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Table of contents

INVITED LECTURES

Recent advances in the treatment of oral *Candida* infections

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Fungi are commensal organisms in the oral microbiome, and whether strains transform from commensal into a pathogen usually depends on changes in the host's immune system. *Candida albicans* remains the most frequently identified *Candida* spp. in these disorders, but other non-*C. albicans Candida* species (NCACs) are also on the rise. Photodynamic inactivation (PDI) using non-toxic dyes and harmless light offers a potential solution to a major drawback associated with conventional topically applied antifungals [1]. In our study, the antifungal susceptibility profile of oral samples (n=21) collected in a Slovakian hospital was determined using the broth microdilution method (EUCAST) and E-test® [2]. The total biomass of biofilms and viability were measured using crystal violet and the XTT reduction method. Consequently, the biofilms of FLC-resistant oral isolates´ were tested with methylene blue (MB) or phloxine B (PhB) by counting the colony forming units (CFU) after 24 hours of cultivation. Of all samples collected, *C. albicans* was the most abundant species (n = 13) and 6 strains were resistant to FLC: *Candida krusei* (n=3), *Candia intermedia* (n=1) and *Candida valida* (n=2). *Galleria mellonella* survival test showed no critical differences in virulence even for the highest biofilm former (*C. krusei*). Biofilms with MB or PhB (15 min pre-incubation in the dark) were irradiated for 120 s by laser. The application of PDI to sessile biofilm cells resulted in a significant reduction of all strains. Microscopy using staining with calcofluor white and propidium iodide confirmed efficacy of PDI and therefore PDI represents valuable therapeutic approach.

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[2] Černáková, L.; Líšková, A.; Lengyelová, L.; Rodrigues, C.F. Prevalence and Antifungal Susceptibility Profile of Oral *Candida* spp. Isolates from a Hospital in Slovakia. *Medicina (Kaunas).* 2022, 58(5):576. doi: 10.3390/medicina58050576.

This abstract was supported by the Slovak Research and Development Agency under contracts of no. APVV-21-0302; The Ministry of Education, Research, Development and Youth of the Slovak Republic under the contract no. VEGA 1/0240/23.

Carriers of multidrug-resistant bacteria in the hospital environment

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In 2019, more than 1.2 million people died from infections caused by resistant bacteria. If fundamental steps are not taken, up to 10 million people will die annually due to antimicrobial resistance (AMR) in 2050. The increasing possibilities of medicine, especially in the field of intensive care, are associated with a higher probability of complications such as bacterial infections.

Currently, the extent of the spread of multidrug-resistant microorganisms (MDR) is a global problem. The prevalence of antibiotic resistance reduces the effectiveness of treatment, leads to the emergence of difficult-to-treat infections and increased mortality.

Eradication of MDR bacterial carriage remains an open question.

The development of new therapeutic solutions, including alternative ones, and the improvement of existing ones, which will reduce the use of antimicrobial drugs and the spread of antimicrobial resistance, is very important. Microbial autovaccines and the use of phage therapy are among the known alternative methods used for the treatment of chronic recurrent diseases. Despite extensive experience with the use of autovaccines and phage therapy, the mechanisms of their action are not sufficiently studied, there are no standardized methods, which limits their widespread use. Using the possibilities of modern immunology and microbiology will allow us to deepen our knowledge of the immune mechanisms of action of autovaccines and phage therapy and will modernize the method of production of autovaccines and phage therapy with the aim of increasing their safety, which will also have a positive impact on reducing the number of MDR bacterial carriers in the hospital environment.

This abstract has been supported by APVV-23-0546 and the EU Next Generation EU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V01-00109

Sequential activation of adaptive mechanisms in filamentous fungi under azole-induced stress

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Antifungal compounds, used to treat fungal infections, trigger detoxification and adaptive response in fungal cells. Based on our observations, the activation of the adaptive response requires the sequential engagement of conserved signalling pathways. These were in the focus of our study, specifically in the filamentous fungus *Neurospora crassa* exposed to azoles. The research focused on the role of conserved signaling pathways, including TOR (target of rapamycin) pathway, calcineurin pathway, HOG (high osmolarity of glycerol) kinase pathway, and UPR (unfolded protein response), as well as the contribution of reactive oxygen species (ROS) to stress response.

Transcriptomic analysis revealed that azole exposure specifically activates the HOG pathway, increasing intracellular glycerol levels. This, in turn, upregulates antioxidant enzymes like catalase and peroxidase, reducing ROS levels. This suggests the dual role of ROS: as damaging agents and signaling molecules inducing compensatory responses.

Increased glycerol likely causes membrane tension, activating the TORC complex. This complex, along with elevated ROS, activates sphingolipid biosynthesis. Azoles inhibit ergosterol synthesis, disrupting membrane lipid rafts. If treated with azoles, *N. crassa* replaces ergosterol with eburicol. Simultaneously with increased sphingolipid content, all these lipids from now stabilize the lipid rafts.

Similar to sphingolipid activation, the transcription factor SREBP-SAH2, crucial for ergosterol biosynthesis, is activated. This activation involves proteolytic cleavage, dependent on *hac1*, a UPR marker. These mechanisms contribute to signal transduction, ultimately facilitating development of resistance. The findings highlight the complex interplay of signaling pathways and lipid metabolism in fungal adaptation to antifungal stress.

This work was supported by the research projects APVV-19-0094 and VEGA 1/0388/22

ADVANCES IN ANTI-DOPING SCIENCE – FROM MOLECULAR DETECTION TO GENE DOPING

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The evolution of anti-doping science has been driven by advances in analytical chemistry, molecular biology, and forensic toxicology, enabling increasingly precise detection of performance-enhancing substances and methods. This work explores the progression of doping detection, from early analytical techniques to emerging challenges in gene doping and contamination-based violations.

Key developments include differentiating exogenous from endogenous substances using GC-MS and IRMS, advancements in blood doping detection through the Athlete Biological Passport, and the growing concern of gene doping, which involves genetic modifications such as erythropoietin expression, myostatin inhibition, and mitochondrial manipulation. Unlike traditional doping, gene doping alters endogenous pathways, making detection significantly more complex.

Beyond deliberate doping, contamination-based violations have become a growing concern, with prohibited substances detected in supplements, pharmaceuticals, and even meat due to veterinary use. Ultra-sensitive analytical techniques have led to trace-level detections, raising ethical questions about fairness in anti-doping sanctions.

The introduction of Dried Blood Spot (DBS) testing offers new possibilities for minimally invasive sample collection, but also presents challenges in standardization and detection limits.

While modern anti-doping methods have reached nanogram-level sensitivity, the scientific, ethical, and regulatory complexities of gene doping, environmental contamination, and biological variability continue to shape the future of anti-doping science. This study examines the balance between scientific rigor, detection capabilities, and fairness in elite sports.

ORAL PRESENTATIONS

Photoactive Nanocomposites: A Promising Approach to Combat MRSA Biofilms

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for biofilm-associated infections connected to the increased morbidity and mortality. The challenge today is to develop modified materials with anti-biofilm properties for medical applications. The aim of the study was to test the effectiveness of photoactive nanocomposites (NC) consist of a layer containing organoclay (OC) functionalized with the phloxine B (PhB) and investigate changes in the expression of genes *sodA, sodM, katA, ahpC*, encoded the enzymes responsible for oxidative stress response.

The standard strain of *S. aureus* CCM3953 (CCM) and two MRSA isolates, namely S61 and L12, were used. NC were prepared from polyurethane (PU) with the layer of OC based on saponite and polydiallydimethylammonium cations (1.5 mmol/g^{-1}) functionalized with different concentrations of PhB (0.05–0.4 mM). The part of the samples with PhB was irradiated with a green laser (λ = 532 nm, 100 mW, duration 120 s). The effectiveness of NC was tested on 24-h biofilms calculated by CFU/mL. The production of reactive oxygen species (ROS) was evaluated by ROS-Glo^{TM} H_2O_2 assay. The expression of genes on the NC was assessed by RT-qPCR. Expression was calculated by the $2^{\triangle\triangle}CT$ method.

The results showed that the effectiveness of the NC depended on the concentration of PhB. The effectiveness of NC with 0.44 mM PhB on MRSA S61 and L12 strains achieved over 2.3 $\log_{10}$ inhibition. The results confirmed significant increase of ROS after irradiation (over 14-fold). RT-qPCR showed only slightly increased expression of the *sodA* gene in CCM and S61 on NC with PhB and after irradiation.

The results confirmed that the primary mechanism of action of the tested NC is the rapid production of ROS. The reaction proceeds so quickly that bacterial genes do not have sufficient time to respond, making this approach highly effective for eradication.

This work was supported by the Slovak Research and Development Agency under contracts of no. APVV-21-0302, APVV-22-0150, Ministry of Education, Science and Research of the Slovak Republic under contract no. VEGA 1/0240/23, GUKE UK/3101/2024, and APD0134. EU Next Generation EU though the Recovery and Resilience Plan for Slovakia under project no. 09I01-03-V04-00022 „Component 9" from the Government Office of the Slovak Republic.

Photodynamic inactivation of *Staphylococcus aureus* by hybrid nanocomposite functionalized with erythrosine B: anti-biofilm effects

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Xanthene dyes of erythrosine B (EryB) and eosin Y (EosY) are potent photosensitizers that has attracted attention for its promising antibacterial properties against *Staphylococcus aureus* and their biofilms. The aim was to analyze the photoactive properties of EryB and EosY and to demonstrate the effectiveness of polyurethane (PU) - based antimicrobial materials with a nanocomposite film based on saponite modified with poly(diallyldimethylammonium) cations and functionalized with EryB. Additionally, a potential impact of the Agr (Accessory Gene Regulatory System) quorum-sensing (QS) system participating in biofilm formation of bacteria was tested.

Preliminary experiments were performed only with the standard strain *S. aureus* CCM 3953 in the Mueller-Hinton broth. Different concentrations of EryB and EosY were tested on planktonic cells. To compare samples, they were irradiated with a green laser (λ = 532 nm, 100 mW) and green LED light (2.42 mW) for 2 or 10 min and for 1.5 h, respectively. The results were evaluated by calculating the colony-forming units (CFU/mL). Then, experiments were conducted with a nanocomposite containing the photoactive molecules EryB, which shoved a higher inhibitory effect compared to EosY.

The Agr system was analyzed using Agr CAMP test and by qRT-PCR, employing the *agr* δ-hemolysin gene, *hld*, encoded by RNA III on a standard strain of *S. aureus* and methicillin-resistant MRSA isolates of S73 and S68.

PU modified with EryB -functionalized hybrid film achieved approximately 1000-fold to10,000-fold reduction in biofilm growth of the standard strain of *S. aureus* and the resistant strains of *S. aureus* S73 and S68 after irradiation with both light sources. Preliminary results showed that while activation of Agr system manifested correlation with biofilm formation, there were shown no impact on PDI.

The developed nanocomposite offers a promising direction for future research, particularly in healthcare, due to the potential of modified polymers with specific surface properties for various applications.

This work was supported by the EU's Next Generation EU package through the Recovery and Resilience Plan for Slovakia under project no. 09I03-03-V01-00105 from the Government Office of the Slovak Republic and the Slovak Research and Development Agency under contracts of APVV-21-0302, APVV-22-0150, and APD0134.

Layered silicate/polymer nanocomposites as carriers of antimicrobial substances

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Polymer nanocomposites represent a wide range of materials that are used in various fields. Much attention has been paid to composites with modified layered silicates, which alter the properties of the polymer matrix but can also serve as carriers for various molecular substances. In recent years, there has been increasing interest in composites containing molecules or groups with antimicrobial activity. Their contact effect or the gradual release of active molecules can influence the survival and growth of microorganisms. Our research has mainly focused on composites containing photosensitizers. Under the influence of visible light, these compounds generate reactive oxygen species that lead to an antimicrobial effect on material surfaces. A sufficient concentration of the active molecules on the surface and their gradual release are crucial to achieve an anti-biofilm effect. Among the different photosensitizers, we have mainly tested methylene blue and halogenated fluorescein derivatives, in particular phloxine B. Ongoing studies are aimed at the properties of novel photosensitizers in such complex systems, for example derivatives of porphyrins and benzothiazoles. An important aspect of the project is also the modification of the photophysical properties of dyes on the silicate surface, in particular their increased photoactivity. In terms of polymer systems, we have been working on poly(urethane) and poly(caprolactone) and have recently started a detailed study of modified hydrogel surfaces. The various stages of development of these complex materials are being addressed in research projects. The progress of the projects will be discussed.

The work was supported by the Slovak Research and Development Agency under contract No. APVV-22-0150 and APVV-21-0302 and the VEGA grant agency, project 1/0484/24.

Towards Nanotechnologies Fighting Resistant Microbial Biofilms

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This abstract summarizes the most important issues aimed on study antimicrobial/antibiofilm properties of bioactive molecules and new nanocomposites based on clay mineral with functionalized photoactive dye. The research will continue in testing antimicrobial nanomaterials and optimizing them to achieve maximal antimicrobial efficacy while minimizing toxicity. This involves parallel studies on available *in vitro*, *ex vivo* and *in vivo* models. In addition to evaluating the antimicrobial effects of various nanomaterials, the research will delve into molecular analyses of genes crucial for biofilm formation. This includes modelling biofilms compose of different microorganisms and their combinations on nanomaterial surfaces. The study of quorum sensing (QS) systems will also progress, particularly their role in forming mixed biofilms on novel materials. The *Galleria mellonella* model will be extensively used to assess the biocompatibility of new nanocomposites and the efficacy of bioactive/photoactive molecules in treating infections caused by the specified microorganisms. This includes exploring treatments for systemic infections and burn wounds using newly designed hydrogels containing photoactive substances. These experiments will be complemented by molecular analyses focusing on the expression of genes encoding key antimicrobial peptides in *G. mellonella*, as well as genes related to biofilm formation and QS systems in microorganisms. Furthermore, the research will investigate efflux transporters, especially those in the multidrug resistant bacteria and yeasts, examining their relationship to the antimicrobial efficacy of nanomaterials. Understanding how nanomaterials interact with these efflux systems could lead to strategies that enhance antimicrobial activity against resistant strains. Overall, this comprehensive approach aims to develop and optimize nanomaterials with potent antimicrobial properties and minimal toxicity, providing effective solutions against resistant microbial infections.

This work was supported by the Slovak Research and Development Agency under contracts of no. APVV-21-0302, APVV-22-0150, Ministry of Education, Science and Research of the Slovak Republic under contract no. VEGA 1/0240/23.

Evaluation of the effect of photodynamic inactivation in the combination with phloxine B against dual biofilms of *Candida albicans-Staphylococcus aureus*

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Candida albicans and *Staphylococcus aureus* are opportunistic pathogens commonly found in the human body. However, if they form biofilms, they can cause life-threatening infections. Despite efforts and advances in antimicrobial therapy, resistance phenomenon has become a global problem. It is therefore important to look for a new ways – other approaches of treating these microbial infections. Photodynamic inactivation (PDI) is one of these methods.

The effectiveness of phloxin B (PhB) were previously tested on 24-h biofilms formed by yeast cells of standard strain *C. albicans* SC 5314 and the clinical isolate *C. albicans* CCY 29-3-164, also on 48-h mixed biofilms formed by standard strains of *C. albicans* SC 5314 and *S. aureus* CCM 3953 using calculation of colony forming units/mL.

In this study were tested different concentrations of PhB (0.5; 0.1 and 0.05 mM) with 2-h of incubation iradiated with a green laser (65 mW/cm^2, λ = 532 nm) for 5 min. PDI in the presence of 0.5 mM PhB significantly reduced cell survival in 24-h single yeast biofilms with 44.9% and 26.22% of survived biofilm cells of *C. albicans* SC 5314 and CCY 29-3-164, respectively. In the case of dual biofilm of *C. albicans-S. aureus*, there were survived 18.87% and 0.64% cells, respectively, compared to the control samples. These results were considered in designing new nanocomposites based on polyurethane modified with organoclay (ODTMA/Saponite=0.8 mmol/g) and with functionalised PhB (n_{PhB}/m_{Sap}=0.21 mmol/g). The effectiveness of the nanocomposite was tested against a 24-h mixed biofilm with growth reduction of both *C. albicans* and *S. aureus*. Scanning electron microscopy confirmed a low density of the irradiated mixed biofilm.

Overall, PDI can be seen as a method with great potential for future applications.

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Analysis and photodynamic inactivation of *Klebsiella pneumoniae* biofilm *in vitro* and *in vivo* using *G. mellonella* model.

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Prevalence of CPE producing *K. pneumoniae* bloodstream isolates has increased during recently among hospitals in Slovakia. Resistance is associated with ability of clinical isolates to form biofilm. One of the alternative approaches to combat biofilms could be photodynamic inactivation (PDI). The main aim was to test ability of biofilm development of *K. pneumoniae* clinical isolates with confirmed CPE production and to determinate presence and regulation of the virulence genes *fimH, mrkD, entB* in single or mixed biofilm of *K. pneumoniae* and *Candida albicans* and *Pseudomonas aeruginosa* respectively. Another part of study was to set up PDI therapy against selected biofilms of resistant isolates *in vitro* and *in vivo* using *Galleria mellonella* model. More than 50% of all *K. pneumoniae* isolates were confirmed as strong biofilm producers with higher metabolic activity. Additionally, PCR confirmed presence of *fimH, mrkD, entB* genes in all tested clinical isolates and qPCR confirmed higher expression of these genes in strong biofilm producers comparing to weak producers. Regulation of gene expression was different in mixed biofilms as well. The effectiveness of PDI was tested on 24h biofilms of selected *K. pneumoniae* isolates. Three concentrations (0.5;0.25;0.125 mM) of methylene blue (MB) as a photosensitizer were tested. The PDI was the most effective with concentration of 0.125 mM MB after 1 min of irradiation with red laser (2-log reduction). Our results provide vulnerable information about correlation of increasing CPE resistance and production of biofilm of *K. pneumoniae* in Slovakia and point to a high potential of PDI against resistant biofilms as well.

This work was supported by the Slovak Research and Development Agency under contracts of no. APVV-21-0302; Ministry of Education, Science, Research and Sport of the Slovak Republic under the contract no. VEGA 1/0240/23 and by EU Next Generation EU though the Recovery and Resilience Plan for Slovakia under project no. 09I01-03-V04-00022 „Component 9" from the Government Office of the Slovak Republic.

Antibiotic resistance and virulence factors of multidrug-resistant staphylococci isolated from dog skin

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Staphylococci are pathogens causing skin, respiratory, and systemic infections in humans and animals. Their resistance to antibiotics is increasing, resulting in complicated treatment. In this study, totally 108 samples taken from dogs´ skin were analyzed, of which 66 strains of *Staphylococcus* spp. were isolated. By antibiotic susceptibility testing methods, 17 isolates were identified as multiresistant: *S. equorum* (2), *S. felis* (2), *S. nepalensis* (1), and *S. pseudintermedius* (12). The highest proportion of resistant strains as a result of the disk diffusion method was observed to doxycycline (100%), clindamycin (71.5%), and amoxicillin/clavulanate (57%), and by the MIC method to ampicillin (93.5%), erythromycin (86.5%) and chloramphenicol (80%). Resistance to oxacillin was confirmed in 26.5% of isolates. Methicillin resistance caused by the expression of *mecA* was detected in 2 isolates of *S. equorum*, while the homologous gene to *mecC* was not confirmed. Selected virulence factors were also determined – lecithinase was produced by 50% of *S. felis* isolates and 8.5% of *S. pseudintermedius* isolates; DNase was produced by all of *S. felis*, *S. nepalensis* and *S. pseudintermedius* isolates; strong production of efflux pumps was recorded in all of *S. felis* and 61.5% of isolates of *S. pseudintermedius*; all isolates were strong biofilm producers except of 50% of *S. felis* and 8.5% of *S. pseudintermedius*, which were weak biofilm producers. The highest prevalence of multidrug-resistant isolates was recorded during summer months. The results indicate a significant representation of multidrug-resistant staphylococci in skin infections of dogs and emphasize the need to monitor their occurrence and resistance.

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Effect of clove and thyme essential oils on growth inhibition and biofilm formation of *Arcobacter* spp.

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Bacteria of the genus *Arcobacter* are Gram-negative bacteria. This genus includes species that occur in a wide range of environments. The biofilm formation ability of these bacteria results in higher resistance to antimicrobial substances.

This study examines the antibacterial effect of essential oils of clove and thyme on the inhibition of *Arcobacter* growth and biofilm formation. The antimicrobial effect of these oils was tested using the volatilization method. The effect of essential oils on growth parameters was monitored using the RTS-8 bioreactor. Furthermore, the influence of oils on biofilm formation significant strains of *Arcobacter* spp. was investigated.

The results showed that both essential oils exhibited antimicrobial activity. No inhibitory effect of thyme and clove EOs at the highest concentration tested (1024 µL/mL) was observed. The testing of oil samples using the volatilization method revealed that the determined antimicrobial activity of thyme and clove oil ranged from 32–1024 µg/ml in the liquid phase and 512–1024 µg/ml in the vapor phase. The results of the antimicrobial effect are also supported by similar conclusions from monitoring growth curves using the RTS bioreactor. Inhibition zones of 37.3–47.5 mm and 25.0–38.0 mm were found for Arcobacter strains for the thyme and clove EOs, respectively. A reduction in biofilm formation was generally observed at higher concentrations (64–1024 µg/mL). In contrast, lower concentrations (1–32 µg/mL) supported biofilm formation.

Overall, the results support the use of clove and thyme essential oils as potential antimicrobial agents against bacteria with pathogenic potential.

This abstract was supported by the Faculty of Chemical Technology, University of Pardubice SGS_2025_004.

Identification and characterization of novel type of bacteriocin in veterinary strains of *Escherichia coli*

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Bacteriocins are antimicrobial substances produced by bacteria. *Escherichia coli* strains produce antimicrobial proteins (colicins) and peptides (microcins) that inhibit the growth of related bacteria [1, 2]. *Escherichia coli* D344 strain isolated from dromedary (*Camelus dromedarius*) was producing inhibitory zones, even though no known bacteriocin was detected. We found that the D344 strain harbours a small bacteriocinogenic plasmid (4,980 bp). The bacteriocin operon consists of three genes responsible for its activity, immunity, and lysis. The activity gene encodes a protein of 408 amino acids (43.51 kDa). The immunity gene encodes a protein of 117 amino acids (13.89 kDa) and is transcribed in the opposite direction, which is typical for pore-forming colicins [2]. The lytic protein gene was identified downstream from the structural and immunity genes, encoding a protein of 46 amino acids (4.83 kDa). The sequence of the new colicin is unique to other known colicins. The N-terminal sequence of 235 amino acids displays 78% similarity to the N-terminus of colicin K, while its C-terminus displays similarity to pore-forming colicin F_Y (46%). In addition to predicted pore-forming activity, translocation mechanism of the new colicin was revealed experimentally. The new colicin binds to sensitive cells via interaction with outer membrane receptor OmpA and translocator OmpF. Cytotoxic activity is then facilitated via cascade of interactions with the Tol-proteins (TolA, -B, -Q, -R). Taken together, a novel colicin type has been identified and partially characterized. It has been designated colicin C_D.

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Practical application of propidium monoazide-qPCR for determination of viable cells of inter-species biofilm of *Candida albicans-Staphylococcus aureus*

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Determining the number of viable cells by calculating colony-forming units is time-consuming. The evaluation of mixed biofilms consisting of different species is particularly problematic. Therefore, the aim of this study was to optimize a molecular method-propidium monoazide quantitative polymerase chain reaction (PMA–qPCR)-for accurate and consistent differentiation between living and dead cells. In the practical experimental example, the number of genome copies representing living cells was determined in a mixed biofilm of *Candida albicans–Staphylococcus aureus* inhibited by photodynamic inactivation. Optimal conditions such as PMA concentration and the duration of light exposure, the optimization of DNA isolation from the mixed biofilm and standardization of PMA–qPCR parameters were tested prior to the main experiment. The genome copy number was calculated based on the known amount of genomic DNA in the qPCR and the genome size of the respective microorganism. The results showed that photodynamic inactivation in the presence of 1mM methylene blue decreased the total genome copy number from 1.65×10^8 to 3.19×10^7, and from 4.39×10^7 to 1.91×10^7 for *S. aureus* and *C. albicans* ($P<0.01$), respectively. The main disadvantage is the overestimation of the number of living cells represented by genome copy numbers. Such cells are unable to reproduce and grow (no vitality) and are continuously dying. On the other hand, PMA–qPCR determines the copy numbers of all microbial species, including a mix of eukaryotic yeasts and prokaryotic bacteria in a biofilm in one step, which is a great advantage.

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The impact of water activity on the growth and enterotoxin production in food isolates of *Staphylococcus aureus*

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Staphylococcal enterotoxins (SE), predominantly produced by *Staphylococcus aureus*, are a major cause of foodborne intoxications. SE production is influenced by several factors, including SE type, strain characteristics, and environmental conditions. Understanding the impact of stress factors (e.g., *aw*, pH, temperature) on *S. aureus* growth and SE production is crucial for food safety.

We evaluated the effect of NaCl at varying water activity (*aw*) levels (aw = 1.00; 0.99; 0.97; 0.95) on bacterial growth and SE production in three enterotoxigenic *S. aureus* isolates from food. Growth rates were strain-dependent, despite an extended lag phase, salt addition enhanced bacterial proliferation, reflecting the halotolerant nature of *S. aureus*.To improve SE detection, we focus on implementing mass spectrometry as a cost-effective alternative to the ELISA method for SE detection. This approach could be implemented as part of routine analytical procedures in laboratory practice. Thus far, we have detected one of the five most prevalent SEs in food, and further optimization is required to expand the detection spectrum. Additionally, we tested the virulence of isolates *in vivo* using the *Galleria mellonella* model, identifying the lowest LD50 in the SEE-producing strain between days 1 and 2 after inoculation.

Knowledge in the field of food safety regarding the presence of *S. aureus* in food continues to progress, however, further investigation into gene regulation mechanisms controlling SE production is essential. Expanding knowledge of *S. aureus* growth and enterotoxin production is critical for optimizing food processing strategies to reduce contamination risks and enhance food safety.

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The impact of dysbiotic microbiota obtained from patient with ulcerative colitis on the pathogenesis of uc by utilizing animal model

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The research aimed to determine whether the microbiota from a UC patient alone is sufficient to induce disease in animals, or whether concomitant damage to the mucosal wall is a prerequisite. In addition, the study seeks to compare the pathogenesis of disease development between chemical induction using dextran sulfate sodium (DSS) and induction through transplantation of fecal microbiota from a patient diagnosed with UC. Due to the individual intake of DSS, we divided the animals of the DSS and DSS-UC groups into mild (DSS/Mi, DSS-UC/Mi), based on their developing individual clinical picture of acute ulcerative colitis, including rectal bleeding and loss of total weight. moderate (DSS/Mo, DSS-UC/Mo) and severe forms (activity) (DSS/S, DSS-UC/S) of the disease. The observed pathology of histological architecture of *tunica mucosa* and *tela submucosa* of the colon, manifested as degeneration of epithelium, loss of mucin, infiltration of neutrophils in *lamina propria* and *submucosa* and cryptitis in the intestine, corresponded to histological picture of induced acute UC in both models (DSS, DSS-UC). Dysbiotic composition of faecal microbiota in our patient with UC showed similarity in trends of increases and decreases in taxonomic representatives of the caecal microbiota associated with UC. Based on our partial results, we can argue that the animal model with combined UC induction appears to be optimal due to its similarity to the histopathology of the intestinal mucosa with UC and the dysbiotic microbiota in UC patient.

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Monitoring the adverse effects of selected pharmaceutical substances in the context of viral infections

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Adverse drug reactions (ADRs) include all unexpected and undesirable responses caused by pharmaceutical substances. They are classified into type A (dose-dependent and predictable, accounting for up to 90% of cases) and type B (dose-independent and unpredictable, accounting for 10% of cases), which includes drug hypersensitivity reactions (DHRs). Various *in vitro* methods are used to detect molecular changes in activated T-lymphocytes for diagnosing drug allergies. In phase 1, T-cell subpopulations (Th, Tc) and antigen-presenting cells (APCs) from peripheral blood can be isolated using immunomagnetic cell separation to minimize interference from other cells. In phase 2, flow cytometry is performed, enabling the identification and quantification of cell populations through monoclonal antibody labeling. This technique provides rapid processing and simultaneous assessment of multiple cellular markers. The early T-lymphocyte activation marker (CD69) plays a crucial role in immune response regulation, cytokine production, and lymphocyte migration. In addition, intercellular adhesion molecule 1 (ICAM-1), also known as CD54, is an immune response marker that regulates adhesion, migration, and activation of immune cells. Monitoring its expression is essential in studying inflammation, infections, and cancer. This research aims to develop a suitable *in vitro* model using various cell lines (THP-1, K562, Caco-2, HUVEC) for monitoring the impact of pharmaceutical substances (antibiotics, contrast agents, nonsteroidal anti-inflammatory drugs, mitogens, and others) and their adverse effects in the context of viral infection (HSV-1, HSV-2, CMV, EBV). This study will provide insights into the interactions between viruses, pharmaceuticals, and human cells in vitro.

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Antimicrobial evaluation of newly synthesized silver-glycine complexes

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The search for alternative antimicrobial agents has intensified due to the increasing prevalence of multidrug-resistant bacteria. Silver has long been recognized for its potent antimicrobial properties, while glycine, as the simplest amino acid, offers several advantages for metal complexation. Glycine not only enhances the solubility and stability of metal complexes but may also play a key role in their transport into bacterial cells, facilitating the silver interaction with intracellular targets. By combining silver with glycine-based ligands, we aimed to develop novel compounds with improved antibacterial efficacy and potential biological compatibility.

In this study, we evaluated the antibacterial activity of newly synthesized silver-glycine complexes (Ag-Gly, Ag-Gly-Gly, Ag-Gly-Gly-Gly) and methylated glycinesilver complexes (AgSar – N-methylglycine, AgDMG – N,N-dimethylglycine, AgBet – N,N,N-trimethylglycine). Their antimicrobial effects were tested against the model gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacterium *Escherichia coli*. Among the glycine-based complexes, Ag-Gly exhibited the highest antibacterial activity, likely due to its smaller molecular size and potential for enhanced cellular uptake. The methylated complexes showed comparable antimicrobial efficacy. None of the tesed complexes has exhibited mutagenic activity evaluated with the Ames test.

To elucidate the mechanism of action, we investigated the effect of metioned complexe on bacterial physiology. Our results indicate that the direct interaction with the membrane was not observed. We have detected increased production of intracellular reactive oxygen species (ROS) and the interaction with bacterial DNA polymerase Preliminary results suggest partial inhibition of DNA polymerase activity, which may contribute to the overall antimicrobial mechanism.

These findings highlight ROS generation as the primary antimicrobial mechanism of silver-glycine and methylated glycine silver complexes, with membrane damage and potential DNA polymerase inhibition as secondary effects. Given the role of glycine in facilitating cellular transport, these complexes may represent promising candidates for the development of novel silver-based antimicrobials.

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Determination of the synergistic effect of azoles and stress-inducing agents on *A. fumigatus* strains

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Aspergilli, including the important opportunistic pathogen *A. fumigatus*, are characterized by an extraordinary ability to adapt to different stress conditions through a network of multiple stress response pathways that serve both to detect changes in the environment and to regulate the response. Activation of these pathways also occurs by the effect of antifungal compounds. As the successful treatment of aspergillosis is threatened by the increasing spread of resistance to commonly used antifungals and limited alternative treatment resources, it requires the continuous development of new therapeutic approaches. One of the ways to increase the efficacy of antifungals could be to block the key stress response pathways that determine the effectiveness of these drugs.

In our work, we focused on the possible synergistic effect of antifungal compounds (azoles) and agents inducing endoplasmic reticulum stress or interfering with the TOR kinase and calcineurin pathways, respectively. The strains used differed both in origin (environmental, clinical) and in the degree of susceptibility to azoles (susceptible, resistant due to the *cyp51A* mutation), which may provide further insight into whether these differences affect the stress response to azoles. Synergistic effect was assessed by comparison of growth in the presence of single or both substances. The most commonly observed reduction in growth was when posaconazole and dithiothreitol combined. In the case of the clinical azole-susceptible isolate, an increased inhibition rate was also observed in combination of posaconazole with other tested agents.

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Evaluation of bacterial composition and viability of faecal microbiota transplant: comparative analysis of its frozen and lyophilized forms

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Faecal microbiota transplantation (FMT) is defined as the transfer of minimally modified and pre-screened healthy donor stool to the patient's gastrointestinal tract, with the aim of improving the dysbiotic condition by increasing the overall diversity by restoring the functionality of the intestinal microbiota. 1 The aim of this study was to confirm the suitability of FMT from a selected donor based on the optimal composition of his fecal microflora and to evaluate the viability of microbiota in frozen and lyophilized forms of processed FMT under different storage conditions. The metabolic activity of the microorganisms of the stored frozen form of FMT (-70 °C), analyzed by flow cytometry, showed that the bacterial activity of FMT was at the same level between 38-44% before storage and up to 2 months of storage, and was significantly higher ($p<0.001$) compared to all lyophilized forms. The number of live microorganisms from the 3rd month of storage of FMT (-70 °C) steadily decreased with increasing storage time, with a significantly higher ($p<0.001$) average viability of microorganisms compared to freeze-dried forms (-70 °C, 4 °C and 20 °C), maintained at a level between 20-30%, until the end of the monitored period of 9 months. It is important to note that the number of live microorganisms was significantly higher ($p<0.001$) during the entire storage period for the frozen form of FMT (-70 °C) compared to the lyophilized forms, and with its viability at the level between 20-30%, it represented the most suitable form from the point of view of the highest metabolic activity of FMT for transplantation purposes.

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Antimicrobial properties and evaluation of cotton materials with antimicrobial treatment

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This study focuses on the dyeing of cotton materials with direct dyes followed by antimicrobial treatment and fixation with ALTHOSAN MB at different concentrations. Dyeing and antimicrobial treatment were performed in a Datacolor Ahiba IR apparatus. The prepared materials were subjected to water, washing and perspiration fastness tests, and antimicrobial properties were also measured. The obtained results were compared with materials dyed with direct dyes without fixation, as well as materials fixed with Sera Fast C-NC. Antimicrobial properties were evaluated by the agar diffusion method on Mueller-Hinton agar. Materials were tested against several microorganisms including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus* and the yeast *Candida albicans*.
The results show that agent ALTHOSAN MB, unlike Sera Fast C-NC, exhibits positive antimicrobial effects, improving protection against bacterial and yeast growth. Further testing and analysis will focus on a more detailed evaluation of the effects of this new material, which could find widespread application. Such applications may be found in military equipment, where protection against pathogens and long-term durability of the material are essential.

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„Effective engagement in Horizon Europe (HE) projects“

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The lecture will offer an idea of building research management and administration excellence at your workplace (RMA). How to do it and what are your experiences? The aim is to present the key actors of the whole process; the importance of writing national and European projects, grants, prizes, and nominations. Through the interactive form of the workshop, we will try to create a strategy for involvement in quality projects relevant to our target group of young scientists.
The workshop will be divided into two categories: young, motivated scientists and senior academic experts. The first three questions are as per following:

1. Why to get involved in HE projects?
2. What are the biggest obstacles to writing and what helps to overcome them?
3. What are the key supporting actors and how is your cooperation with them?

The basis of success consists of education, writing in foreign languages, quality of publishing, attending international conferences, and establishing business contacts mainly in research areas. In this context, two more questions occurred in addressing to more experienced scientists:

4. What is the key to a successful management?
5. Providing with your tips and recommendations to your colleagues and scientific teams.

An overview of these basic questions will help to create the above-mentioned strategy in the short-term as well as in the long-term scale. What do the following terms mean: "scouting" people within institutions; creating a „keywords“ database; and enhancing the basic structure of the EU projects will be the subject of this lecture. The main idea will be to ease the burden of the administration at your workplace, to persist in so called “fight for survival" with one's own institution, and to participate in “the promising EU train” furthermore.

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Adhesion and invasion capacity of probiotic *Escherichia coli* strains *in vitro*

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Probiotics are beneficial microorganisms with therapeutic potential. Several probiotic products used in medicine contain strains of *Escherichia coli*. While *E. coli* is usually a harmless gut resident, certain strains can cause infections. Since adhesion (attachment to cells) and invasion (entry into cells) are critical steps in bacterial infection, analyzing these characteristics in probiotic strains is essential for assessing their safety. Although some level of adhesion is desirable for probiotic efficacy, invasion is a hallmark of pathogenicity and raises safety concerns. In this study, we determined the adhesion and invasion capacities of commercial probiotic strain *E. coli* Nissle 1917 and four experimental *E. coli* probiotic strains isolated from the gut microbiota of healthy individuals[1,2]. Adhesion and invasion properties were assessed by *in vitro* methods based on co-cultivation with three different colorectal cancer cell lines (Caco-2, HT-29 and SW620). We found that all four experimental *E. coli* strains had similar adhesion capacity compared to established commercial strain *E. coli* Nissle 1917. In addition, two of them (582, B771) exhibited equal or lower level of invasion into epithelial cells compared to *E. coli* Nissle 1917. According to this data, strains 582 and B771 exhibit comparable safety profiles in terms of adhesion and invasion as probiotic strain *E. coli* Nissle 1917. Therefore, they appear to be promising candidates for novel probiotic applications.

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Methicillin-resistant staphylococci in animals – characteristics and susceptibility to bacteriocins

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Antibiotic resistance (AR) is a growing issue in both medicine and animal farming, as the constant gene transfer between bacteria leads to the spread of resistant bacteria. The presence of methicillin-resistant (MR) staphylococci (MRS) in livestock and their products can pose a health risk to consumers. Research is increasingly focused on reducing AR and looking for new naural antimicrobial substances, such as bacteriocins (antimicrobial peptides produced mostly by lactic acid bacteria belonging to postbiotics). Our aim was to form a target of MRS strains based on the basis of MR (using phenotypic tests and PCR for *mec*A, *mec*C, *bla*Z, *msr*A gene detection). The susceptibility of MRS to 9 enterocins (Ents; bacteriocins produced by beneficial *Enterococcus faecium* and *E. durans* strains of animal, feed and environmental origin: EntA/P, EntM, Ent7420, Ent9296, Ent55, Ent412, EntEF9a, EntKI2b and DurancinED26E/7) was tested using agar spot test. Among 118 MRS, tested genes were detected in low rate (*mec*A in 4 strains, *mec*C-6, *bla*Z-5, *msr*A-4). Most MRS were sensitive to tested Ents (100-25,600 AU/mL). The most tested MRS was inhibited by EntA/P (70% of strains), Ent412 (68%) and Ent7420 (66%) and the highest activity was observed in the case of Ent9296 and Ent7420, both inhibiting 20% of MRS strains in the range 12,800–25,600 AU/mL. Fourteen strains showed resistance to Ents. These results suggest that Ents have significant potential to inhibit animal-derived MRS and could offer a promising approach to avoid diseases caused by these bacteria, with prophylactic and medicinal effect.

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