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LAMBDA LIFE

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Newly Synthesized BODIPY Dyes as Promising Photosensitizers Targeting *Staphylococcus aureus*

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Photodynamic inactivation (PDI) represents a promising strategy for combating biofilm-associated infections. *Staphylococcus aureus* is a major nosocomial pathogen belonging to the ESKAPE group of microorganisms. In this study, newly synthesized boron-dipyrromethene (BODIPY) dyes were evaluated as potential photosensitizer against *S. aureus.*

Standard strain *S. aureus* CCM3953 was used for experiments. All tested BODIPYs were prepared at the tested concentrations in MHB. The antibacterial activity of all BODIPYs was tested on planktonic cells according to the modified protocol of Bugyna et al. [1]. The anti-biofilm activity of selected BODIPYs was tested on 24-h biofilms of *S. aureus* CCM3953 formed in 96-well plates. The samples were illuminated with a blue-green LED lamp for 1.5 h. The number of cells was counted as colony-forming units (CFU) per mL. The toxicity of selected BODIPYs was tested *in vivo* using the *Galleria mellonella* model and survival was observed 5 days after BODIPY injection. Tested BODIPYs did not showed antibacterial activity without illumination. For testing of anti-biofilm activity was selected BDP with the highest activity, more than 95% inhibition of bacterial cells after illumination compared to the control cells: BDP 2, BDP 3, BDP 4 a BDP 7 and BDP 17. Selected BODIPYs were evaluated as non-toxic on *in vivo* model.

The results demonstrate that selected BODIPY dyes exhibit significant antimicrobial activity against *S. aureus* after illumination. In addition, the absence of toxicity suggests promising biocompatibility and supports further investigation for antibiofilm applications.

[1] Bugyna, L., Bilská, K., Boháč, P., Pribus, M., Bujdák, J., and Bujdáková, H. Anti-Biofilm Effect of Hybrid Nanocomposite Functionalized with Erythrosine B on *Staphylococcus aureus* Due to Photodynamic Inactivation, Molecules 29 (2024) 3917. https://doi.org/10.3390/molecules29163917.

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Complex Photoactive Hybrid Systems Based on Layered Silicates and Photosensitisers

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Hybrid systems based on layered silicates and photosensitizers represent a promising platform for the development of advanced photoactivated antimicrobial materials and drug delivery systems. Biofilm-associated infections pose significant challenges in modern medicine due to their high resistance to conventional antibiotics and the limited penetration of active compounds through the extracellular matrix. Photoactivated antimicrobial strategies that leverage reactive oxygen species (ROS), particularly singlet oxygen generation, offer a compelling non-antibiotic alternative; however, their efficacy is highly dependent on the stability, aggregation state, mobility, and molecular organization of the photosensitizer.

This contribution provides an overview of hybrid systems utilizing synthetic smectites in conjunction with various photosensitizers, emphasizing how interactions with layered silicates affect photophysical properties and photoactivity. Special attention is given to the surface-induced structural organization of photosensitizers, including emission effects prompted by surface fixation, suppression of non-radiative deactivation, and regulation of molecular aggregation on silicate surfaces.

Additionally, more complex multicomponent systems employing Förster resonance energy transfer (FRET) will be examined. These systems integrate ROS-generating photosensitizers with light-harvesting dyes, facilitating more efficient utilization of electromagnetic radiation and enhancing photoactivation efficiency. The role of layered silicates as functional regulators that control molecular arrangement, mobility, and intermolecular interactions within these systems will be underscored.

This work combines spectroscopic characterization, chemometric analysis, and examinations of hybrid material organization to elucidate the relationship between photosensitizer structure, surface interactions, and ROS-generating performance. Overall, the presented systems illustrate the potential of layered silicates as adaptable platforms for the rational design of advanced photoresponsive antimicrobial materials.

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Photodynamic Anti-biofilm Inactivation of *Staphylococcus aureus* Using an Eosin Y-Based Nanocomposite

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The photosensitizer eosin Y (EosY) is a synthetic xanthene dye that has attracted attention for its promising antibacterial properties against *Staphylococcus aureus*. This study addresses the photoactive properties of EosY and evaluates the efficacy of polyurethane (PU)-based antimicrobial materials using a nanocomposite film composed of saponite modified with poly(diallyldimethylammonium) cations and functionalized with EosY. Additionally, the potential impact of PDI on the Agr (Accessory Gene Regulatory System) quorum-sensing (QS) system, which participates in biofilm formation in Gram-positive bacteria was studied. In preliminary experiments, different concentrations (1.0μM-0.1mM) of EosY were tested on planktonic cells of the standard strain *S. aureus* CCM 3953. The results were assessed by calculating the colony-forming units (CFU/mL). It was confirmed that EosY affected cell survival after irradiation, resulting in a 100-fold reduction at 0.1 mM. Then, experiments were conducted using a nanocomposite containing EosY. PU modified with EosY-functionalized hybrid film achieved approximately 10-fold in the standard strain of *S. aureus* and the resistant strain *S. aureus* S66 of biofilm growth reduction after irradiation with both irradiation sources. To improve the anti-biofilm efficacy, another green light LED source with varying light intensities was used. At the intensity of 25%, biofilm growth on the nanocomposite was reduced by more than 100-fold in the standard *S. aureus* CCM 3953 strain and the resistant *S. aureus* S66 isolate. The functionality and impact of PDI on the Agr system was tested using the Agr CAMP test and qRT-PCR, respectively, employing the *hld* gene encoding δ-haemolysin within the RNAIII transcript. Preliminary results showed that activation of the Agr system correlated with biofilm formation; however, although PDI exhibited significant activity against biofilm formed on nanocomposite, no effect on *hld* gene expression was observed.

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Design of Photoactive Polymer–Clay Nanocomposites for Biofilm Control

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One of the key challenges in microbial resistance is developing materials with antimicrobial properties that can prevent the spread of infectious diseases. A major issue in this context is biofilm formation, which significantly complicates the treatment of infections related to medical devices such as catheters and implants.

Polymeric engineering materials are widely used in healthcare, and surface modification of polymers is an effective strategy to inhibit biofilm formation. Nanocomposites containing layered silicate particles represent a promising class of materials for biomedical applications. These particles can act as carriers for bioactive molecules, and an appropriate design ensures their accessibility at the polymer surface, which is essential for antimicrobial performance.

An emerging strategy for preparing antimicrobial polymer surfaces involves the fusion of thin films of organically modified silicates with polymers, forming composite layers on polymer substrates. This can be achieved using liquid precursors or polymer melts, enabling the fabrication of surface coatings only a few micrometers thick. Functionalization with photosensitizer molecules enables both photodynamic antimicrobial activity and controlled release of active compounds.

Our studies have focused on photosensitizers based on halogenated xanthene dyes and polymer matrices such as polyurethane, low-melting-point polycaprolactone, and, more recently, hydrogels. Polyurethane systems show efficient release of active photosensitizers, whereas hydrogels exhibit stimulus-responsive antimicrobial behavior. Structural analysis confirmed that polymer intercalation within silicate films leads to strong interfacial coupling between phases. Upon irradiation, these materials effectively eliminated *Staphylococcus aureus* biofilms, achieving up to a 10,000-fold reduction in bacterial growth.

Biological studies have further explored mechanisms of action and pathogen responses to photodynamic treatment, including effects on resistance and virulence factors. Overall, these results demonstrate the strong potential of photoactive nanocomposite surfaces for advanced biomedical applications.

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Towards nanotechnologies using bioactive particles/molecules in the fight against microbial biofilms

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The project addressed current scientific challenges through an interdisciplinary approach focused on microbial biofilms and their prevention and eradication using newly designed hybrid materials. The research integrated basic and molecular microbiology with nanomaterial chemistry. The study focused on multispecies bacterial and yeast biofilms, including those formed by ESKAPE pathogens, Escherichia coli, and yeasts of the genus *Candida,* all of which are highly relevant in biofilm-associated infections. Advanced microbiological methods were used to investigate biofilm formation, interspecies interactions, the role of quorum sensing molecules, and the efficacy of bioactive compounds in biofilm prevention and eradication. The project tested hybrid materials based on layered nanoparticles acting as carriers for bioactive molecules, particularly photosensitizers such as methylene blue, phloxine B, and erythrosine B. It also included the study of virulence gene expression associated with microbial adhesion processes. *In vivo* studies using the model *Galleria mellonella* were applied to assess nanomaterial toxicity and host–pathogen interactions, including immune responses to mixed biofilm infections. These analyses involved the expression of antimicrobial peptide genes and hemocyte-based immune responses. In addition, developed materials, including hydrogels, were characterized in terms of their physicochemical, photochemical, and photophysical properties in order to optimize their anti-biofilm efficacy.

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Elimination of mixed biofilm formed by C*andida albicans* and E*nterococcus faecium*

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Vancomycin-resistant enterococci are widespread in hospitals worldwide; however, compared with *Enterococcus faecalis*, relatively little is still known about the virulence and pathogenesis of *Enterococcus faecium*. Effective treatment of persistent nosocomial infections and diseases associated with recurrent contamination by polymicrobial biofilms is both essential and challenging. In order to eliminate these microorganisms from surfaces or prevent their adhesion, their properties must be thoroughly investigated. Currently, natural compounds such as fungal secondary metabolites (e.g., farnesol) are attracting increasing attention. Another effective alternative appears to be photodynamic inhibition. The aim of this study was to characterise the interaction between *Candida albicans* and *Enterococcus faecium* in the formation of dual or mixed biofilms. Standard methods, including crystal violet staining, colony-forming unit enumeration, and microscopy, were used to assess morphogenesis and biofilm formation. Subsequently, the expression of genes associated with adhesion, a key factor in biofilm development was investigated. The virulence of pathogens was assessed *in vivo* using *Galleria mellonella,* In addition, changes in the immune response were studied via expression of genes coding larval antimicrobial peptides following infection with single and multiple pathogens. Photodynamic inhibition using phloxine B was also successfully applied for eradication of biofilms *in vivo.*

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Photodynamic inactivation as an effective tool for biofilm eradication and quorum sensing regulation in mixed biofilms *Klebsiella pneumoniae and Candida albicans*

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Mixed biofilms represent complex microbial communities composed of various microorganisms embedded within an extracellular matrix. Among clinically significant pathogens capable of forming mixed biofilms are *Candida albicans* and *Klebsiella pneumoniae*, both associated with increased morbidity and mortality in hospitalized patients. Due to the growing resistance of microorganisms to conventional antimicrobial therapies, the development of novel therapeutic approaches has become essential. Antimicrobial photodynamic inactivation (aPDI) represents a promising alternative based on the combination of a photosensitizer, light of an appropriate wavelength, and oxygen, resulting in the generation of reactive oxygen species and subsequent microbial cell damage. The aim of this study was to analyze mixed biofilms formed by *C. albicans* and *K. pneumoniae*, characterize interspecies interactions and quorum sensing (QS), and evaluate the efficacy of aPDI with Methylene blue and Phloxine B as photosensitizers, in the eradication and genetic regulation of these biofilms. aPDI proved to be an effective method for the eradication of mixed biofilms, with the strongest effect observed after the application of methylene blue (1mM) followed by irradiation with a red laser (660 nm). After 5 minutes of irradiation, an approximately 2 log10 reduction in viable cells of bacteria and 0.5 log10 reduction of yeasts was achieved, accompanied by decreased expression of virulence genes *fimH* and *mrkD* and the *luxS* gene that is main QS regulator *in K. pneumoniae*. Additionally, biofilm and QS associated genes *HWP1* and *ERG20* of *C. albicans* were downregulated as well. In conclusion, qPDI effectively eradicated mixed biofilm of *C. albicans* and *K. pneumoniae* and can be considered as promising strategy to combat clinically relevant resistant biofilms.

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Colonization of the gastrointestinal tract by VRE in patients with *Clostridioides difficile* infection

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Colonization by vancomycin-resistant enterococci (VRE) represents an increasing concern in patients with *Clostridioides difficile* infection (CDI). Antibiotic therapy and disruption of the intestinal microbiota predispose these patients to colonization by opportunistic pathogens, particularly *Enterococcus faecium* and *Enterococcus faecalis*. The rising prevalence of VRE is alarming due to their role as reservoirs of antibiotic resistance genes and their contribution to resistance spread in healthcare settings. The aim of this study was to evaluate the presence of enterococci in stool samples from patients with confirmed CDI, determine their antibiotic susceptibility, and assess the prevalence of vancomycin-resistant strains. A total of 94 stool samples from patients with culture-confirmed CDI were analyzed. Enterococci were isolated on selective media and identified using MALDI-TOF MS. Antibiotic susceptibility was determined by disk diffusion test using ampicillin, doxycycline, linezolid, vancomycin, trimethoprim–sulfamethoxazole, and ciprofloxacin. Vancomycin-resistant isolates were further tested by E-test to determine minimum inhibitory concentration (MIC). Enterococci were detected in 77 (81.9%) samples, with 121 isolates identified. The most prevalent species were *E. faecium* (n = 64) and *E. faecalis* (n = 36). High resistance was observed to trimethoprim–sulfamethoxazole (73%), while the highest susceptibility was to doxycycline (79.34%). Vancomycin resistance was identified in 28 isolates (23.1%). MIC values > 256 µg/mL were detected in 18 isolates, all confirmed to carry the *vanA* gene by PCR. These findings highlight CDI patients as an important reservoir of antimicrobial resistance and emphasize the need for continuous surveillance.

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Comparative genomic analysis of *Escherichia coli* isolates from patients with colorectal neoplasia and healthy controls

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Escherichia coli is an important part of the human intestinal microflora. While many *E. coli* strains are considered commensal, others are important pathogens responsible for diarrheal conditions and extraintestinal infections. In recent years, the role of *E. coli* in colorectal cancer has also gained increasing attention. In this study, we analyzed genomes of 218 *E. coli* strains isolated from biopsies of patients with colorectal neoplasia and healthy controls (n = 61 and 20, respectively). The assembled draft genomes had 344x median coverage. The Prokka software was used for genome annotation, with 4344-5881 predicted coding sequences per genome. The annotated sequences were subsequently analyzed by Roary to infer core genes, defined as genes present in 99 % of isolates. The core genome comprised of 2741 genes. Genes unique to neoplasia patients compared to healthy controls were selected for further analysis. In addition, the genomes were screened for the presence of virulence-associated genes (VAGs) using the VFDB database. No significant difference was observed in the number of VAGs between the analyzed groups, with median 74 genes in each. However, several VAGs showed positive associations with neoplasia diagnosis ($p < 0,05$). These included genes encoding the *E. coli* common pilus, F1C and S fimbriae, type IV pili, EaeH surface protein, the invasin IbeA, the salmocheline siderophore, enterohemorrhagic autotransporters, TPS pathway genes, as well as α-hemolysin, the uropathogenic specific protein and cytotoxic necrotizing factor 1.

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Comprehensive analysis of enterotoxigenicity, antimicrobial resistance and persistence of *Staphylococcus* spp. using combined analytical approaches

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Staphylococci represent a major food safety concern due to their ability to produce enterotoxins, persist under environmental stress, and exhibit antimicrobial resistance. This study provides a comprehensive evaluation of *Staphylococcus* spp. from food, animal, and human sources, integrating analyses of enterotoxigenicity, resistance, persistence mechanisms, and detection approaches.

Enterotoxin production was confirmed in 59% of isolates, including 20% coagulase-negative staphylococci, highlighting their underestimated role. Classical *se* genes (*sea–see*) were detected in 46% of isolates, with 16% carrying multiple genes. Discrepancies between toxin production detected by ELISA and the presence of *se* genes identified by PCR highlight the complexity of toxin expression in *S. aureus*. Nearly half of the isolates were multidrug-resistant, and all exhibited biofilm-forming ability, with higher production in enterotoxin-positive food isolates. To overcome limitations of ELISA, HPLC-DAD and MALDI-TOF MS were applied for protein-level toxin analysis. Characteristic peaks (20–35 min) and mass signals (m/z 20–23 kDa and 27–29 kDa) suggested the presence of TSST-1 and staphylococcal enterotoxins, supported by PCR, although definitive identification was limited by the absence of reference standards.

NaCl-induced stress (a_w 1.00–0.95) prolonged *lag* phases but promoted growth, confirming the halotolerant nature of *S. aureus*. Transcriptomic analysis of enterotoxins A–D under salt stress revealed time- and condition-dependent variability, indicating complex regulatory mechanisms.

Overall, enterotoxigenicity, antimicrobial resistance, and persistence traits are closely interconnected and influenced by environmental conditions. The findings highlight the need for integrated analytical approaches and improved surveillance strategies addressing both *S. aureus* and coagulase-negative staphylococci in the food chain.

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From the Laboratory to Safe Foods: The Impact of Environmental Factors on Staphylococcal Enterotoxin Production

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The aim of this study was to evaluate the effect of reduced water activity (aw), induced by sodium chloride (NaCl) addition, on the expression of selected enterotoxigenic genes (*sed*, *sec*, *sea*, and *seb*) in collection strains *Staphylococcus aureus* CCM 5971 and CCM 5755 as a function of incubation time. Gene expression was analysed using RT-qPCR at aw values of 1.00, 0.99, 0.97, and 0.95 at different incubation intervals, with the housekeeping gene *rho* used as a reference. In parallel, colony-forming unit (CFU) counts were determined to assess the relationship between bacterial growth phase and gene expression dynamics. The presence of enterotoxins was verified by ELISA. In *S. aureus* CCM 5971, *sed* expression was detected only at early incubation times, with a mild induction observed after 6 h at aw 0.99, followed by a decrease to non-detectable levels at later time points. Expression of *sec* was detected only after 10 h of incubation and showed reduced expression at lower aw values. In *S. aureus* CCM 5755, a pronounced time- and aw-dependent expression pattern of *sea* was observed, including significant upregulation at moderately reduced aw after 10 h of incubation. Expression of *seb* was not detected under any of the experimental conditions tested. ELISA analysis confirmed the presence of enterotoxins in all samples, highlighting the distinction between transient gene transcription and accumulation of stable toxin proteins in the culture medium. These results indicate that reduced aw within the tested range does not represent a uniform regulatory stimulus for enterotoxigenic gene expression in *S. aureus*, and that expression dynamics are strongly influenced by incubation time and bacterial growth phase.

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Molybdenum oxide nanoparticles functionalized to target lung and pancreatic tumor cells caused disruption of tumor microenvironment

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Molybdenum oxide (MoOx) is a material with photothermal activity potential, able to induce apoptosis after irradiation with 800 nm laser. The presence of molybdenum oxide can also significantly increase expression of M1 phenotype macrophage – related genes like TNF-alpha, which has important antiviral activity. Specificity of MoOx nanoparticles (NPs) in targeting hypoxic lung and colorectal cancer cells was achieved by their functionalization with anti-carbonic anhydrase IX (CAIX) antibody. Carbonic anhydrase IX is predominantly expressed in cancer cells on their cellular membrane. MoOx NP functionalized with anti-CAIX antibody bind to CAIX present on the cell surface, followed by the internalization of NPs and their transport by endocytic pathways. Lung carcinoma cells bound functionalized MoOx with highest affinity and with lowest viability disruption. However, the irradiation of cancer cell monolayer with 800 nm laser significantly decreased viability while increasing caspase activity. Irradiation in 3D cell cultures and in *in vivo* experiments led to decrease in the size of spheroids and xenografts, to increased caspase activity and higher presence of double strand DNA breaks. These results show very promising effectiveness of targeted photothermal therapy of lung and colorectal carcinoma. Also, molybdenum oxide could play an important role in viral diseases.

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Study of the effects of bioactive substances based on glycosaminoglycans and sulfated heteropolysaccharides

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Pro-inflammatory activity of macrophages is essential for the natural anti-tumor defense of the organism. This pro-inflammatory activity is also induced by the macrophage-activating factor GcMAF, which is formed through the enzymatic modification of the Gc protein. The enzyme α-N-acetylgalactosaminidase deglycosylates GcMAF and its precursor, thereby preventing macrophage activation. Increased activity of this enzyme has been recorded in various types of cancer and viral infections. Tumor and infected cells secrete α-N-acetylgalactosaminidase into the extracellular space and the bloodstream, leading to immunosuppression of the organism. In such cases of immunosuppression, it is not a matter of total immune failure, but rather a specific inhibition of a particular component of the immune system, which creates the prerequisites for a therapeutic approach referred to as immunonormalization. Sulfated polysaccharides, chondroitin sulfate and fucoidan demonstrate the ability to interfere with the pathological activity of α-N-acetylgalactosaminidase, where chondroitin sulfate competitively inhibits its activity and fucoidan reduces its expression in tumor cells. In the research we will study the antiproliferative, antitumor and immunomodulatory effects of these substances. The essence of the immunomodulatory effect of substances based on glycosaminoglycans-low molecular weight chondroitin sulphate and fucoidan-sulphated heteropolysaccharide containing fucose and galactosamine is the in vivo increase of GcMAF protein production by inhibition of nagalase activity. Initially, we will start testing substances on 2D and 3D cell cultures. Following targeted subcutaneous administration of tumor cells to laboratory mice, we will monitor the effect of the oral drug on progression as well as inhibition of xenografts formed. We anticipate that experimental studies will bring medically significant markers to GcMAF immunotherapy.

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Contribution of Oxidative Stress to the Mode of Action of Azole Antifungal Compounds in *Neurospora crassa*

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Azole antifungals are widely used inhibitors of ergosterol biosynthesis; however, their broader cellular effects and their contribution to fungal stress signalling remain incompletely understood. In this study, we investigated the involvement of oxidative stress in the early adaptive response of the model filamentous fungus *Neurospora crassa* following short-term (2 h) exposure to azole compounds. Surprisingly, azole exposure did not lead to a significant increase in ROS production compared with control conditions. To further examine whether redox-sensitive defence mechanisms were nevertheless activated, we performed transcriptional analysis of genes encoding key antioxidant enzymes, including catalase, peroxisomal catalase, and superoxide dismutase, as well as the transcription factor Nap1. The results revealed strong upregulation of catalase genes and moderate induction of superoxide dismutase expression, whereas *nap1* expression remained unchanged. In parallel, azole exposure caused a significant increase in intracellular glycerol concentration, consistent with activation of the high-osmolarity glycerol (HOG) pathway and the involvement of Hog1 which has been previously linked to mild oxidative stress as transcription factor for antioxidant genes. Despite the elevated transcript levels of antioxidant genes, no corresponding increase in enzymatic activity was observed. These findings indicate that the basal antioxidant capacity of *N. crassa* is probably sufficient to maintain redox homeostasis during the early phase of azole exposure. To further characterize the oxidative status, the glutathione and thioredoxin systems were analyzed. Structure-dependent alterations in the ratio of reduced to oxidized glutathione were detected, indicating subtle redox modulation rather than pronounced oxidative stress. Together, these results suggest that ROS are unlikely to act primarily as damaging agents during the early response of *N. crassa* to azoles. Instead, redox-related changes may contribute to the activation of adaptive signalling pathways.

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Early Adaptive Response of *Aspergillus fumigatus* to Azole Antifungal Compounds: Links Between Gene Expression, Growth Behaviour and Antioxidant Capacity

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Azole antifungals are widely used in the treatment of aspergillosis; however, resistant and tolerant *Aspergillus fumigatus* isolates represent a serious clinical challenge. While resistance is frequently associated with genetic alterations in the ergosterol biosynthetic pathway, fungal survival under azole exposure may also depend on early adaptive mechanisms. This study investigated the early response of azole-sensitive and azole-resistant *A. fumigatus* strains to voriconazole, itraconazole and posaconazole, with emphasis on growth behaviour, transcriptional regulation and antioxidant capacity.

Growth characteristics were monitored during the exponential phase, and gene expression was analyzed after short-term azole exposure. The analysis focused on genes involved in ergosterol biosynthesis (*cyp51A*, *cyp51B*, *erg25*, *erg24*, *erg3*), regulatory factors (*atrR*, *srbA*) and the transport-related gene *cdr1*. Basal expression profiles were complemented by assessment of superoxide dismutase and catalase activities and glutathione levels.

The results revealed marked strain-dependent variability. The reference strain showed growth inhibition together with increased expression of ergosterol-related genes, indicating a compensatory response to azole-induced sterol disruption. In contrast, isolates carrying the same *cyp51A* mutation displayed different transcriptomic profiles: AF6600 showed a downregulated response, whereas AF6601 has shown upregulation of all analyzed genes which correlated with increased tolerance to azole compounds demonstrated by growth assay. Notably, this enhanced tolerance was observed across multiple azoles, not limited to voriconazole, despite the shared target-site mutation. Other isolates showed selective or variable transcriptomic profiles.

Importantly, the basal antioxidant capacity, including the overall antioxidant system as well as catalase, superoxide dismutase, and glutathione activities, was comparable between the susceptible reference strain and both resistant isolates under control conditions.

Overall, the findings suggest that early azole adaptation in *A. fumigatus* involves not only resistance-associated mutations, but also involves strain-specific transcriptional plasticity.

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Preparation of Photoactive Nanocomposites Based on PDDA-modified Saponite and Phloxine B for Anti-biofilm Materials

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Nanocomposites composed of layered silicates, a polymer matrix, and a photosensitising dye are a promising class of materials for anti-biofilm applications. This work focuses on the preparation and photophysical characterisation of polyurethane-based nanocomposites containing saponite modified with poly(diallyldimethylammonium chloride) (PDDA) and functionalized with the photosensitizer Phloxine B (PhB). The first step in the preparation of the material was the modification of saponite using PDDA, which enabled the effective binding of the anionic PhB. A series of samples with varying loadings (mmol of PhB per gram of saponite) was prepared in the form of dispersions, from which thin films were subsequently prepared by filtration and coating with polyurethane. These samples were used to test antimicrobial activity. The photophysical characterisation was performed on the sample with the highest dye concentration (0.43 mmol g^{-1}), where UV-Vis and fluorescence spectra as well as dye release were studied. The results confirmed that the antibacterial activity of the nanocomposite is primarily determined by the loading of PhB. The modification of PDDA enabled high adsorption of PhB while maintaining photoactivity and minimising dye aggregation, representing a significant improvement compared to other surfactant-modified systems.

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Evaluation of synergistic interactions between simvastatin and antifungal agents against yeasts causing candidiasis

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Yeast infections, along with increasing resistance to antifungal agents, present a serious clinical problem that significantly affects treatment efficacy. The main causative agents include *Candida albicans*, *Candidozyma auris* (formerly *Candida auris*), *Nakaseomyces glabratus* (formerly *Candida glabrata*), and *Candida parapsilosis*. Current treatment options are limited, highlighting the need for new strategies. Recent studies have focused on compounds such as antipsychotics, antihistamines, analgesics, and statins. Statins, commonly used to lower LDL cholesterol and treat cardiovascular diseases, have also been shown to exhibit potential antifungal activity, particularly against resistant strains. This study aimed to evaluate the antifungal potential of simvastatin and its possible synergistic effects in combination with fluconazole against standard and azole-resistant clinical isolates of pathogenic yeast. Phenotypic growth analysis was performed by cultivation on agar plates with various concentrations of simvastatin, determination of the minimum inhibitory concentration (MIC) of fluconazole, and *in vitro* evaluation using a checkerboard assay. The degree of synergism was quantified by calculating the fractional inhibitory concentration index (FICI). Based on FICI values, combinations showing the most pronounced synergistic effects were selected and further analyzed using growth curves for individual *Candida spp.* strains. These experiments confirmed the adjuvant effect of simvastatin with fluconazole in inhibiting yeast growth. The results indicate a synergistic to additive effect of simvastatin combined with fluconazole, particularly in resistant isolates, suggesting the potential of statins as an adjuvant strategy in antifungal therapy.

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The circadian clock-controlled gene *ccg-8* modulates stress tolerance in *Neurospora crassa*

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In the study of filamentous fungi, both in biocontrol and pathogenesis, chronobiology remains an under-explored frontier. Fungi contain an internal circadian clock that governs such processes, synchronizing them to a 24-hour period. Yet the downstream effects on stress adaptation are not fully understood. In this study, we investigated the role of the clock-controlled gene *ccg-8* in *Neurospora crassa*. While previously linked to ketoconazole susceptibility, we aimed to determine its broader impact on tolerance to environmental stimuli. Our results reveal that the transcription factor *CCG-8* regulates the expression of proteins involved in ribosome biogenesis and fatty acid biosynthesis. Deletion of *ccg-8* renders the fungus hypersensitive to a wide array of stressors, including various azole and echinocandin antifungals. Furthermore, proteomic changes in the cell wall of the *Δccg-8* mutant led to enhanced recognition of conidia by *Galleria mellonella* larvae. These findings highlight the critical role of circadian regulation in chemical tolerance and validate *G. mellonella* as a robust model for studying fungal physiology and antifungal resistance in non-pathogenic systems.

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Determination of the Viability of *Klebsiella pneumoniae* and *Candida albicans* Biofilms after Antimicrobial Photodynamic Inactivation

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Klebsiella pneumoniae and *Candida albicans* represent significant threats to public health due to increasing antimicrobial resistance and their ability to form biofilms, which contribute to persistent and chronic infections. Among alternative therapeutic approaches, antimicrobial photodynamic inactivation (aPDI) has emerged as a promising strategy. This method is based on the application of a photosensitizer that, upon light activation, generates reactive oxygen species (ROS) capable of damaging essential cellular structures and inducing microbial cell death.

Viability Polymerase Chain Reaction (vPCR) is a molecular method that employs the intercalating dye propidium monoazide (PMA) for the selective quantification of viable cells by determining the number of genomic copies. Importantly, vPCR also enables the detection of VBNC (Viable But Not Cultivable) forms of microorganisms, which retain metabolic activity and may contribute to recurrent or chronic infections despite being undetectable by conventional cultivation techniques. Another method used for the determination of microbial viability and metabolic activity is the BacTiter-Glo™ Microbial Cell Viability Assay, which is based on ATP quantification through a luminescent luciferase reaction following cell lysis.

The aim of this study was to compare these methods in the practical application of photodynamic inactivation against mono-species biofilms of *K. pneumoniae* and *C. albicans*, as well as mixed K. *pneumoniae* and *C. albicans* biofilms. The obtained results were compared with the conventional colony-forming unit method (CFU/mL), and statistical analysis was performed to evaluate their validity. The results indicate notable differences between CFU/mL and PMA-based vPCR analysis, with PMA-vPCR demonstrating higher sensitivity in detecting viable microbial cells after aPDI treatment.

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Assessment of genotoxicity of *Escherichia coli* strains isolated from patients with colorectal neoplasia and healthy controls

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Colorectal cancer (CRC) represents a significant global health burden. Current experimental evidence shows that gut-resident microorganisms actively participate in CRC pathogenesis. Genotoxin-producing bacteria might contribute to the accumulation of genetic changes, which are one of the key aspects of neoplastic process. *Escherichia coli* strains, coding for genotoxic colibactin (*pks*+), are among the most studied bacteria associated with CRC. These strains have been proven to induce DNA damage in experimental models and are enriched in gut microbiota of patients with CRC compared to healthy individuals. In addition to colibactin, various *E. coli* strains harbor other candidate genes (*cdt, cnf1, ibeA, usp*) encoding proteins with potential genotoxic or other pro-tumorigenic properties. Our study utilized a collection of 218 *E. coli* isolates from a cohort of 61 individuals, encompassing patients at various stages of colorectal neoplasia (non-advanced adenoma, advanced adenoma, and carcinoma), as well as healthy controls (n = 20). We focused on assessing genotoxicity potential of the *E. coli* isolates using *in vitro* DNA degradation assay. This approach allowed us to monitor the extracellular DNase activity by observing the rate of exogenous DNA cleavage over time. We have observed significantly higher amount of strains with extracellular DNase activity in carcinoma group compared to healthy group (31.6 % vs 55.0 %, p = 0.037). These results along with planned additional assays aimed at detecting DNA cross-linking activity and molecular response triggered in eukaryotic cells, will contribute to a better understanding of the role of *E. coli* in neoplastic transformation.

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