT, and spatial transcriptomics of murine tumors and matched colorectal-cancer biopsies before and after neoadjuvant 5×5 Gy RT, followed by immunofluorescence and immunohistochemistry. In addition, a dorsal skinfold window chamber with intravital microscopy was applied to visualize vascular changes and lymphocyte interactions with activated endothelium in real time.

Results: In murine (bEnd.3, 2H11, SVEC4-10) and human (HUVEC, EA.hy926, HULEC-5a) EC lines, IR (2–10 Gy) reduced proliferation and increased cell death in a dose- and time-dependent manner. RNA sequencing of irradiated HUVECs revealed down-regulation of cell-cycle and DNA-replication pathways, with up-regulation of p53 and FoxO signaling and early activation of innate (*NF-κβ, TNFα*) and adaptive (Th1/Th2/Th17) immune-response pathways. In vasculature-on-a-chip models, 5 Gy enhanced VWF and ICAM-1 expression, nuclear IRF9, and IFNα/β secretion, indicating IR-induced endothelial activation. Furthermore, lymphocyte–endothelium interactions increased 24 h after both 2 and 5 Gy compared to controls. In vivo, both regimens reduced tumor volume; however, fractionated 5×5 Gy induced the strongest activation of Vwf, Icam-1, and Irf9, accompanied by elevated CD8 and CD4 expression. Spatial transcriptomics showed enrichment of TA-HEV markers within TEC-positive regions co-localizing with immune-response signatures in murine tumors. Similar findings were observed in patient biopsies after neoadjuvant RT, with TA-HEV-associated genes colocalizing with immune transcripts, confirmed at the protein level by immunohistochemistry. Ongoing intravital imaging in dorsal skinfold window chambers is expected to further clarify IR-driven immune cell trafficking.

Conclusion: IR induces activation and functional reprogramming of tumor endothelium toward a pro-adhesive, immune-supportive phenotype in a time- and dose-dependent manner. RT also promotes TA-HEV formation, potentially facilitating lymphocyte infiltration into tumors.

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MOSAIC: A HIGHLY HETEROGENEOUS *SACCHAROMYCES CEREVISIAE* OUTBRED POPULATION TO UNVEIL THE GENETIC BASIS OF NATURAL DIVERSITY

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Recombination and allele reshuffling are key mechanisms driving genetic diversity and adaptation, providing a powerful approach to dissect the genetic architecture of complex traits. To investigate the genomic and phenotypic consequences of extensive recombination in *Saccharomyces cerevisiae*, we generated MOSAIC (Multiparent Outbred *S. cerevisiae* for Advanced Investigation of Complex traits), a highly heterogeneous outbred population. This population was initiated through systematic pairwise crossing of 23 genetically diverse strains, each representing a distinct phylogenetic clade from a global collection of 1,011 natural isolates.[1] The pairwise crossing resulted in 506 distinct hybrids, which were then subjected to 12 rounds of bulk sporulation and mating. Our outcrossing strategy was designed to reduce linkage disequilibrium and generate novel allele combinations of the genomic diversity of the parental strains, resulting in segregants with unique mosaic genomes. We identified 858,307 genetic variants segregating among 23 parental strains, the majority of which are SNPs (90.3%), along with short INDELs (9.1%) and larger structural variants (0.6%), representing the full spectrum of variation potentially segregating in the outbred population. To phenotypically characterize the population, we isolated over 2,000 segregants from the final generation and phenotyped them alongside the 1,011 natural isolates. We measured their growth in optimal non-stress condition as well as their resistance to antifungal drug ketoconazole. Our results show that the phenotypic variation in the MOSAIC population exceeds that of the 1,011 natural isolates as well as that of the parental strains, especially under antifungal stress. We observed a substantial number of transgressive segregants, highlighting the potential of MOSAIC to uncover novel genetic variants contributing to natural phenotypic diversity. Whole-genome sequencing of individual segregants and pooled selections will further elucidate genomic patterns of recombination, haplotype structure, allele frequency shifts, and structural variation across generations. By integrating high-resolution genomic data with extensive phenotyping, MOSAIC will provide a powerful resource for future association studies, complex trait dissection, and investigations into the genetic basis of adaptation.

Reference

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THE ROLE OF *tisB* IN PROTECTING *Escherichia coli* AGAINST ENVIRONMENTAL THREATS

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Bacteria use various strategies to survive in stressful environments.[1] In *Escherichia coli*, DNA damage triggers the SOS response, which activates the *tisB* gene. The product of this gene is a small protein TisB that damages the cell membrane, causing depolarisation and reducing cell survival. However, in some environments, TisB-dependent depolarisation can be beneficial, as it may reduce the uptake of some harmful agents into the SOS-induced cells, for example colicins and bacteriophages, that require PMF to enter the cells.[2,3] The aim of our study was to investigate the effect of *tisB* on the survival of *E. coli* after treatment with trimethoprim (TMP) or mitomycin C (MMC) and the role of *tisB* in the defence against colicin E7 and bacteriophage T4 infection. We compared wild-type (WT) and Δ*tisB E. coli* strains by measuring survival under different concentrations of MMC or TMP, the extent of membrane depolarisation, and survival outcomes in environments with the colicin E7-producing strain BZB2110. In addition, we examined the effect of *tisB* on bacteriophage T4 infection by measuring the burst size with and without prior SOS induction. The results showed that the survival differences following *tisB* induction depended on both the type of antibiotic used for SOS induction and its concentration. When exposed to colicin E7, TisB-dependent depolarisation can either protect cells by reducing membrane transport or reduce survival by limiting the import of nutrients. In addition, upon SOS induction, the Δ*tisB* strain exhibited larger T4 burst sizes than the WT strain, but this difference was not significant when the potential effects of differential cell survival to TMP were taken into account. In conclusion, the results obtained provide a better understanding of the complexity of bacterial defence systems and their role in survival against environmental threats such as TMP, MMC, colicin E7 and bacteriophage T4.

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VIRUS -INDUCED CAHNGES IN miRNA EXPRESSION IN GRAPEVINE (*Vitis vinifera* L.)

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Grapevine (*Vitis vinifera* L.), one of the most widely cultivated fruit crops, is highly susceptible to viral infections. Over 100 viruses have been identified in grapevines, with several causing significant disease symptoms that negatively affect both yield and fruit quality (1). Viral infections are also known to modulate the expression of microRNAs (miRNAs)—small, non-coding RNAs that regulate gene expression at the post-transcriptional level (2, 3). In this study, we analyzed the impact of viral infections on miRNA expression in the grapevine cultivars Refošk and Zeleni Sauvignon.

Small RNAs were extracted from virus-free and virus-infected grapevine plants. Sequencing was conducted on the Ion Proton™ platform (Ion Torrent™, Life Technologies). Viruses were identified in sRNA-seq datasets using the VirusDetect pipeline. We analyzed miRNA expression with three miRNA prediction tools: miRador, ShortStack and miRDeep2. The combined results from all three tools were used for differential expression analysis using DESeq2 in R. Differential expression of miRNAs was validated by stem-loop RT-qPCR. Potential targets of differentially expressed miRNAs were predicted by psRNATarget, followed by gene ontology (GO) enrichment analysis to determine their biological functions. To confirm the cleavage of target transcripts, degradome sequencing was performed.

By small RNA sequencing we obtained between 6.3 to 25 million reads per library. In virus-free samples, the absence of viruses was confirmed, while infected samples contained the expected viruses GPGV, GRSPaV, and GRVFV. Differential expression analysis revealed 19 differentially expressed miRNAs in Refošk and 17 in Zeleni Sauvignon, with 3 miRNAs differentially expressed in both cultivars. Target prediction identified 131 potential targets for Refošk and 123 for Zeleni Sauvignon. Gene Ontology (GO) enrichment analysis revealed that these targets are involved in diverse biological processes and molecular functions. By stem-loop RT-qPCR we were able to confirm differential expression for 9 out of 12 tested miRNAs in Refošk and 5 out of 15 in Zeleni Sauvignon. Results of degradome sequencing confirmed cleavage of certain predicted target transcripts. The function of differentially expressed miRNAs will be further validated through virus-induced gene silencing method (VbMS) (4).

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GLUCOSINOLATE PROFILING OF CABBAGE (*BRASSICA OLERACEA* VAR. *CAPITATA*) LINES AND HYBRIDS REVEALS PREDICTIVE POWER OF PARENTAL PHENOTYPES

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Glucosinolates (GSLs) are sulphur- and nitrogen-rich metabolites derived from amino acids. Upon tissue disruption they are hydrolysed by endogenous thioglucosidases (myrosinases) to isothiocyanates, thiocyanates and nitriles (the so called "mustard oil bomb"), which play a key role in plant responses to abiotic stress and defence against herbivores and pathogens.[1] Owing to their health benefits, most notable anticancer activity[2] and shifting breeding goals that include biotic and abiotic stress resilience and nutritional quality alongside yield[3], elevating GSLs in cabbage is a promising breeding objective. Our study evaluated GSL composition, variation, heterosis, and parent–hybrid correlations in 14 cabbage (*Brassica oleracea* var. *capitata)* doubled haploid lines and 11 derived hybrids. Thirteen GSLs were detected and quantified as desulfoglucosinolates by UHPLC–MS/MS. Total contents ranged from 19.7 to 67.8 µmol g^{-1} dw, with profiles dominated by aliphatic GSLs (72.9%), followed by indolyl (26.9%) and aromatic (0.1%). The most abundant GSLs in most genotypes were glucobrassicin, sinigrin, glucoiberin and progoitrin. Marked genotypic variation in both content and profile was observed, but no consistent differences emerged between lines and hybrids for individual or total GSLs. Hierarchical clustering revealed two major genotype groups, differentiated mainly by 3-carbon (3C) and 4-carbon (4C) aliphatic GSLs, with correlation analysis indicating trade-offs in their accumulation. Principal component analysis (PCA) showed that most hybrids grouped closely with their parental lines, indicating that glucosinolate profiles are at least partly genetically determined and inherited. Heterosis for GSLs varied by compound and parental combination. Median mid-parent heterosis (MPH) was positive for gluconapin, gluconasturtiin and glucoiberverin, and negative for glucobrassicin and neoglucobrassicin. For most aliphatic glucosinolates, linear regression models explained a large proportion of the variability in hybrid content based on mid-parent values, indicating that parental phenotyping can reliably predict hybrid performance. In parallel, agronomic traits (head weight, diameter, height, inner core length and compactness) showed uniformly positive MPH, with head weight exhibiting the highest heterosis (473.8%). Collectively, these findings show the potential for integrating GSL profiling of inbred lines into cabbage breeding programs to improve defence-related and nutritional traits alongside yield. Validation in larger populations

and targeted crosses among high-GSL lines will help generalise and exploit the predictive value of parental phenotypes for hybrid development.

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A PHASED GENOME RESOURCE FOR POTATO CULTIVAR DÉSIRÉE

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Potato cultivar Désirée is one of the most widely used red-skinned potatoes in both research and breeding, valued for its agronomic traits and for being amenable to genetic studies. Until now, most potato studies have relied on the *Solanum tuberosum* Phureja DM haploid reference. While useful, DM does not capture the full complexity of cultivated tetraploid potatoes and misses a large fraction of genes that are relevant for their biology and agronomic traits. We assembled a haplotype-resolved, chromosome-level genome of potato cultivar Désirée using PacBio HiFi reads and Hi-C scaffolding. To annotate genes, we developed a comprehensive pipeline combining five complementary approaches: de novo prediction, homology-based, transcriptome-guided, deep-learning, and reference-based methods. The merged annotation provides a highly complete gene set, supported by a BUSCO score of 99.4% for Solanaceae-specific orthologs. A genome-wide methylation profile is also available as a resource for the community. Importantly, when comparing the Désirée annotation with the most complete DM annotation (UniTato), almost 20% of genes are specific to Désirée. This shows that phased assemblies of cultivated tetraploids recover substantial gene space absent from DM, giving us a more complete reference for comparative genomics, breeding program design, and functional studies in *Solanum tuberosum*. Including Désirée and other phased tetraploid genomes will help fill major gaps in the potato pangenome, providing breeders with a stronger toolkit for developing more resilient and productive cultivars. At the same time, working with a phased tetraploid genome comes with challenges: downstream analyses such as

transcriptomics are more complex, and many tools are not yet designed to properly handle polyploid references. I will highlight some examples of these challenges at the colloquium.

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TRAIT-LINKED DNA MARKERS REVEAL ENHANCED PATHOGEN RESILIENCE IN COMPOSITE POPULATIONS OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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Heterogeneous/composite populations represent a valuable source of phenotypic and genetic variability, which enhances tolerance to biotic and abiotic stresses, maintains nutritionally important traits, and provides a reservoir of functional alleles for breeding. However, such populations have not been extensively studied in common bean (*Phaseolus vulgaris* L.). Therefore, we collected 50 composite populations of common bean from diverse sources, including genebanks, European and Slovenian project collections, as well as the ongoing breeding program at the Agricultural institute of Slovenia. Each composite population consisted of two to five distinct seed phenotypes differing in coat colour and pattern. Phenotypes were individually assessed and cultivated in field trials in Jablje, Slovenia, over two consecutive years (2022 and 2023), enabling the observation of intra-population variability under natural growth conditions. Leaf samples were collected and DNA was extracted for molecular analysis. Genotyping within and among composite populations was performed using 14 functional DNA markers associated with resistance to important common bean pathogens including bean rust, angular leaf spot (ALS), anthracnose (ANT), and bean common mosaic virus (BCMV).

Genetic diversity analysis based on binary genotyping revealed substantial genetic variation across the composite populations. The average number of bands per population was 32, ranging from 21 to 47. Private bands, indicative of unique alleles, were detected in only five composite populations, highlighting the specificity of certain genetic backgrounds. Analysis of molecular variance (AMOVA) revealed that the majority of genetic diversity (72%) was present within composite populations, whereas 28% was attributable to differences among them, confirming pronounced intra-population diversity. Shannon's information index and the proportion of polymorphic loci (3.7 – 45%) further supported this variability.

Given the functional relevance of these markers, we further examined the distribution of pathogen resistance genes within the analysed populations. Most composite populations contained alleles conferring resistance to ALS and ANT across both years, whereas resistance to BCMV and rust was more variable, particularly in 2023. During the 2022 growing season, the number of phenotypes within 36 composite populations changed due to the emergence of new phenotypes and loss of some existing ones. This was particularly pronounced in composite populations INCBN_01609 and INCBN_03267, where seven new phenotypes were identified. The increase in phenotypes during the growing season further enhanced the overall resilience of the populations, even though the loss of some phenotypes did not exclusively preserve resistant alleles. Notably, composite population 359x417 consistently retained resistance alleles against all studied pathogens in both years.

These findings demonstrate that composite populations of common bean harbour high levels of variability within population pathogen resistance genes. Such variability ensures reliable yield and also reduces the spread of pathogens, enhancing the adaptive potential of such populations. Maintaining and studying these populations is therefore essential.

MODELING THE TUMOR MICROENVIRONMENT AND RESPONSE TO TREATMENT OF AGGRESSIVE CANCERS

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Tumor heterogeneity and the microenvironment play a critical role in therapy resistance and relapse in aggressive cancers. Our goal is to model patient-specific tumor ecosystems in the laboratory and use these models as tools to investigate the pathobiology of therapy resistance and explore novel therapeutic strategies, including combinations of standard treatments and immunotherapy (1). In this talk, I will present advanced tumor models that closely mimic the tumor microenvironment and immune compartment observed in glioblastoma patients, as well as mechanisms of resistance to radiotherapy and chemotherapy (2). We employ spatial biology techniques—particularly in situ sequencing—to characterize the glioblastoma microenvironment and organoid models under various treatment conditions, all within a spatial context. The in situ sequencing (ISS) platform provides spatially resolved gene expression data for curated gene panels at subcellular resolution, enabling high-throughput mapping of up to 5,000 genes. I will discuss the application of ISS in identifying cancer cell niches and cellular communities that leads to therapy resistance, highlighting its major advantages and the challenges involved in characterizing the tumor ecosystem.

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POSTER ABSTRACTS

IDENTIFYING NOVEL HOST GENES INVOLVED IN VIROID REPLICATION AND MOVEMENT IN PLANTS

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Viroids represent the smallest infectious agents known to affect plants. They are circular, highly structured RNA molecules, typically between 246 and 401 nucleotides in length, and lack protein-coding capacity. As a result, their replication and spread rely entirely on host-encoded factors. Several plant proteins involved in viroid biology have already been identified, including:

1. proteins mediating nuclear import, such as VirP1, which facilitates transport via an importin-dependent pathway;
2. components of the RNA polymerase II complex, with a splice variant of transcription factor IIIA contributing to viroid replication through its role in Pol II organization;
3. enzymes linked to RNA processing, such as DNA ligase 1, which is implicated in circularization;
4. proteins associated with nuclear export, cytoplasmic trafficking, and cell-to-cell movement, potentially involving the 5S rRNA pathway.

This doctoral research, conducted under ViroiDoc doctoral network, is designed to uncover additional host factors participating in viroid replication and movement, while also validating those already characterized. To achieve this, publicly available RNA-seq datasets from diverse plant–viroid interactions will be analyzed to identify conserved and species-specific transcriptional responses, as well as enriched biological pathways. From this analysis, up to six candidate host genes will be chosen for functional testing. These genes will be silenced in hop (*Humulus lupulus*) or a model host such as *Nicotiana benthamiana* using virus-induced gene silencing (VIGS), after which plants will be challenged with selected viroid species (e.g., *Cocadviroid rimocitri*, CBCVd). Disease development and viroid accumulation will be assessed over a six-month period, with RNA samples collected at multiple stages post-inoculation for RT-qPCR quantification of viroid levels using a TaqMan assay. Symptom severity will be scored systematically to monitor progression. By elucidating host-viroid interactions at the molecular level, this doctoral project will broaden our understanding of the processes underpinning viroid infection. The outcomes are expected to provide a foundation for future breeding strategies or preventive measures aimed at enhancing viroid resistance in economically important crops.

DEVELOPMENT OF PLASMIDS EXPRESSING BACTERIAL TOXINS FOR *IN SITU* CANCER VACCINATION

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In situ cancer vaccination presents a promising strategy to transform the tumor into an autologous source of antigens, thereby enhancing immune-mediated tumor elimination. We are developing a plasmid-based gene therapy platform that utilizes secretory forms of bacterial toxins delivered via gene electrotransfer (GET) to stimulate antitumor immune responses directly within the tumor microenvironment. Our approach combines pore-forming toxins (PFTs), intended to lyse tumor cells and promote antigen release, with superantigen toxins (SAgs), aimed at amplifying local immune activation. To identify optimal toxin candidates and suitable eukaryotic secretion signals, we conducted a comprehensive literature review. Coding sequences of selected bacterial toxins were retrieved from nucleotide databases, codon-optimized for expression in eukaryotic cells, and fused with secretion signals positioned upstream of the toxin genes. In parallel, reporter constructs containing green fluorescent protein (GFP) downstream of the secretion signals were generated to assess secretion efficiency. All constructs were synthesized de novo, cloned into mammalian expression vectors, and validated by restriction analysis and whole-plasmid sequencing. For functional evaluation, transfection-grade plasmid DNA was delivered into B16-F10 murine melanoma cells using GET. At 48 hours post-transfection, secretion signal functionality was assessed by quantifying intracellular and extracellular GFP fluorescence, and toxin gene expression was evaluated by qRT-PCR. We successfully generated five PFT encoding plasmids: Escherichia coli cytolysin A and Shiga toxin 2, *Streptococcus pyogenes* streptolysin O, *Aeromonas hydrophila* aerolysin, and *Staphylococcus aureus* alpha-hemolysin; and three SAg encoding plasmids: *S. aureus* enterotoxins A and B, and toxic shock syndrome toxin-1 (TSST-1), each fused to the secretion signal from perforin-1. Following GET into target cells, toxin gene expression was confirmed by qRT-PCR, and functionality of the secretion signal was verified by GFP signal localization in the culture medium. AlphaFold-based three-dimensional modeling predicted preserved functional conformations for all toxins, suggesting preservation of biological activity. Ongoing functional assays aim to determine whether the toxins secreted from transfected tumor cells, rather than their bacterial counterparts, retain effective pore-forming and superantigenic activities. The identification of the most potent toxin genes and effective secretion signals represents a critical milestone in the development of our plasmid-based in situ cancer vaccination approach. The results will lay the foundation for advancing this novel gene therapy strategy into preclinical tumor models, with the ultimate goal of translating it into a clinically viable approach for eliciting robust and durable systemic anti-tumor immunity.

STRATEGIES FOR A NATIONAL CORE COLLECTION TO PRESERVE THE GENETIC DIVERSITY OF SLOVENIAN APPLES

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The apple (*Malus x domestica* Borkh.), is the economically and culturally most important fruit crop in the temperate zones of the world and also in Slovenia. Nowadays, apple production and breeding programmes are based on a reduced number of varieties, although there are more than 10,000 varieties all around the world. The diversity once found in orchards with old and traditional varieties has been largely replaced by well-adapted modern varieties, many of which are genetically linked. In recent decades, increasing attention has been paid to the conservation and utilization of these genetic resources. The Slovenian apple germplasm collections are essential for preservation, research and breeding. Despite their importance, no national assessment of their genetic diversity and structure has yet been carried out. For this purpose, 233 accessions from three different locations in Slovenia were analysed using 15 internationally recognized SSR markers.[1] This analysis identified 182 unique genotypes and 51 synonyms (22% redundancy). Remarkably, 62% of the genotypes were represented by only one accession, highlighting their vulnerability. A total of 228 different alleles were amplified, with an average of 15.2 alleles per locus. With an average power of discrimination of 0.94, all markers proved to be highly discriminating and powerful for variety identification. The results showed that the germplasm is highly diverse with a mean expected heterozygosity of 0.83 and a mean observed heterozygosity of 0.86. Using flow cytometry, 23% of the genotypes were identified as triploids. Structural analysis using UPGMA clustering revealed three main groups. In addition, genetic analysis, using the Bayesian model, revealed a very strong differentiation into two main groups, mainly corresponding to the geographical origin of the genotypes. The first group (G1) comprised 85 genotypes, including four reference varieties and seven local varieties. The second group (G2) comprised 105 genotypes, including four reference varieties and two local varieties. Moreover, nested clustering was performed for these two groups, and four and two subgroups were found, respectively. This core collection will be an essential resource for genetic research, conservation, and preservation of Slovenian apple biodiversity, as it represents a valuable resource for improving adaptability, fruit quality and disease resistance under future climatic conditions.

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IMPACT OF ANTIBIOTIC RESISTANCE MARKER STABILITY ON CONJUGATION FREQUENCY IN TWO *Escherichia coli* LABORATORY STRAINS, DH5α AND Top10

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Laboratory strains used in genetic research usually carry genetic markers, generally antibiotic resistance genes. The stability of these resistance markers is crucial, as it enables reliable tracking of strains during experiments. Conjugation is a process of horizontal bacterial gene transfer from a donor cell to a recipient cell mediated by a conjugative plasmid. Conjugation assays rely on selection and counter-selection, usually based on antibiotic resistance genes, to identify and isolate bacteria that have successfully undergone conjugative gene transfer, the so called transconjugants (recipients that have received the plasmid).[1] Based on our observations from previous experiments, we assumed that the stability of chromosomal antibiotic resistance markers might differ in *Escherichia coli* strains DH5α and Top10. The aim of this study was to investigate the impact of antibiotic resistance marker stability on conjugation frequency in these two *E. coli* strains. Stability was tested for strains DH5α (resistant to nalidixic acid, 25 µg/mL) and Top10 (resistant to streptomycin, 150 µg/mL), in growth assays determining CFUs on LB agar plates with and without the antibiotic, nalidixic acid and streptomycin, respectively. In quantitative conjugation assays strain KP possessing the pOX38:Cm plasmid (chromosomally-encoded gentamicin resistance, 20 µg/mL, and plasmid-encoded chloramphenicol resistance, 30 µg/mL) was used as the donor and strains DH5α and Top10 were used as the recipients. Conjugation was performed according to the protocol described by Starčič Erjavec and Žgur-Bertok.[1] The presence of the antibiotic had no detectable effect on the number of CFUs for Top10 strain. However, on the plates supplemented with nalidixic acid, the number of DH5α CFU was decreased by an order of magnitude. In addition, a prolonged growth time of DH5α colonies was observed on these plates. The conjugation frequency calculated per recipient as well as the conjugation frequency calculated per donor in mating assays with DH5α was lower than those from mating assays with Top10 and varied greatly between independent experiments. These results suggest that the antibiotic resistance marker in the DH5α strain is unstable, and that this instability affects the calculated conjugation frequency, leading to erroneous data and an underestimation of conjugation frequency. Taken together, results once again demonstrate the importance of stable genetic markers for obtaining correct results.

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INVESTIGATING dsRNA-MEDATED SIGS FOR THE SUPRESSION OF *VERTICILLIUM NONALFALFAE*

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The fungus *Verticillium nonalfalfae* (*V. nonalfalfae*) poses a serious challenge in hop (*Humulus lupulus* L.) production, as it causes verticillium wilt disease of hop, characterized by plant wilting and dieback. Due to the pathogen's resistance to available plant protection products and its ability to survive for extended periods in the form of melanized mycelium, current control methods, such as removal of infected plants, chemical disinfectants, and machinery sanitation, are only partially effective.[1] RNA interference (RNAi) is a natural regulatory mechanism that enables the silencing of specific gene expression through the action of small interfering RNAs (siRNAs). Modern approaches, such as spray-induced gene silencing (SIGS), employ synthetically produced double-stranded RNAs (dsRNAs) to specifically silence genes that are crucial for the survival or virulence of pathogens. This environmentally friendly method offers a sustainable strategy for plant disease management.[2] In our study, we applied the SIGS approach to control two isolates of V. nonalfalfae, a highly virulent isolate (T2) and a less virulent one (Rec). We synthesized dsRNAs complementary to selected target genes responsible for fungal growth and pathogenicity. Using an in vitro fungal growth inhibition assay on solid media, we evaluated the impact of dsRNA treatment on fungal growth, followed by qPCR-based analysis of target gene expression. Our results showed that treatment with dsRNA targeting the DES gene and dsRNA targeting the NOX gene led to a reduction in fungal growth, indicating the effectiveness of specific gene silencing. Furthermore, we tested dsRNA by directly treating spores of the highly virulent *V. nonalfalfae* isolate, which were then used to inoculate tobacco plants. We monitored wilting symptoms in plants infected with treated versus untreated fungal spores. The results demonstrate that SIGS is a promising tool for sustainable control of hop diseases.

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ISOLATION OF EXTRACELLULAR VESICLES FROM *VERTICILLIUM NONALFALFAE* AND INVESTIGATING THEIR RNA CARGO AND POTENTIAL IMPLICATION IN HOP-PATHOGEN INTERACTIONS

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Hop (*Humulus lupulus* L.) is a crop of major economic importance in Slovenia, primarily cultivated for brewing. The cultivation of hops is increasingly challenged by *Verticillium* wilt, a disease caused by the soil-borne fungi *Verticillium dahliae* and *Verticillium nonalfalfae*. *Verticillium nonalfalfae* is considered particularly aggressive and destructive due to its persistency, longevity and survival in soil, while also being resistant to conventional phytosanitary products. Understanding the mechanisms that underly the host-pathogen interactions is therefore essential to develop new combat strategies. Our research aims to investigate the interaction between hops and V. nonalfalfae at the level of cross-species RNA communication. Recent studies suggest that fungal pathogens, including the *Verticillium* species, may exploit RNA delivery pathways, such as vesicle-mediated RNA interference, to promote infection. In order to investigate this hypothesis, we focused on the isolation and characterization of extracellular vesicles (EVs) from *Verticillium nonalfalfae*. Our initial objective was to establish a reliable and efficient protocol for the isolation of fungal extracellular vesicles. For this purpose, we compared two growth mediums, a commonly used fungal medium Czapek-Dox and a xylem-simulating medium, which is intended to provide conditions more closely resembling those found in plant tissues. Following the optimization of the EV isolation protocol, RNA was isolated from the fungal extracellular vesicles. Preliminary sequencing of the EV-associated RNA has been conducted, and the results will be presented. Our next steps will include sequencing RNA from fungal EVs from a highly virulent strain that causes lethal symptoms in hops, as well as from a less virulent strain that only causes mild symptoms. Our research aims to advance and deepen the understanding of the interactions between hops and *Verticillium nonalfalfae*, with potential implications for the development of innovative approaches to plant disease management and defense.

EVALUATING THE RESPONSIVENESS OF DIFFERENT *Cannabis sativa* GENOTYPES IN TEMPORARY IMMERSION SYSTEMS

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Cannabis sativa L. is a plant that has recently seen a rise in demand, especially within the pharmaceutical and cosmetic sectors. It is mainly grown to produce cannabinoids, which are secondary metabolites that have been used in numerous clinical trials for alleviating various medical issues.[1] Depending on concentrations of major cannabinoids, cannabis is divided into 5 significant chemotypes.[2] Traditional cultivation of seed-grown plants is time-consuming and produces plants with a high heterozygosity. *In vitro* propagation (micropropagation) presents a more favourable alternative as it requires less space, offers better control of growing conditions, and enables the infinite production of genetically identical plants.[3] Plantlets are usually grown in individual growth containers partially submersed in semi-solid media. However, this process is still extremely laborious and generates a large amount of waste as the containers are mainly single use.

We studied the growth of plantlets grown in temporary immersion system (TIS) with different cannabis chemotypes. TIS allow the growth of multiple plantlets in liquid media in an automated system. We compared growth rates and shoot quality of different chemotypes grown in TIS and in semi-solid media from two separate subcultivations. Number of newly grown shoots, the lengths of the longest shoots, degrees of hyperhydration, and other morphological traits were assessed. Four different genotypes were cultivated, all presenting different chemotypes. A higher number of shoots per explant and the longest shoots were observed in plantlets grown in TIS, most prominently chemotype II, while chemotype I was proven to be the least responsive. Total chlorophyll concentrations were found to be reduced in chemotypes I and III, while II and IV did not show any significant change in concentrations. Moreover, all chemotypes grown in TIS had a greater weight, due to increased shoot number and length, as well as due to showing signs of mild hyperhydricity.

Our research shows that plantlets grown in TIS experience genotype specific responses, reinforcing the need for different cultivars that can positively respond to growth in liquid media. Overall TIS presents a possible alternative for traditional cannabis micropropagation and warrants further research of their use for this purpose.

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TOWARDS IMPROVED FUNCTIONAL TESTING IN HEREDITARY ERYTHROCYTOSIS

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Hereditary erythrocytosis (ECYT) is a rare hematologic disorder with a complex genetic background, defined by a ≥25% increase in red blood cell mass relative to age and sex-specific reference values. Although often asymptomatic, ECYT can result in severe thrombotic events.[1] This study provides a multidisciplinary overview of the clinical, biotechnological, and economic aspects involved in the ongoing search for effective detection methods of gene variants of unknown significance (VUS). Using analytical and quantitative research approaches, data were collected from multiple databases, including ClinVar[2], MalaCards[3], LOVD[4] and Ensembl[5]. The results reveal that while hundreds of pathogenic gene variants are known, over a thousand remain of uncertain origin, underscoring the need for a deeper understanding of the disease's pathomechanism. It was observed that a major obstacle hindering progress in the field of rare diseases is the lack of applicable functional assays to confirm the clinical relevance of newly identified VUS from individual patients. To address this, an in-depth literature review and direct interviews with active researchers were conducted, offering first-hand knowledge about practical aspects of gene variant research, as well as an introduction to novel perspectives on testing strategies. The results of this study will lay the groundwork for developing a universal functional testing method, with the potential to enhance the reliability of Hereditary Erythrocytosis diagnosis.

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GLIOBANK: A SLOVENIAN TRANSLATIONAL BIOBANK UNVEILING DAB2 AS A PROGNOSTIC MARKER IN GLIOBLASTOMA

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The Slovenian translational platform GlioBank is an academic biorepository that aims to improve brain tumor research, especially on glioblastoma (GB), which is one of the most lethal solid tumors in humans. It has been established to address the challenges arising from the heterogeneity of GB and to facilitate the identification of clinically relevant biomarkers and effective therapeutic strategies. The main goal of GlioBank is the systematic collection of fresh tumor tissue samples together with the corresponding histopathological, molecular, and clinical data, as well as the establishment of cell models, including primary glioblastoma cells, glioblastoma stem cells (GSCs), and glioblastoma organoids (GBOs). Using GlioBank datasets and materials, we investigated molecular signatures associated with GB progression and patient survival. In a cohort of 91 patient tissues and 23 primary GBM cells we performed targeted qPCR and bioinformatic analyzes of clinical and research data to investigate genes associated with GBM stem cells, epithelial-to-mesenchymal transition (EMT), immunosuppression and GBM subtypes. We identified DAB2 as an independent prognostic marker, with high *DAB2* expression associated with poor overall patient survival. Furthermore, comparative analysis of tumor core and rim samples from 27 patients revealed significant differences in gene expression. Genes such as *STMN4, ERBB3,* and *ACSBG1* were upregulated in the tumor rim, suggesting their involvement in the invasive behavior of GB. These findings highlight the importance of considering regional tumor heterogeneity in the development of targeted therapies.[1]

GlioBank represents a significant advance in GB research by fostering collaboration between key institutional players in the biomedical field, i.e. hospitals, research institutes, universities and small and medium-sized enterprises. It advances our knowledge of the disease and opens the door to more personalized treatment plans.

Keywords: GlioBank, glioblastoma, DAB2, molecular signatures, tumor core, tumor rim, personalized medicine.

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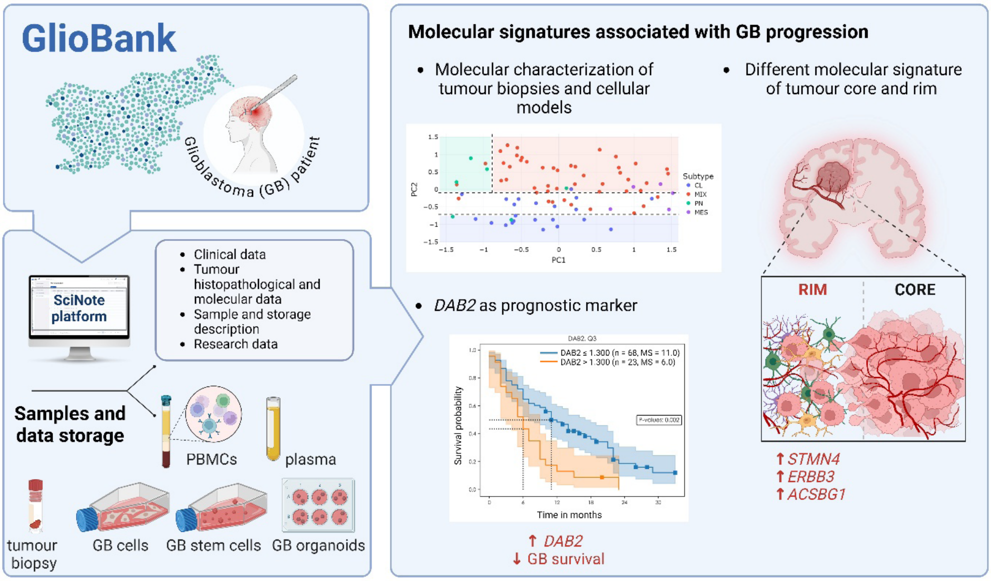

GENE ELECTROTRANSFER OF mRNA CODING FOR IL-12 IN CANCER CELLS: INFLUENCE OF CAP MODIFICATIONS ON PROTEIN EXPRESSION AND CYTOTOXICITY

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Interleukin 12 (IL-12) is a potent immunostimulatory cytokine with great potential for cancer therapy, as it modulates various mechanisms that counteract the immunosuppressive tumor microenvironment. Systemic administration of IL-12 is associated with toxicity, which has shifted preclinical research towards local delivery approaches, such as gene electrotransfer (GET).[1,2] To improve safety and efficacy, mRNA-based GET represents an alternative to plasmid DNA (pDNA), as mRNA enables high transfection rates, avoids nuclear entry, and eliminates the risk of insertional mutagenesis. Its stability and translational efficiency can be further enhanced by chemical modifications, including capping.

In our study, we evaluated the efficiency of therapeutic IL-12 mRNA electrotransfer in murine melanoma (B16F10) and colon carcinoma (CT26) cell lines. Two versions of IL-12 mRNA were used, differing in cap modification (Cap0 and Cap1), along with a non-coding scrambled mRNA control (Cap0 and Cap1). Cell viability was assessed three days after electrotransfer, while IL-12 protein expression was measured at multiple time points. The results showed that IL-12 expression peaked 24 hours after GET. In B16F10 cells, IL-12 levels were significantly higher after mRNA Cap1 electrotransfer compared to Cap0, consistent with the improved cytosolic stability of Cap1-modified mRNA. In CT26 cells, however, no significant difference was observed between Cap0 and Cap1. Importantly, electrotransfer of IL-12 mRNA reduced cell viability compared to controls.These findings demonstrate that therapeutic IL-12 mRNA can be effectively delivered by GET, with Cap modifications influencing expression efficiency in a cell line–dependent manner. The results provide an important basis for further *in vivo* studies and the optimization of IL-12 mRNA-based cancer immunotherapy.

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ANALYSIS OF THE EFFECTS OF SPIRULINA AND ITS COMPONENTS ON IMMUNITY AND REPRODUCTION OF FRESHWATER FISH

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Arthrospira platensis (spirulina) is a blue-green microalga widely used in aquaculture due to its high nutritional value, containing 60–70% protein, essential amino acids, polyunsaturated fatty acids, vitamins, minerals, and bioactive pigments. It is recognized for its antioxidant, immunostimulatory, anti-inflammatory, antiviral, and antibacterial properties.[1] Incorporating spirulina into aquafeeds improves growth performance, strengthens immune function, and supports reproduction, while serving as a sustainable protein-rich alternative to conventional feed ingredients.[2] This study aims to design a nutritional trial, develop a database of spirulina's effects on immunity and reproduction of freshwater fish, and perform in silico functional and docking analyses to predict the molecular roles of spirulina components in key pathways. A nutritional trial is being conducted on European grayling (T*hymallus thymallus*), a freshwater salmonid experiencing population declines in Slovenia due to habitat degradation and overexploitation. The trial evaluates the effects of a high-spirulina diet on immune responses and reproductive performance. A literature review shows consistent positive effects of spirulina supplementation on hematological, biochemical, and immunological parameters. Reported responses include increased erythrocyte and leukocyte (activating innate immune cells) counts, as well as a higher hemoglobin, and hematocrit levels. Biochemical changes involve higher serum protein, albumin, and globulin concentrations. Immunological benefits include elevated lysozyme activity, enhanced leukocyte phagocytic capacity, and increased serum immunoglobulins, indicating improved innate and adaptive immunity. Antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are consistently upregulated, reducing oxidative stress and improving overall health. Transcriptomic studies report upregulated expression of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) along with regulatory cytokines (IL-10), suggesting balanced immune activation and modulation. Reproductive improvements are also evident. In males, spirulina supplementation enhances sperm motility, viability, and density. In females, spirulina-enriched diets increase gonadosomatic index, fecundity, egg quality, and vitellogenesis, reflecting enhanced reproductive efficiency and offspring quality. To explore the molecular mechanisms, in silico analyses were performed. STRING-based functional analyses identified potential interactions of spirulina bioactive compounds with genes involved in immune and reproductive pathways. Docking studies using the ProBiS-Dock platform predicted favorable binding of spirulina components to protein targets within these pathways, supporting reported in vivo effects and suggesting mechanisms through which spirulina may promote immunity and reproduction. These findings highlight the potential of spirulina as a functional feed additive for freshwater fish, providing practical insights for sustainable aquaculture and conservation strategies for species such as European grayling.

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TRANSCRIPTOMIC ANALYSIS OF THE HYPOTHALAMUS IN MOUSE LINES DIVERGENTLY SELECTED FOR OBESITY AND LEANNESS

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Obesity is a complex metabolic condition in which the central nervous system, particularly the hypothalamus, plays a key role by regulating feeding behavior, metabolism, and hormonal balance.1 The link between gene expression in the hypothalamus and phenotypic differences in body mass has been observed repeatedly, yet many underlying mechanisms remain unclear. Previous studies have focused on SNP analysis2 and alternative polyadenylation3, but a comprehensive analysis of gene expression at the hypothalamic level using RNA-seq technology has not yet been performed. In this study, we analyzed transcriptomic differences between the FLI (obese) and FHI (lean) mouse lines, divergently selected for obesity and leanness, by comparing RNA-seq data from the hypothalamus. Differential expression analysis revealed 19 statistically significant differentially expressed protein-coding genes located within known quantitative trait loci (QTL) associated with obesity. We subsequently obtained functional annotations from publicly available databases and performed a literature review to investigate the roles of these genes. Six of them (*Phf6, Ifrd1, Bub1b, Spint1, Scg5, Ly6a*) are, according to published literature, clearly linked to processes such as adipocyte differentiation, feeding regulation, insulin signaling, and metabolic homeostasis. These findings support the initial hypothesis that differentially expressed hypothalamic genes coincide with the locations of known QTLs and may contribute to the pathogenesis of obesity.

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IN VITRO CHARACTERIZATION OF CELL DEATH PATHWAYS INDUCED BY ELECTROCHEMOTHERAPY

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Electrochemotherapy (ECT) is a local ablative technique that uses electric pulses to transiently increase cell membrane permeability, therefore enhancing the uptake of chemotherapeutic agents such as Bleomycin (BLM), Oxaliplatin (OXA) and Cisplatin (CDDP) into the cell, potentiating their cytotoxic activity. In addition to its direct cytotoxic effects, ECT can also promote anti-tumor activity by inducing immunogenic cell death (ICD). ICD is a cell death modality that elicits an adaptive immune response against neo-antigens released from dying tumor cells, effectively converting them into an in situ cancer vaccine.[1] The immunogenic characteristics of ICD are mainly mediated by the release of molecular signals, called damage-associated molecular patterns (DAMPs). Two types of DAMPs have been described: constitutive DAMPs (cDAMPs), which are normally present inside healthy cells and released upon plasma membrane rupture, and inducible DAMPs (iDAMPs), which are synthesized in response to stress or cell death. Key cDAMPs include the "find me" and "eat me" signals such as calreticulin (CALR), adenosine triphosphate (ATP) and high-mobility group box 1 (HMGB1), while iDAMPs mainly comprise NF-kB-induced cytokines, type I interferons and the IL-1 family members. For cell death to be considered immunogenic, both types of DAMPs have to be released in a coordinated manner to successfully activate adaptive immunity.[2] To fully exploit the potential of ECT-induced ICD, it is essential to define the type and temporal dynamics of cell death, as well as the release of DAMPs, for each chemotherapeutic agent individually. To address this, we have generated cell lines deficient in specific cell death pathway genes (Bax and Bak for apoptosis, RIPK3 and MLKL for necroptosis, GSDME and GSDMD for pyroptosis) using the CRISPR-Cas9 system to elucidate their role in cell death after ECT in combination with BLM, OXA or CDDP. Using these cell lines, we aim to determine the type of cell death and their importance for the observed ICD after ECT.

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SYNERGISTIC EFFECT OF DEBIO 1143 AND ELECTROCHEMOTHERAPY WITH CISPLATIN IN MURINE OROPHARYNGEAL SQUAMOUS CELL CARCINOMA CELL LINES

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Head and neck squamous cell carcinoma remains a major health concern worldwide due to its resistance to conventional therapies and challenges associated with tumor accessibility. The incidence of oropharyngeal cancer, in addition to tobacco and alcohol consumption, is linked to infection with human papillomavirus (HPV). The main aim of this study was to evaluate the therapeutic potential of the inhibitor of apoptosis (IAP) antagonist DEBIO 1143 in combination with electrochemotherapy (ECT) using cisplatin (CDDP) or bleomycin (BLM) in HPV-negative and HPV-positive murine oropharyngeal squamous cell carcinoma cell lines (MOC1 and MOC1-HPV). DEBIO 1143 significantly enhanced the cytotoxic effects of electrochemotherapy only in combination with CDDP, indicating a synergistic interaction between the two therapies. When combining ECT with DEBIO 1143, RT-qPCR analysis showed that cIAP (one of the IAPs) expression was upregulated in both cell lines, likely reflecting a compensatory survival response to IAP inhibition. This study suggests that DEBIO 1143 may potentiate the cytotoxic effects of ECT and supports further investigation of the mechanisms underlying their combined action.

Keywords: electrochemotherapy, DEBIO 1143, head and neck cancer, HPV, cisplatin

COMPARISON OF GROWTH CHARACTERISTICS OF *Escherichia coli* STRAINS N4i, MSE259, AND ŽP: THE IMPACT OF PLASMID LOAD

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Plasmids are extrachromosomal, circular DNA molecules whose presence in *Escherichia coli* represents a dynamic balance between metabolic burden and potential adaptive benefits.[1] While replication and expression of plasmid-encoded genes can, in the absence of selective pressure, slow growth and reduce competitive fitness, plasmids may also enhance host performance under specific environmental conditions.[2] This study compared the growth characteristics of three *E. coli* Nissle 1917 derivatives: N4i (plasmid-free), MSE259 (pOX38::Cm), and ŽP (pOX38::Cm carrying the *colE7* gene). Growth curves, specific growth rate (μ), generation time (g), and colony morphology were determined under selective conditions, i. e. growth in medium with antibiotics (N4i, 15 μg/mL gentamicin, Gm; MSE259, 15 μg/mL gentamicin, Gm and chloramphenicol 50 μg/mL, Cm; ŽP 15 μg/mL gentamicin, Gm and chloramphenicol 50 μg/mL, Cm). Results showed that MSE259 achieved the highest μ and shortest g, suggesting that its plasmid does not impose a detectable metabolic burden and may even confer a selective advantage. N4i grew more slowly than expected for a plasmid-free strain, while ŽP displayed reduced growth but formed the largest colonies. These findings challenge the common assumption that plasmids invariably impair growth due to metabolic load, indicating instead that their impact is determined by a combination of genetic content, copy number, gene expression, and integration with host metabolism. Understanding these dependencies is relevant to microbial ecology, where plasmids facilitate horizontal gene transfer, and to biotechnology, including the development of probiotic strains and recombinant protein production. Our findings suggest that some plasmids may be metabolically neutral or even advantageous under conditions where they confer a selective benefit, underscoring the need to re-evaluate the simplistic cost model of plasmid carriage.

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DNA EXTRACTION PROTOCOLS FOR GENOTYPING OF HOP CONES AND PELLETS

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The cultivar of hops (*Humulus lupulus*) strongly affects their economic value and authentication of cones and pellets by means of DNA detection remains challenging due to abundant PCR inhibitors such as polyphenols and bitter acids. To address this, we compared four CTAB-based DNA extraction protocols: (1) CTAB extraction[1], (2) CTAB with PVP40, (3) CTAB with PVP40 and activated charcoal[2], and (4) CTAB with PVP10 and liquid nitrogen homogenisation[3]. In addition, pre-treatment of cones or pellets with hexane was tested to lower bitter acid content prior to DNA isolation. All methods were tested on both hop cones and processed pellets from several varieties with different levels of alpha-acids, beta-acids and essential oils. Isolated DNA was evaluated by spectrophotometry (NanoDrop), fluorometry (Qubit), and agarose gel electrophoresis. Among the first three protocols, no clearly superior method was found, but all gave DNA of sufficient yield and quality for microsatellite genotyping. The fourth method (PVP10 with liquid nitrogen) produced low yields and poor purity, and was not suitable for downstream use. Genotyping by fluorescent microsatellite markers [4] showed consistent allele patterns across the first three protocols. In some cases, pellets produced slightly weaker signals of peaks compared to cones, but there was no systematic connection between extraction method and PCR success. Rare unexpected alleles were detected in some varieties, suggesting either previously unreported alleles or possible contamination, which will require further study. In summary, traditional CTAB-based methods remain reliable for genotyping from hop cones and pellets, but they are slow and require considerable manual work. Based on these results, we are now working on developing a faster DNA extraction protocol for hop cones, with the goal of reducing both time and labour while keeping DNA quality suitable for reliable genotyping.

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Pseudomonas PHYLOGENY: RETHINKING THE COMMON GENETIC BIOMARKERS FOR PHYLOGENETIC ANALYSIS OF PHYTOPATHOGENS OF MULBERRY

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Pseudomonas is a genus of Gram-negative bacteria with members capable of colonizing a wide range of hosts. Phytopathogenic isolates cause a variety of plant diseases. Among virulence-associated genes (VAGs) that play an important role in phytopathogenesis is the *hrpZ* gene encoding a type III effector protein causing plant hypersensitivity response.[1] Identification of bacterial species and pathovars is essential and is based mostly on common genetic biomarkers (16S ribosomal RNA gene and sigma-70 RNA polymerase subunit gene, *rpoD*).[2,3] Gomila et al.[4] discovered that the majority of GenBank deposited genome sequences of phytopathogenic Pseudomonas isolates were misclassified and they proposed a new classification based on 11 nomenspecies.

The aim of our study was to evaluate the ability of two common housekeeping genes, 16S rDNA and *rpoD*, and the VAG *hrpZ*, for correct species and pathovar identification and phylogenetic resolution in order to identify a yet unidentified *Pseudomonas* isolated from a diseased white mulberry tree (*Morus alba* L.). One hundred twenty whole genomes of *Pseudomonas* phytopathogens and 2 outliers, *P. aeruginosa* and *P. lutea*, deposited in the GenBank were used to perform a phylogenetic analysis with MEGA 12 software's MUSCLE algorithm using 16S rDNA, *rpoD* and *hrpZ*. Gene trees were constructed using Maximum likelihood method. Both, 16S rDNA and *rpoD*, resulted in poorly resolved phylogenetic trees that failed to match the whole genome phylogenetic analysis baseline obtained by Gomila et al.[4] and were hence not useful for identifying the yet unidentified *Pseudomonas* pathovar isolated from the diseased mulberry. However, the *hrpZ* gene, which was detected in 83 of the 120 (69%) analysed whole-genome sequences and produced a higher resolution, enabled clustering of our unidentified isolate among *P. amygdali* pv. *mori*. The VAG *hrpZ* was suitable for identifying our *Pseudomonas* pathovar isolated from the diseased mulberry, however it is not suitable for genus-wide phylogeny studies, as it lacks the resolution required for reliable identification for *Pseudomonas* phytopathogens due to its insufficient prevalence and/or probable horizontal transfer. In order to overcome these limitations alternative biomarkers for *Pseudomonas* are still needed.

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ESTABLISHMENT OF ELECTROTRANSFER OF *TRANS*-AMPLIFYING mRNAs IN B16-F10 MOUSE MELANOMA *IN VITRO* AND *IN VIVO*

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Gene therapy of cancer is one of the highly promising approaches to cancer treatment. As vectors, mRNA-based therapeutics could be advantageous over plasmid DNA as vectors due to their higher transfection efficiency and the absence of insertional mutagenesis but have been hindered by intrinsic instability and short-lived protein expression. In our study, we aimed to establish gene electrotransfer (GET) of a bipartite, *trans*-amplifying mRNA system (taRNA) to locally deliver and amplify mRNAs intracellularly (*in vitro*) and intratumorally (*in vivo*, permit number U34401-3/2022/17) and thus enhance and prolong protein expression in a B16-F10 mouse melanoma model to create a new platform for cancer gene therapy.

The taRNA (1) consists of a non-replicating mRNA encoding the alphaviral replicase (nrRNA-REPL) and a second mRNA encoding green fluorescent protein (trRNA-EGFP) or luciferase (trRNA-LUC2), which can be amplified by the replicase. We measured reporter gene expression using flow cytometry after GET of taRNA with trRNA-EGFP on B16-F10 cells and IVIS ® bioluminescence imaging following introtrumoral GET of taRNA with trRNA-LUC2 into B16-F10-tumor-bearing C57BL/6 mice. In both experimental settings, we compared protein expression after GET of several trRNA and nrRNA-REPL concentrations (20 ng to 20 µg) and using 4 distinct electroporation protocols (EP1: 1300 V/cm, 1 Hz, 100 µs, 8 pulses; EP2: 1300 V/cm, 5 kHz, 100 µs, 8 pulses; EP3: 600 V/cm, 1 Hz, 5 ms, 8 pulses; EP4: 120 V/cm, 1 Hz, 20 ms, 2 pulses).

Upon GET of taRNA using EP1, EP2 or EP3 *in vitro,* we detected a population of high GFP$^+$ cells, which was non-existent upon GET of trRNA-EGFP alone. This population was the most notable and reached the highest fluorescence intensity when EP3 was used. In the *in vivo* setting, we confirmed that intratumoral GET is an effective method for delivering naked mRNAs across a wide concentration range. We confirmed successful intratumoral *trans*-amplification, with luciferase expression increasing up to 4-fold upon co-delivery with nrRNA-REPL. Notably, strong amplification was observed at low (tens of ng) doses of the reporter mRNA, whereas increasing the dose resulted in a diminished amplification effect. Both 10 µg and 20 µg of nrRNA-REPL yielded comparable bioluminescence intensities, possibly indicating a saturation effect at higher replicase concentrations. Transgene expression was dependent on both mRNA dosage and electroporation parameters, where EP3 has proven to be the most effective protocol to deliver taRNA *in vivo*.

We have developed a promising mRNA-based platform for cancer gene therapy that enables sustained transgene expression through trans-amplification. Ongoing studies are aimed at further enhancing the platform's transgene expression potential.

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GENETIC AND PHENOTYPIC CHARACTERIZATION OF SOIL BACTERIA WITH ANTIMICROBIAL ACTIVITY AGAINST UROPATHOGENIC *Escherichia coli*

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Uropathogenic *Escherichia coli* (UPEC) is the major cause of urinary tract infections, with multidrug-resistant (MDR) strains posing a major therapeutic challenge.[1] The search for novel antimicrobial compounds is increasingly focusing on soil microorganisms, which are known to produce a variety of bioactive metabolites.[2] The aim of this study was the genetic and phenotypic characterization of bacterial soil isolates possessing antimicrobial activity against uropathogenic *Escherichia coli*. Eleven bacterial soil isolates collected from five sampling sites in the rhizosphere of trees on Maribor Island, Slovenia possessing antimicrobial activity against the uropathogenic *Escherichia coli* strains DL 92 (non-MDR) and DL 100 (MDR) were used.[3] Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit, the16S rRNA gene was amplified by PCR and sequenced. Sequences were analysed using NCBI BLAST and aligned with MEGA software for phylogenetic comparison. All 11 isolates were then subjected to ERIC-PCR to confirm non-clonality. Phenotypic studies included assessment of colony morphology, antibiotic susceptibility testing, and growth assessment at 25 °C, 28 °C, and 37 °C. Sequencing of the 16S rRNA gene revealed nine *Streptomyces* spp., one *Bacillus* sp. and one *Pseudomonas* sp. The ERIC-PCR profiles obtained demonstrated that two *Streptomyces* isolates, No. 24 and No. 26 were clones of the same strain, while all the other *Streptomyces* isolates displayed unique patterns, and thus belonged to different *Streptomyces* strains. Antibiotic susceptibility testing demonstrated that several isolates remained susceptible to multiple clinically relevant antibiotics. Growth profiling revealed that most isolates grew at 25 °C and 28 °C, but not at 37 °C, with only three isolates (*Streptomyces* No. 33 and No. 46 and *Bacillus* No. 22) growing at all temperatures tested. In conclusion, our results confirmed that soil bacteria from the natural environment of Maribor Island comprise genetically diverse isolates with antimicrobial activity against both MDR and non-MDR UPEC. The combined use of genotypic and phenotypic characterization provided a robust framework for the discrimination of unique strains, that may represent valuable candidates for future studies aimed at discovery of novel potential antimicrobial agents.

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