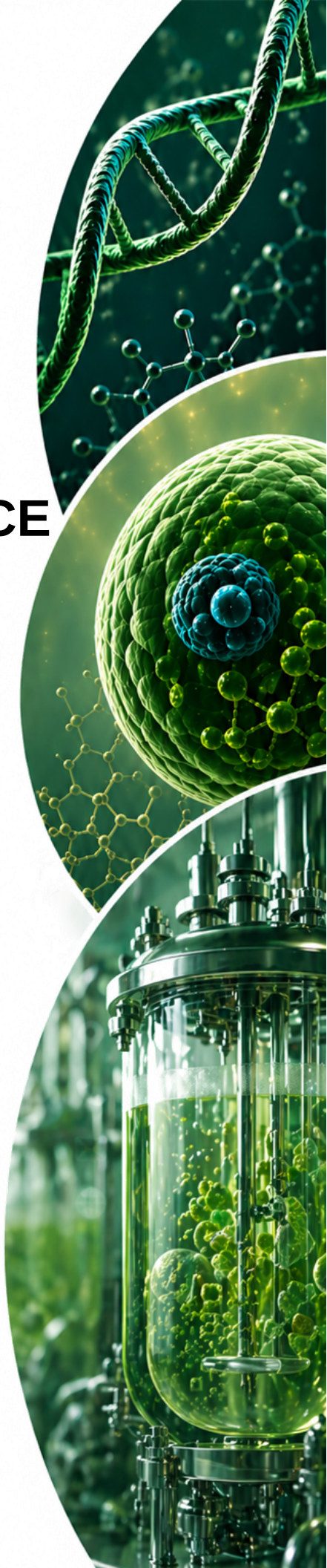
ABSTRACT BOOK



INTERNATIONAL CONFERENCE OF YOUNG SCIENTISTS

of the Institute of Cell Biology and the University of Rzeszów

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Lviv
May 21, 2026

The program of International Conference of young of the Institute of Cell Biology and the University of Rzeszow
Lviv, May 21, 2026

12:00	**Andriy Sibirny**	**Opening Ceremony**
12:10	Roksolana Vasylyshyn	Developing a novel transformation protocol for the yeast *Ogataea polymorpha*
12:20	Nazar Manko	Chitosan-based hydrogels with antimicrobial agents against reference and clinical strains
12:30	Gabriela Żywczak	Assessment of the biotechnological potential of microorganisms isolated from environmental samples (online)
12:40	Iryna Ivasechko	Antifibrotic effect of thiazole and 4-thiazolidinone derivatives targeting tgf-β signaling in tgf-β1-activated fibroblasts
12:50	Yuliia Kozak	In vivo toxicity profile of novel heterocyclic derivatives les-6287 and les-6294 in c57bl/6 mice
13:00	Nikita Polishchuk	Prostate cancer cells are sensitive to combined metabolic treatment based on amino acid deprivation and glycolysis inhibition (online)
13:10	Uliana Pelypyshyn	Promoter-dependent regulation of β-galactosidase degradation in the methylotrophic yeast *Komagataella phaffii*
13:20	Serhiy Romanov	The novel transcription factor is involved in negative regulation of riboflavin biosynthesis in *Candida famata* yeast

*The programme is shown in Ukrainian time zone

Scientific Committee

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DEVELOPING A NOVEL TRANSFORMATION PROTOCOL FOR THE YEAST *OGATAEA POLYMORPHA*

Roksolana Vasylyshyn[1], Oleksandr Feshchuk[1], Dominik Wojdyła[2], Justyna Ruchała[2], Andriy Sibirny[1,2]

[1] Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine;
[2] Institute of Biotechnology, University of Rzeszow, Poland.

Methylotrophic yeast *Ogataea polymorpha* is the model organism for studying the mechanisms of thermotolerance, peroxisome homeostasis, methanol metabolism, production of heterologous proteins on industrial scale and of high temperature alcoholic fermentation. Still, the new tools for continue molecular research are needed, namely additional selective markers for the selection of recombinant strains with the desired physiological characteristics. The aim of this study was to develop the new selective approach for *O. polymorpha* usable in metabolic engineering experiments.

As a potential selective agent, metformin was used - a substance that suppresses gluconeogenesis and, as shown in our previous studies, increases the level of ethanol production from xylose, but not from glucose during high-temperature alcoholic fermentation. The minimal toxic concentration for *O. polymorpha* NCYC495 strain was found to be 50 mg/ml of metformin. The introduction of a vector carrying the overexpressed *CAT8* gene restores the growth of *O. polymorpha* yeast on YNB medium containing glycerol and metformin. The presence of the vector for overexpression in the transformants was confirmed by the PCR method. In summary, the introduction of pUC19/pGAP/CAT8/NTC (Ruchala et. al., 2017) into genome of *O. polymorpha* wild-type strain was sufficient to obtain transformants with restored expression of genes involved in gluconeogenesis and can be used as a new method of selecting recombinant strains.

This work was supported by the National Research Foundation of Ukraine (Grant No.2025.07/0152).

CHITOSAN-BASED HYDROGELS WITH ANTIMICROBIAL AGENTS AGAINST REFERENCE AND CLINICAL STRAINS

Nazar Manko[1], Iryna Ivasechko[1], Iryno Kovalenko[2], Tetyna Rumynska[2], Dmytro Demash[1], Rostyslav Stoika[1]

[1]Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine;
[2]DPN Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.

Effective wound healing remains a highly relevant challenge in modern medicine, particularly due to the growing incidence of chronic, infected, and difficult-to-heal skin lesions. In our previous studies, we developed chitosan-based gel films (up to 1 mm thick) that demonstrated pronounced wound-healing potential together with high biocompatibility and biodegradability. However, several limitations remained, including insufficient stability under humid storage conditions and difficulties associated with sterilization procedures.

To overcome these drawbacks, we designed a biocompatible scaffold based on a chitosan–polyvinyl alcohol (PVA) complex that is suitable for UV sterilization, exhibits improved storage stability, and possesses favorable sorption properties. The obtained hydrogel was further modified by coating it with a hyaluronate–chitosan gel film to enhance its functional characteristics. Initially, the biocompatibility of the developed gels was evaluated using normal cell cultures and chitosan samples of different molecular weights. In parallel, changes in the gel surface morphology were analyzed by SEM.The next stage involved enrichment of the hydrogel with antimicrobial agents using two different approaches: sorption and synthesis. Antimicrobial activity was first assessed against reference strains, including *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231, and *Candida parapsilosis* ATCC 22019. The results demonstrated efficient diffusion of antimicrobial substances from the gel matrix while preserving their biological activity. Importantly, glucose oxidase incorporated into the hydrogel also retained its enzymatic activity. Considering the substantial differences in susceptibility between reference strains and pathogenic clinical isolates, additional studies were performed using gels containing betadine and glucose oxidase against clinical isolates of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus haemolyticus*. These experiments confirmed the sensitivity of the tested isolates to the antimicrobial products generated by glucose oxidase.

Overall, the developed multifunctional hydrogels containing antimicrobial agents or enzymes represent a promising strategy for improving conventional wound-healing therapies and reducing the risk of resistant wound infections.

ASSESSMENT OF THE BIOTECHNOLOGICAL POTENTIAL OF MICROORGANISMS ISOLATED FROM ENVIRONMENTAL SAMPLES

Gabriela Żywczak, Justyna Ruchala

Faculty of Biotechnology, Medical College, University of Rzeszów, Cwiklinskiej 2D, Rzeszów 35-601, Poland.

Microorganisms isolated from environmental samples can be identified and characterized to evaluate their potential applications in various industrial sectors. The aim of this study was to determine the taxonomic identity of the isolated microorganisms and to characterize their morphological and physiological properties.

Identification of the isolates was performed using MALDI-TOF mass spectrometry, and the obtained results were verified by microscopic and cytometric analyses. Growth kinetics studies were also conducted to determine the optimal culture conditions. As a result of the analyses, two Gram-positive bacterial species, *Staphylococcus warneri* and *Rhodococcus fascians*, were identified. These microorganisms may have potential industrial applications, including bacteriocin production and biodegradation of environmental pollutants.

ANTIFIBROTIC EFFECT OF THIAZOLE AND 4-THIAZOLIDINONE DERIVATIVES TARGETING TGF-B SIGNALING IN TGF-B1-ACTIVATED FIBROBLASTS

Iryna Ivasechko[1,2], Yuliia Kozak[1,2], Nataliya Finiuk[1,2], Olha Kluchivska[1], Rostyslav Stoika [1], Roman Lesyk[2]

[1]Institute of Cell Biology of the National Academy of Science of Ukraine, Lviv, Ukraine; [2]Molecular Design Center, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.

Fibrosis is characterized by an excessive deposition of extracellular matrix components, primarily collagen, leading to tissue scarring and organ failure. Transforming growth factor beta 1 is the systemic profibrotic cytokine that drives fibroblast-to-myofibroblast differentiation, marked by α-smooth muscle actin expression and enhanced ECM synthesis. Here, we report about anti-fibrotic effect of thiazole derivatives targeting TGF-β1 signaling.

Materials and Methods. BALB/3T3 mouse embryonic fibroblasts and BJ human fibroblasts were cultured in DMEM supplemented with 1 % fetal bovine serum. Fibrotic phenotype was induced via stimulation with recombinant human TGF-β1 (2 ng/mL) for 48 h.

The tested compound was added simultaneously with the pro-fibrotic stimulus or 24 h later in 0.1–10 μM concentrations. Cell viability was assessed using MTT assay. Collagen deposition was quantified by Sirius Red staining and by measurement of hydroxyproline content using a colorimetric hydroxyproline assay kit. Myofibroblast differentiation and intracellular protein expression were evaluated by the Western blot analysis. Morphological changes were visualized by fluorescence microscopy.

New derivatives in 0.1-0.5 μM doses reduced Sirius Red staining in TGF-β1 stimulated fibroblasts, while maintaining high cell viability. Higher dose (10 μM) of this compound reduced the viability of these cells. Taken microphotographs revealed fewer elongated, stress fiber-rich myofibroblasts in the treated group.

Compounds in low concentrations exhibits potent antifibrotic effects *in vitro* by suppressing TGF-β1-mediated collagen synthesis and myofibroblast differentiation without compromising fibroblast viability.

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IN VIVO TOXICITY PROFILE OF NOVEL HETEROCYCLIC DERIVATIVES LES-6287 AND LES-6294 IN C57BL/6 MICE

Yuliia Kozak[1,2], Nataliya Finiuk[1,2], Iryna Ivasechko[1,2], Nazar Manko[1], Olha Klyuchivska[1] Roman Lesyk[2], Rostyslav Stoika[1]

[1]Institute of Cell Biology of the National Academy of Sciences of Ukraine, Lviv, Ukraine; [2]Molecular Design Center, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.

Novel heterocyclic derivatives are actively explored as anticancer candidates with the potential to improve the efficacy-toxicity balance compared with conventional chemotherapeutics. Doxorubicin remains a widely used reference drug but its clinical utility is limited by pronounced systemic and organ-specific toxicities, including myelosuppression and hepatotoxicity. Therefore, early preclinical toxicological evaluation with a focus on delayed toxic effects is essential to identify safer lead structures before further development.

To assess the *in vivo* short-term repeated-dose toxicity and delayed toxicological effects of the novel heterocyclic derivatives Les-6287 and Les-6294 using doxorubicin as a toxic reference anticancer agent.

C57BL/6 mice received Les-6287 or Les-6294 intraperitoneally for 3 consecutive days at a cumulative dose of 500 mg/kg, while doxorubicin was administered at a cumulative dose of 20 mg/kg. Toxicity was evaluated over a 22-day study period based on body weight changes, hematological parameters, and organ weight indices.

Both test compounds demonstrated favorable safety profiles with minimal body weight changes (≤6% variation), preserved white blood cell counts, stable neutrophil-to-lymphocyte ratios, and normal organ-to-body weight ratios. In contrast, doxorubicin induced severe progressive weight loss (37% reduction), significant myelosuppression (2.4-fold decrease in leukocytes), marked elevation in neutrophil-to-lymphocyte ratio (4.5-fold increase by day 22), and hepatotoxicity (1.4-fold reduction in liver-to-body weight ratio).

The heterocyclic derivatives Les-6287 and Les-6294 exhibit favorable preclinical safety profiles without the systemic, hematological, or organ-specific toxicities typically associated with conventional cytotoxic agents, supporting their further development as therapeutic candidates with improved tolerability.

The work was supported by the National Research Foundation of Ukraine under grant agreements No. 2023.03/0104 (biological studies) and No. 2023.05/0021 (chemical studies).

All animal procedures were reviewed and approved by the Bioethics Committee of the Institute of Cell Biology, National Academy of Sciences of Ukraine (protocol No. 2025-1, 05 February 2025).

PROSTATE CANCER CELLS ARE SENSITIVE TO COMBINED METABOLIC TREATMENT BASED ON AMINO ACID DEPRIVATION AND GLYCOLYSIS INHIBITION

Nikita Polishchuk[1,2], Oleh Stasyk[1]

[1]Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine;
[2]Ivan Franko National University of Lviv, Lviv, Ukraine.

More than 1.4 million new cases of prostate cancer are diagnosed annually worldwide with mortality exceeding 370,000 cases per year. Despite advances in radiotherapy and chemotherapy, treatment efficacy remains limited by insufficient cytotoxicity and the development of resistance. Therefore, there is a huge interest in novel therapeutic strategies targeting tumor-specific metabolic vulnerabilities.

Arginine Deprivation Therapy (ADT) has demonstrated promise as potential anticancer therapy. However, its long-term efficacy as a monotherapy is often limited by insufficient cytotoxic effect. In this study, we investigated the effects of recombinant human arginase (rhARG1) combined with bacterial asparaginase with glutaminase activity, or the glutaminase 1 (GLS1) inhibitor CB-839 on the proliferation and viability of human prostate cancer PC-3 cells and murine prostate cancer RM1-BM cells. Lactate production was analyzed as a marker of the Warburg effect under amino acid starvation conditions.

Treatment with CB-839 (1 μM), or bacterial asparaginase, induced a cytostatic effect in PC-3 cells, while the effect of CB-839 was significantly enhanced when combined with arginine deprivation. In contrast, the combination of recombinant human arginase and bacterial asparaginase showed only moderate effect in PC-3 cells. Additional analysis of amino acid metabolism inhibitors demonstrated that azaserine, a glutamine antagonist, exhibited selective cytotoxicity under glutamine-deficient conditions. In RM1-BM cells, glutamine deprivation or recombinant human arginase treatment completely inhibited proliferation within 24 h. Importantly, the combination of recombinant human arginase and bacterial asparaginase induced elevated time-dependent tumor cell death. Unexpectedly, amino acid deprivation also significantly increased lactate production, tentatively indicating activation of the Warburg effect and enhanced glycolytic metabolism under metabolic stress. Furthermore, inhibition of glycolysis significantly enhanced the cytotoxic effects of ADT after 48 h of the treatment.

Our findings suggest that combining ADT with inhibitors of glutamine metabolism and/or glycolysis may represent a promising strategy for improving metabolic cancer therapy and warrant further investigations *in vitro* and *in vivo*.

PROMOTER-DEPENDENT REGULATION OF β-GALACTOSIDASE DEGRADATION IN THE METHYLOTROPHIC YEAST *KOMAGATAELLA PHAFFII*

Pelypyshyn Uliana[1], Dmytruk Kostyantyn[1], Dmytruk Olena[1], Sibirny Andriy[1,2]

[1]Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine;
[2]University of Rzeszow, Rzeszow, Poland.

The methylotrophic yeast *Komagataella phaffii* is a convenient model for studying the selective degradation of cytosolic proteins. The investigation focused on evaluating how different transcriptional regulatory modes influence protein inactivation and degradation of the heterologous β-galactosidase (Lac4) protein fused to GFP (green fluorescent protein). Protein expression was controlled by methanol-inducible (*AOX1* - alcohol oxidase, *FLD1* - formaldehyde dehydrogenase, *DAK1* - dihydroxyacetone kinase, and *FBP1* - fructose-1,6-bisphosphatase), ethanol-inducible (*ICL1* - isocitrate lyase), and constitutive (*GAP1* - glyceraldehyde-3-phosphate dehydrogenase) promoters.

To create recombinant strains, plasmid vectors with the *LAC4* gene under selected promoters were constructed and integrated into the yeast genome. Construct accuracy was confirmed by restriction analysis and PCR. Preliminary screening of yeast transformants on X-Gal medium visually assessed β-galactosidase activity through colony blue colour intensity. Cultivating recombinant strains in induction media, then transferring to glucose-containing medium, will allow us to evaluate enzyme inactivation rates depending on promoter regulation type.

THE NOVEL TRANSCRIPTION FACTOR IS INVOLVED IN NEGATIVE REGULATION OF RIBOFLAVIN BIOSYNTHESIS IN *CANDIDA FAMATA* YEAST

Serhii Romanov[1], Dariia Volkova[1,3], Liubov Dzanayeva[1], Andrii Tsyrulnyk[1], Liubov Fayura[1], Dariya Fedorovych[1], Kostyantyn Dmytruk[1], Andrii Sybirny[1,2]

[1]Institute of Cell Biology, Lviv, Ukraine;
[2]Rzeszow University, Rzeszow, Poland;
[3]Ivan Franko National University, Lviv, Ukraine.

Yeasts possess numerous transcription factors that control the expression of genes involved in metabolism, including both structural and regulatory genes. The expression of the same gene may be activated or repressed by different transcription factors, while the same transcription factor may activate or repress different target genes. Such complex interactions efficiently orchestrate the metabolic state of the yeast cell in accordance with environmental conditions.

A good example of transcription factor interplay is the regulation of riboflavin biosynthesis in the so-called flavinogenic yeasts of the genera Candida and Debaryomyces. The well-known phenomenon of vitamin overproduction under iron starvation is mediated by the positive regulator Sef1. Negative regulators have also been studied, including Sfu1 and the recently discovered Sef2. Sef2 was shown to repress riboflavin biosynthesis fivefold when the encoding gene was overexpressed in the wild-type strain VKM Y-9. A similar effect was observed in the overproducing strain AF4 harboring a defective version of the SEF2 gene lacking 130 amino acid residues. Deletion of this gene led to a sixfold and fourfold increase in riboflavin production in iron-replete and iron-depleted media, respectively.

To study potential interactions between Sef1 and Sef2 proteins, we performed relative expression analysis in the wild-type strain L20105 and the sef2Δ mutant grown in Fe+ and Fe− media. The expression of the SEF1 gene was found to be 2.5 times lower in the mutant strain compared to the wild type under iron starvation and 2 times higher under normal iron supply. The expression of the structural gene RIB6, encoding 3,4-dihydroxy-2-butanone-4-phosphate synthase, was 8 times higher in the sef2Δ mutant.

These results strongly indicate the negative effect exerted by Sef2 on riboflavin biosynthesis and demonstrate its possible interaction with the previously studied Sef1, thereby expanding current knowledge of the yeast regulome. Further studies are required to fully elucidate the iron-dependent signaling pathway in flavinogenic yeasts.

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