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FROM ACADEMIA TO START UP ATTEMPTS IN THE FIELD OF DIAGNOSTICS

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An academic career innovating in the field of biosensors and diagnostics has led to the usual output in publications (https://orcid.org/0000-0002-9697-3805) and patents, most of which were abandoned by the institution, save those that led to the creation of the start-ups I founded. Some companies closed usually for lack of funding or team complications, some still in operation, but no serious investments nor buyouts. In short no true successes, so far. The lecture will cover the landscape and issues the academic encounters in making the jump into the cut-throat world of business. It is a worthwhile challenge for some, but the academic must be aware: he is on his own with truly little from his own institution save cosmetic help.

CARBAPENEM RESISTANCE IN THE ENVIRONMENT

Jasna Hrenovic

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Bacterial resistance to β-lactam class of antibiotics, carbapenems, is found as intrinsic in environmental autochthonous species. Emergence of bacteria with acquired resistance to carbapenems gains much attention in clinics during the past 20 years. In Croatia, carbapenem resistance of two clinically important bacteria increased from 2008 to 2022 from 10 % to 99 % for *Acinetobacter baumannii* and from 0.3 % to 24 % for *Klebsiella pneumoniae*. During the last decade One Health approach opened the problem of the presence of clinically relevant carbapenem-resistant bacteria in environment. Here, the overview of the presence of these problematic bacteria in natural environment in Croatia is given.

Clinically relevant carbapenem-resistant gram-negative bacteria were recovered from polluted environment influenced by human solid and liquid waste, but not from the pristine environment. Dissemination of carbapenem-resistant bacteria was found via untreated hospital wastewaters, to urban sewage, wastewater treatment plant and Sava River as natural recipient of treated wastewater. Input of untreated urban and hospital wastewater resulted in the presence of carbapenem-resistant bacteria in water and sediment of Krapina River. Carbapenem-resistant bacteria were found in soils at illegal dump sites, swine manure and agricultural soils fertilized with manure.

Clinically relevant bacteria with acquired resistance to carbapenems in the environment are the source of community-acquired and consequently, nosocomial infections. Disinfection of potentially infective waste prior to its discharge in environment is obligatory to avoid a public health risk.

"*FOOTPRINTS*" – SOCIAL MEDIA MEETS KNOWLEDGE DISCOVERY IN A DATA SHARING ENVIRONMENT

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Three trends appear ripe for consolidation and mutual benefit: use of social media in daily life, use of artificial intelligence technology for knowledge discovery in data repositories, and the sharing of raw data to promote advancement in research. The advantages to consolidating these three trends under a single umbrella are significant.

- Reusing raw data for the knowledge discovery potential that is within it promotes growth and development of vital research areas like biotechnology and biomedicine. It further promotes advancement in these areas for diagnostics and remediation and enables more researchers to perform more research without the expense of new, time-consuming and expensive experimental procedures.
- The application of artificial intelligence technology is proving to be transformative across many industries especially in biotechnology and biomedicine. Tools, like AlphaFold that can predict protein folding patterns from amino acid sequences, as well as molecular AI models like Umol promise to expedite drug design and discovery. There are many further tools in use to perform chemical analysis of compounds, sequence strands of RNA and DNA, and to perform enzyme studies. Cancer therapeutics, and visual diagnostics of radiological images are also standout examples.
- Surrounding these tools and data with an easy-to-use social media interface places the knowledge discovery potential of the tools within a framework that is common, easy to use, and familiar. It further enables widespread sharing of useful techniques within groups of users who share similar interests and on a global scale. It also encourages a 24/7/365 conference-like environment where users can turn for help, assistance, or can share their own techniques and tools that have benefitted them.

"*Footprints*" is a platform, which brings these three trends together and holds promise to reinvent how research work might be supplemented and advanced within a social media user supported environment, where data sharing supports knowledge discovery.

HEALTHY GRAPEVINE – FIRST PATENT AT THE FACULTY OF SCIENCE IN ZAGREB

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Grapevine (*Vitis vinifera* L.) is one of the most important fruit species globally, offering both economic and nutritional value to numerous countries, including Croatia. The grapevine hosts about 80 different types of viruses, some of which cause serious economic problems including lower yield, shorter lifespan of vinyards and lower wine quality. Due to uncontrolled propagation of infected planting material in the past decades the majority of Croatia's cultivars are infected with some of the most harmful viruses: grapevine leafroll-associated virus 1 and 3 (GLRaV-1 and GLRaV-3), arabic mosaic virus (ArMV), grapevine fleck virus (GFkV) and grapevine fanleaf virus (GFLV). The most important elimination technique of viruses and other pathogens from infected plants is apical meristem culture. However, apical meristem isolation is very demanding, since the size of the explant must be below 0.5 mm for *V. vinifera* in order to reduce the risk of contact with the vascular system and the virus. In addition, a rather low survival rate of such small explants in tissue culture further decreases the overall success of the method. An alternative method used for virus elimination is somatic embryogenesis. Here, we report the results of a study in which somatic embryogenesis was initiated and standardised for a set of seven indigenous Croatian cultivars. Field-grown donor plants and SE-derived plantlets were analysed for the presence of 6 typical viruses GFLV, ArMV, GLRaV-1 -2, -3 and GFkV. The results showed that viruses GFLV, GLRaV-1, -3 and GFkV present in donor plants were successfully eliminated by the somatic embryogenesis. With this patented procedure it is possible to obtain a large number of healthy individuals from infected plant material in a relatively short time.

CHANGES IN MICROBIAL CONSORTIUM COMPOSITION DURING THE CELL IMMOBILIZATION ON NATURAL CARRIERS

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The aim of this study was to determine which bacterial species from microbial consortium, previously isolated from activated sludge, have a greater affinity for immobilization on different natural carriers. A microbial consortium was isolated and conditioned for bioaugmentation of biogas-producing reactors using *Miscanthus X giganteus* as a substrate, and chosen carriers were natural zeolitized tuff, ZeoSand®, perlite, and crushed corncob. The identification of grown colonies from the bacterial suspension and the colonies obtained after immobilization was achieved through the cultivation on nutrient agar plates and subsequent usage of MALDI-TOF mass spectrometry method. The results indicate that certain bacterial species from the bacterial suspension did not exhibit a strong affinity for specific carriers, whereas *Enterobacter cloacae* demonstrated an ability to be immobilized in largest numbers on each tested carrier. Other dominant species in the consortium were *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter asburiae*, *Leclercia adecarboxylata* and *Exiguobacterium indicum*.

Furthermore, the goal was to determine the number of immobilized bacteria on each natural material. The highest rate of immobilization of microbial consortium was obtained on perlite, followed by ZeoSand®, crushed corncob and natural zeolitized tuff. Due to their porous structure, suitable surface for immobilization, and non-toxicity, all natural materials could be appropriate carriers of the microbial consortium.

NATURAL AND PRIMED HT TOLERANCE AT WHEAT - CAN WE SPEAK OF TRANSGENERATIONAL MEMORY?

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Despite the continuous global interest on the creation of transgenic abiotic stress resistant wheat cultivars, yet there are not approved lines for consumption and trade. This has triggered the need to analyse the molecular basis of natural resistance, as well as to find out possible trans-generational primed stress memory mechanisms. Wheat cultivars produced via selective breeding, and others of foreign origin, in use in Albania are being studied in order to discriminate the ones which display tolerance to environmental stresses (HT, draught, salinity), and to test conditions which may trigger plant's epigenetic memory. For these, morphometric parameters of growth, physiological phenomena (apoptotic cell death through *in vivo* AL-PCD "*corpse morphology*" and fluorescence microscopy), biochemical synthesis (chlorophyll pigments, GSH, carbohydrates) and gene expression regulations (for Rubiso rbcL/S, rubisco activase Rca1β, glutathione transferase GST), are investigated. Rubisco activase, which is the catalytic chaperone regulating the carbon assimilatory pathway in wheat, is reported to modify the catalytic efficiency of *Rubisco* even though the last is produced normally under specific stress conditions, while glutathione transferases are involved in detoxification of endogenous and exogenous substrates having the glutathione as co-enzyme. Based on the group's results, the possibility that the modified response of plants to repeated stress conditions could be considered as epigenetically regulated (primed) stress memory, is also discussed.

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ESTIMATION OF GROWTH PARAMETERS FOR A STOCK OF OHRID TROUT (*SALMO LETNICA* KARAMAN, 1924) BASED ON THE ASSESSMENTS OF COMMERCIAL CATCHES IN THE ALBANIAN PART OF OHRID LAKE

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Ohrid trout (*Salmo letnica*) is a endemic fish species from Ohrid Lake (South-East of Balcan Penisula). In this paper are presents some informations about growth features of this species, based on assessments of the commercial catches in the Albanian part of the Lake. Were assessed the coefficients *a* and *b* in the allometric relationship between total length (TL,cm) and total weight (W,g) (LWR), the allometric condition factor (*K*') and the parameters ($L\infty$,*K*,*t*o and *t*max) in the Von Bertalanffy's growth function (VBGF). The values of coefficients in LWR were: initial growth coefficient *a*=0.0161 and the slope *b*=2.956. The value for allometric condition factor was *K*'=1.624. For the parameters of VBGF were calculatet these values: asymptotic length $L\infty$=53.46 cm, annual growth coefficient *K*=0.192/year, the "age" at length 0 *t*o=-1.41 years and the maximum theoretical age *t*max=15.6 years. The obtained values for LWR coefficients shows almost isometric growth for the assessed stock of Ohrid trout. The walues for VBGF shows a greater similarity with the limnophilic species of the genus Salmo than with the species that live in the rivers.

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ASSESSMENTS ON THE GROWTH PERFORMANCE OF MEDITERRANEAN MUSSEL (*M. GALLOPROVINCIALIS* MOLLUSCA, BIVALVIA) REARED IN BUTRINTI LAKE (SOUTH-WESTERN ALBANIA), ACCORDING TO EVALUATION OF THE PARAMETERS IN VBGF

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In Albania, since the 60s of the last century, the commercial aquaculture of Mediterranean mussel (*M. galloprovincialis*) is located only in Butrinti Lake. In the period between April and December 2023 we have estimated the parameters in the VBGF, the instantaneous rate of natural mortality (*M*), the predicted length at first maturity (*L*m), and some growth performance indices for mussels reared in the panel-structures of aquaculture system. In April the average value of shell length was *SL*=34.91±1.821 mm, while in December this parameter has the value *SL*=63.11±4.273 mm. The maximum value of *SL*, found during sampling in the aquaculture farm was *SL*max=66.9 mm. The average value of specific growth rate index was *SGR*=0.210±0.039. The following values were estimated by as for the parameters in VBGF: asymptotic length $L\infty$ = 76.2 mm; annual growth coefficient *K* = 0.23/yr; theoretical or expected age at length zero *t*o = -0.566 yr; the theoretical lifespan *t*max = 7.04 yr. For the shell length was found this Von Bertalanffy's growth function: *L*t = 76.20 [1- $e^{-0.230\ (t+0.566)}$]. The value of the predicted length at first maturity was *L*m = 45.15 mm. The value of instantaneous rate of natural mortality was *M* = 0.457/yr. The overall growth performance index had the value *OGP* = 5.008, while the value of ϕ'-index, or the growth performance index, was ϕ' = 3.12. The value for theoretical age at which the length achieved 50% of $L\infty$ (*t*50%) was 2.54 yr. This study marks the start of the involvement of stock assessment procedures in Albanian aquaculture.

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IN HOUSE VALIDATION OF AN LC-MS/MS MULTI METHOD FOR THE DETERMINATION OF MYCOTOXINS IN WHEAT

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Mycotoxins are secondary metabolites secreted by many fungal species present in plants during their pre-and post-harvest, transportation, processing and storage, and often found in food and feed. In particular wheat grains are susceptible to contamination with various *Fusarium* mycotoxins such as Deoxynivalenol (DON), Zearalenone (ZEN), T-2 toxins, HT-2 toxin, Fumonisin B1, Fumonisin B2 etc, As their presence can cause disease and death both in humans and livestock, a fundamental step for ensuring public health is the development of highly sensitive, robust, selective, quick, easy, and multi-analyte extraction and detection method. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was the method of choice for the quantification of the main mycotoxins, inclusive of some mycotoxins that are currently regulated acc to EU 2023/915, and emerging mycotoxins such as Enniatins (not included in the previous EU regulation), in plant material (wheat seeds of 20 *Triticum aestivum* L. cultivars in use in Albania). Extraction of mycotoxins was performed using a modified QuEChERS extraction in the presence of the acidified aqueous extraction and organic solvent. Matrix-matched calibration curves were established, and limits of quantification were below the maximum levels (0.5 µg/kg (each of the total Aflatoxins) to 50 µg/kg (DON). According to 2782/2023_EU, LOQ shall be ≤0.5*ML or should preferably be ≤0.2*ML). Recoveries ranged between 70 and 120%, fulfilling the EU legislation (SANTE 11312-2021). Results demonstrated that the procedure was suitable for determining 23 mycotoxins in wheat grains.

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LENGTH FREQUENCY DISTRIBUTION, GROWTH PARAMETERS AND MORTALITY RATES FOR A STOCK OF BLEAK (*ALBURNUS SCORANZA* HECKEL AND KNER, 1857) FROM OHRID LAKE.

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The bleak (*A. scoranza*) is a native fish species within the Ohrid-Drin-Skadar drainage. Aiming the bleak's stock assessment in the Ohrid Lake, we have studied the length-frequency distribution and have estimated some growth parameters from VBGF, the theoretical age at which the total length achieves 50% of $L\infty$ (t50%), and the instantaneous rate of natural mortality (M). The average values for total length and total weight were: TL, cm=11.71±2.45 (Var%=20.92) and W, g=12.87±6.58 (Var%=51.11). The values of growth parameters in VBGF were: the asymptotic length $L\infty$=20.76 cm; the annual growth coefficient K=0.198/yr and the hypothetical age the fish would have had at zero length to=-1.023 yr. The value of t50% was 2.4 yr and the value of natural mortality parameter was M = 0.484/yr. It resulted that in comparison to other studies, carried out for the stocks of *A. scoranza* in the Skadar Lake, the stock of Ohrid Lake is distinguished by smaller size individuals as well as by the lowest values of growth parameters.

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PHYSICAL-BIOLOGICAL INDICATORS AND SINGLE-CELL TOXICITY SENSING AS A NEW MONITORING MODEL APPLIED AT LAKE BUTRINT, ALBANIA

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The assessment of surface and underground water quality in Albania has been carried out for years based on standardized methods for measuring physical-chemical indicators, trophic level (ISO 17025), and bacteriological indicators (ISO16649-3:2015), and lately is implemented the use of cellular biosensors to assess the cytotoxicity of waters (ISO 16649-3:2015).This paper will refer to the results on the quality of waters of Butrint Lake in Albania and the problems encountered, using conventional standardized methods (ISO) and advanced methods of biotechnology (single-cell biosensors, CARD-FISH, fluorescence microscopy, Flow Cytometry, Factorial analysis of bacterial DNA to environmental factors, Genetic diversity of phytoplankton under conditions of pollutants of different categories, etc.).The quality of waters is among the most discussed issues in the context of climate change, and so do the methodologies used to assess it. In this context, we believe that a combined use of monitoring protocols with those of scientific research, can provide more complete and reliable results on water quality and their complex relationship with the geological content of soils, hydrology, climatic conditions and human interactions.

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CRYPHONECTRIA HYPOVIRUS 1 SPREAD ACROSS ITS HOST POPULATION IN EUROPE

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Invasive species are usually a significant stressor for the native species in an ecosystem. When these alien species are parasitic or even pathogenic, invasive species cause direct harm to the native ones, instead of just outcompeting and replacing them. A sac fungus *Cryphonectria parasitica* has been a prime example of an unintentionally introduced phytopathogenic species that has devastated native chestnut forests across North America and Europe since the beginning of the 20th century. It causes a disease called chestnut blight characterised by the appearance of the cankers: wounds and necrotic lesions appear on the bark of the trees and can cause dieback of the affected branches or entire stems. Fortunately, an infectious RNA, later determined to be an unencapsidated virus named *Alphahypovirus cryphonectriae* (CHV1) was discovered in 1960s and shown to have an attenuating effect on its fungal host's virulence, making the blight disease less severe. Several subtypes and many unique genotypes of CHV1 have been detected across Europe with highly variable effects on *C. parasitica*. Subtype F1 (French) usually has a severe effect on the host's physiology (e.g. growth rate) and fertility (sporulation), while the effect caused by the infection with subtype I (Italian) usually has milder symptoms. This is reflected in the population structure of CHV1 in Europe – the much more "severe" F1 subtype has been found thus far only in a few locations in France, despite being often artificially introduced by humans as a means for biological control of the disease. On the other hand, the "milder" I subtype is widespread across many *C. parasitica* populations in Europe, as the virus is often transmitted via the conidia, the production of which is not as strongly affected by the I subtype.

REPORTER BACTERIA STRAINS ENCAPSULATED WITHIN POLY-LYSINE-REINFORCED ALGINATE MICROBEADS AS VERSATILE BIOSENSORS FOR THE DETECTION OF QUORUM SENSING MOLECULES

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Human *Pseudomonas aeruginosa* infection remains a significant public health challenge due to its extensive antibiotic resistance. The pathogenesis of *P. aeruginosa* is closely linked to quorum sensing (QS), which involves the communication of bacteria through signaling molecules called autoinducers. The QS molecules have emerged as important biomarkers for detecting *P. aeruginosa*. To this end, a whole-cell biosensor was developed to detect a broad range of bacterial autoinducer (homoserine lactone, HSL) concentrations. The bioreporter bacteria-based biosensor was developed by immobilizing two genetically modified strains (LasR and RhlR) of *P. aeruginosa* within poly-lysine-reinforced alginate microbeads. When the immobilized reporter bacteria were incubated with bacterial culture or growth media spiked with synthetic autoinducer or quorum sensing inhibitor (QSI, furanone-C-30), HSL and QSI molecules could diffuse into the microbeads, eliciting a dose-dependent biological response. This whole-cell bacteria biosensor offers a direct and quantitative detection of sub-nanomolar concentrations of synthetic C_4-HSL and C_{12}-HSL that are common in the QS signaling molecules of *P. aeruginosa*. Notably, the biosensor is versatile and can detect bacterial QS molecules in real-life samples (such as different stages of biofilms and bacterial cultures) without requiring additional sample preparation steps. The microbeads exhibit high operational and storage stability for over 40 days at 4 °C. Upon 30 minutes of preincubation at 37 °C, the previously stored (-80 or 4 °C) biosensor microbeads are ready to use, making it a robust on-demand biosensor for the detection of *P. aeruginosa* with potential applications in clinical and environmental settings.

ESSENTIAL AND ADDITIONAL STRATEGIES TO ACHIEVE AND MAINTAIN QUALITY IN GENETIC LABORATORIES: INSIGHTS FROM A HIGH-THROUGHPUT DIAGNOSTIC LABORATORY

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The delivery of high-quality laboratory medical services empowers physicians to make confident decisions regarding disease prevention, diagnosis, and treatment. Several factors are crucial for ensuring the effective functioning of a laboratory, as they intersect and influence one another. Based on our experience, any faults in these factors can have negative impact on result quality.

Careful planning and optimal space design are considered to be a foundation of any laboratory. The layout of the space must align with appropriate biohazard standards to protect both personnel and the environment, while also maintaining sample integrity. Moreover, efficient lab design will also limit unnecessary workflow steps, enhancing personnel productivity while minimizing errors. We have implemented three variations of space design, customized to specific lab protocols and expected sample volumes, each of which will be presented in more detail.

Another important aspect affecting quality is the laboratory personnel. Therefore, prioritizing investment in staff training is fundamental. Basic training in laboratory protocols is mandatory, complemented by specialized education tailored to the specific diagnostic procedures and individual team members.

Investing in automation and digitalization can further reduce error rates and enhance quality. Our laboratory management has adopted various levels of automation, balancing cost-effectiveness with rapid sample turnaround times. Examples ranging from manual to fully automated protocols will be showcased.

Molecular diagnostic laboratories require additional precautions to prevent sample cross-contamination, given the abundance of DNA fragments generated during amplification procedures. We will explore approaches that combine different strategies to prevent nucleic acid contamination, along with internally derived protocols for quality monitoring.

To ensure constant delivery of high-quality results over time, every laboratory must assess the necessary investments in space, personnel, automation, and contamination prevention measures.

LISTERIA MONOCYTOGENES DETECTION: FROM PLATE COUNT TO AN ELECTROCHEMICAL BIOSENSOR METHOD

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Listeria monocytogenes is a high-risk food pathogen that can cause infections in weak and immunocompromised individuals. Listeriosis is a foodborne invasive disease, which occurs following ingestion of contaminated food (RTE products, dairy products, meat, raw ham, fish, smoked fish, and vegetables). The currently recommended ISO 11290-1:2017 standard method (horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp.) although sensitive and able to ensure the compliance with microbiological criteria, requires a long time, up to 7 days for identification confirmation and is not quantitative. Molecular methods generally are more cost-effective than conventional culture-based methods, due to the reduced detection time, are more specific, but requires the extraction of DNA from food samples before the utilization of specific primer for application in PCR.

Nowadays, specific, rapid and sensitive detection for pathogens is possible by the utilization of biosensors, devices which combine fundamental biological, chemical, and physical sciences with engineering and informatics to satisfy needs of a wide range of sectors, including food safety. Food industries require rapid protocols that can provide results in short times to avoid recalls with economic losses. Among the various bioreceptors used for target detection biosensors which utilize ssDNA probes are called genosensors and are successfully used in food analyses. Moreover, other ssDNA short sequences of DNA able to fold in a 3D- structure (aptamers) can be used to build aptasensors, biosensors used for the specific detection of proteins, toxins, metals and whole cells.

THE EFFECTS OF MUTATIONS IN THE *antiCas* TRANSCRIPT AND DELETION OF *anti-cas* GENE ON RESISTANCE TO PHAGE INFECTION

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The CRISPR-Cas adaptive immune system protects many bacteria and most archaea from invading DNA. In *E. coli,* the CRISPR-Cas system is silenced by the global repressor H-NS under normal laboratory growth conditions. However, in cells lacking H-NS and containing anti-lambda spacers, CRISPR-Cas-mediated resistance to phage λ*vir* is highly temperature dependent. It is active at 30 °C and inactive at 37 °C. A short anti-cas transcript of 373 nt, which is controlled by the divergently oriented anti-Pcas promoter, is also regulated by the H-NS repressor. When this gene is overexpressed from the plasmid, it inhibits CRISPR-Cas-mediated resistance to phage λ*vir* at 30 °C by an unknown mechanism. In this study we investigated the effects of different mutations in the *antiCas* transcript and deletion of the first 153 nt of the *anti-cas* gene on CRISPR-Cas-mediated resistance to λ*vir* infection. The indel mutations in the *antiCas* transcript had a slightly reduced inhibitory effect on CRISPR-Cas activity. Deletion of the *anti-cas* gene resulted in smaller plaque size and lower plaque forming units (PFU) count at 37 °C, suggesting that *antiCas* transcript contributes in part to reduced CRISPR-Cas activity at elevated temperature.

'*Candidatus* Phytoplasma solani': CHASING FOR PROTEINE INTERACTION

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'*Candidatus* Phytoplasma, formally known as phytoplasmas, are diverse, pleomorphic pathogen bacteria that live inside plant phloem sieve cells and cause various plant diseases. They can infect a wide variety of plants, including many economically important crops around the world. These bacteria rely on plants and insects for their survival and are spread by insects - vectors that feed on plant sap. Despite efforts, phytoplasma *in vitro* cultivation has not been established yet, which makes studying them difficult.

'*Ca.* P. solani', has a large and highly variable genome compared to other phytoplasmas. Understanding how '*Ca.* P. solani' interacts with its hosts on protein level and what are the protein effectors that contribute to its ability to adapt to different environments and hosts, requires application of different biotechnological scientific approaches. Combining different methods such as floral dip, yeast-2-hybrid and agroinfiltration could provide valuable insights into management and assessment the risks associated with this pathogen. By studying its genetic diversity and how it spreads, we can develop better strategies for controlling and preventing its impact on crops.

PHYTOCHEMICAL ADAPTATIONS OF BROCCOLI TO ELEVATED AND DECREASED ENVIRONMENTAL TEMPERATURES: IMPLICATIONS FOR ITS NUTRITIONAL VALUE

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This work aimed to investigate the impact of low (LT) and high (HT) temperatures on the phytochemical composition of broccoli microgreens (*Brassica oleracea* L. convar. *botrytis* (L.) Alef. var. *cymosa* Duch.) and to assess the biological effects of their extracts. Our goal was to identify sensitive phytochemical parameters as potential markers of LT/HT stress and provide insights for optimizing temperature conditions to enhance the concentration of specific compounds and antioxidant effects for producers and consumers of microgreens. We measured the effects of LT and HT on different groups of phenolics, total glucosinolates, proteins, and soluble sugars, photosynthetic pigments, plant hormones, vitamin C, and individual phenolic acids and flavonoids, and on antioxidant capacity of broccoli extracts. The data collected were statistically analyzed using one-way analysis of variance, Pearson's correlations, and principal component analysis to evaluate differences between samples and visualize relationships among parameters. The results showed that LT increased total phenolics and tannins in broccoli. Total glucosinolates were also increased by LT; however, they were decreased by HT. Soluble sugars, known osmoprotectants, were increased by both types of stress, considerably more by HT than LT, suggesting that HT causes a more intense osmotic imbalance. Both temperatures were detrimental for chlorophyll, with HT being more impactful than LT. HT increased hormone indole-3-acetic acid, implying an important role in broccoli's defense. Ferulic and sinapic acid showed a trade-off scheme: HT increased ferulic, while LT increased sinapic acid. We suggest that parameters responsive to one type of temperature stress, but not the other could be potential mediators crucial for plants' adaptation to LT/HT stress. These findings contribute to understanding the physiological responses of broccoli microgreens to temperature stress and could aid in optimizing growing conditions to enhance their phytochemical composition and bioactivity.

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May 28th to June 4th
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POSTER PRESENTATIONS

Preliminary version, will be updated every week

POSTER

COVALENTLY SYNTHESIZED ALGINATE-PYRROLE HYDROGEL AS A 3D PRINTABLE ELECTROCONDUCTIVE INK

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Electrically conductive hydrogels are investigated actively for potential applicability in biosensing, cellular interface, and tissue engineering. Because conventional hydrogels lack electrical conductivity, efforts are being made to incorporate conductive materials into such hydrogels in a facile but stable manner. Hydrogels of alginate composite are particularly attractive because of their tunable viscoelasticity, excellent biocompatibility, and ease of preparation. In this study, the alginate-pyrrole composite was covalently synthesized via EDC/NHS mediated conjugation between the carboxyl group of the alginate and amino group of the synthesized aminopropyl pyrrole monomer, followed by the spectroscopic characterization of the composite using UV-visible, NMR and FTIR spectroscopies. The hydrogels having variable alginate/pyrrole ratio were prepared by physical crosslinking using Ca^{2+} ion, while $FeCl_3$, $(NH4)_2S_2O_{8,}$ and H_2O_2 were evaluated for oxidative chemical synthesis of polypyrrole from the pyrrole monomer. The resulting hydrogels were subjected to rheological assessment, electrical conductivity study, and morphological characterization using a rheometer, 4-point probes, and SEM. Finally, the composite hydrogels were processed and used as 'ink' for extrusion-based 3D printing in optimized chemical composition, partial crosslinking, printing speed, and pressure conditions. The obtained composite hydrogel exhibited excellent electrical conductivity and high printability with optimal extrusion pressure and rheological properties. Thus, the alginate-pyrrole composite has potential applicability in the tissue engineering of excitable cells and in the biofunctionalization of electrodes in developing electrochemical biosensors.

POSTER

UTILIZING ENVIRONMENTAL SAMPLING, BLANKS AND ASSAY CONTROLS FOR RAPID TROUBLESHOOTING IN GENETIC LABORATORIES

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Next-generation sequencing (NGS) laboratories demand stringent cleanliness levels to avoid contamination events. External contaminants can lower enzymatic activity and affect sensitive reactions. Additional sources of nucleic acid, such as foreign material introduced via testing materials or laboratory personnel, pose additional contamination risks. Cross-contamination may occur when DNA fragments from previous sample analyses contaminate subsequent samples.

From January 2019 to January 2024, NGS laboratory at Polyclinic Breyer issued over 75,000 reports for noninvasive prenatal testing (NIPT). Each analysis, conducted in a 96-well plate format, included blank and procedural assay controls (positive and negative). Regular environmental monitoring, involving air and surface sampling every 1.5 months, was implemented. Blank and environmental samples were assessed midway through the protocol, while positive and negative controls were evaluated post-completion of the laboratory procedure and bioinformatic analyses.

Data obtained from control wells facilitated fast troubleshooting in cases where deviations were noticed: values were over recommended limits or outliers when compared to average laboratory values. First example included elevated concentrations of positive control that prompted investigation and subsequent communication with the reagent manufacturer. Second issue appeared with elevated blank values, persistently increasing despite several rounds of cleaning procedures. A couple of isolated instances of unusually high blank values were also detected.

Despite elevated positive control levels, real sample results remained unaffected, permitting continued use of the reagent lot. On the other hand elevated blank values were traced to human DNA contamination in a specific reagent lot, leading to its immediate discontinuation by the manufacturer. Isolated events of high blanks were resolved through thorough cleaning of automated liquid handling machine, preventing further cross-contamination.

Regular monitoring of environmental cleanliness and reagent quality enables prompt and informed decision-making, saving time and mitigating potential expenses. Preparedness, through the development of standardized operating procedures (SOPs) for cleaning and addressing adverse events, significantly enhances response times.

ASSESING HEAT STRESS TOLERANCE OF ARABIDOPSIS SEEDLINGS WITH ALTERED *DMS3* EXPRESSION

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The DEFECTIVE IN MERISTEM SILENCING 3 (DMS3) protein plays a pivotal role in the RNA-directed DNA methylation (RdDM) mechanism. RdDM is essential for maintaining genome stability and regulating gene expression, ensuring adaptation to environmental challenges throughout the plant's life cycle. To investigate the role of DMS3 in the response of *Arabidopsis thaliana* (L.) Heynh. to heat stress, the wild type (wt), a line overexpressing the *DMS3* gene (*oeDMS3*), and a line with a mutated *DMS3* gene (*dms3-1*) were exposed to 37 °C for 6 hours. Photosynthetic efficiency, proline content, and HSP90 protein level were evaluated immediately after the treatment and after 24-hour recovery at optimal temperature. All three lines showed reduced photosynthetic rate immediately after the treatment, with the *dms3-1* line displaying a decline even after recovery. Proline content decreased in all three lines immediately after the treatment and returned to control levels in the wt and *oeDMS3* line. However, the *dms3-1* line showed significantly increased proline content after recovery compared to the corresponding control. HSP90 protein accumulated in treated seedlings of all three lines at both time points, with the *dms3-1* line showing the lowest basal expression under control conditions and the highest accumulation after the treatment. These results suggest that heat stress had a stronger effect on the *dms3-1* line than on the wt and *oeDMS3* line, highlighting the importance of a functional DMS3 protein for heat tolerance at the seedling stage.

POSTER

BPM1 PROTEIN MEDIATES *DE NOVO* DNA METHYLATION DURING EMBRYOGENESIS OF *ARABIDOPSIS THALIANA*

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The Arabidopsis BPM1 protein is a member of the widespread MATH-BTB protein family. MATH-BTB proteins participate in numerous plant developmental processes, including stress responses and embryogenesis. In addition to the important role of some MATH-BTB proteins in ubiquitin-dependent proteasomal degradation of specific proteins involved in flowering, seed development, embryogenesis and abiotic stress response, BPM1 establishes ubiquitin-independent interactions with the important components of the RdDM mechanism, the RDM1 and DMS3 proteins, suggesting its possible role in de novo DNA methylation. RNA-directed DNA methylation (RdDM) is one of the key mechanisms for epigenetic reprogramming during the onset of plant embryogenesis, regardless of whether embryogenesis is induced by fertilization of the egg cell (zygotic embryogenesis) or by fertilization-independent stimulation of the somatic cell (somatic embryogenesis, SE). In this work, we have shown that the potential for SE is mediated by the presence of BPM1 and DMS3. Furthermore, common binding regions of BPM1 and DMS3 in the Arabidopsis genome were identified by chromatin immunoprecipitation. Of the identified target genes, FBW2 and RKP were selected for further analysis, while CML41, a gene known to be regulated by RdDM, was selected as a control gene. DNA methylation profile and gene expression were analyzed in zygotic and somatic embryos of lines overexpressing BPM1 (oeBPM1) or DMS3 (oeDMS3), in line with downregulated BPM1, 4, 5 and 6 (amiR-bpm) and in line with impaired RdDM function (*dms3-1*). The results suggested a stimulatory role of the BPM1 protein in RdDM, and the mechanism was more pronounced in zygotic embryogenesis than in somatic embryogenesis.