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INFLUENCE OF MICROPLASTICS AND HERBICIDES ON MICROALGAE

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Nowadays, plastics are a big problem, causing enormous pollution of the environment. The production and use of plastic increase every year, which means that more and more plastics in the form of micro and / or nanoplastics are released into the environment. Due to its durability, there is an accumulation in individual elements of the environment and living organisms. Microplastics are small pieces of plastic less than 5 mm in size that are found in fresh and marine waters. It can come from a variety of sources, such as packaging, personal care products, clothing, and the breakdown of large plastic particles. There are many types of plastics that differ in properties. The most common polymers found in seawater are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA), polyethylene terephthalate (PET) and polyvinyl alcohol (PVA).

The presence of this type of micropollutants is a significant problem in the aquatic environment, affecting the living organisms. In addition to the toxicity of the polymers or the additives they contain, microplastics are capable to interact with other pollutants, including pesticides, with implications for potential toxic effects to aquatic organisms. Moreover, microplastic particles and stable xenobiotics susceptible to adsorption to microplastics (especially non-polar ones) can accumulate in subsequent elements of the trophic chain.

Microplastics can interact with algae in several ways, including through physical damage: it can mechanically damage the algae, which can impair their function and make them more susceptible to pathogens, and through toxin adsorption: where it can bind and carry harmful chemicals such as toxins to aquatic organisms.

Issues related to the presence of microplastics in the environment are a new topic. Due to the very large number of pollutants and the interaction of these compounds with microplastics, there is a need to undertake research in this field

VIABILITY AND INVASIVE POTENTIAL OF HUMAN GLIOBLASTOMA CELLS AFTER TREATMENT WITH HAWTHORN FRUIT EXTRACTS (*CRATAEGUS* L.)

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Numerous studies have shown that the polyphenolic compounds of hawthorn fruit extracts may be responsible for the anticancer effect. To answer this question, we conducted studies on extracts obtained from hawthorn berries, evaluating their effect on viability, morphology and invasive potential on the U87MG human glioblastoma cell line derived from an aggressive primary malignant brain tumor. Material from six species of hawthorn (*Crataegus* L.) was used in the study, namely the fruits of *C. monogyna, C. rhipidophylla, C. x subsphaericea, C. laevigata x rhipidophylla x monogyna, C. macrocarpa* and *C. laevigata*, collected in October in Błażowa and Piątkowa near Rzeszów (Poland).

First, the cytotoxic effect of increasing the concentrations (10-750 μg/ml) of the extracts on the U87MG human glioblastoma cell line was analyzed. All tested types of extracts inhibited the viability of glioblastoma cells, mainly in a concentration-dependent manner. A dramatic decrease in cell viability was observed at 250 μg/ml. A concentration-dependent decrease in full-length PARP1 (poly (ADP-ribose) polymerase) was demonstrated during treatment with hawthorn fruit extracts, indicating that the extracts may promote apoptotic cell death. Also, all Crataegus berry extracts significantly affected glioblastoma cell morphology. and did not form lamellipodia, the actin-rich frontal ridges characteristic of migrating cells. In addition, an increased number of cells was observed after the procedure, with features of apoptotic cell death, such as cell shrinkage and formation of apoptotic bodies (blebs).A significant decrease in Akt phosphorylation in glioblastoma cells was observed during incubation of cells with almost all extracts with the deepest effect of *C. laevigata x rhipidophylla x monogyna* berry extracts FAK inhibition, together with a decrease in Akt activity after treatment with the tested extracts, suggested that the obtained extracts can profoundly reduce cell growth and migration, and thus invasiveness U87MG glioblastoma cells.

In conclusion, it was shown that blueberry extracts, in particular extracts from *C. laevigata x rhipidophylla x monogyna*, had the strongest cytotoxic effect, resulting in a significant reduction in cell viability, induction of processes leading to apoptotic cell death and inhibition of prosurvival signaling pathways.

EXPRESSION OF HETEROLOGOUS LACTATE DEHYDROGENASE GENES IN THE YEAST *OGATAEA POLYMORPHA*

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Lactic acid is one of the most commercially valuable compounds with a wide range of application in food, chemical and pharmaceutical industries. The production cost of lactic acid remains relatively high despite all the recent developments in this field. Most of the lactate obtained worldwide comes from the microbial fermentation process where yeast appear as one of the most promising producers. Most yeasts usually do not produce lactic acid, although introducing lactate dehydrogenase genes from other organisms allows to obtain strains for lactate production. Advantages of using yeast for lactic acid production include their ability to utilize cheap and renewable substrates, and tolerance to environmental factors such as low pH and in the case of thermotolerant yeasts like *Ogataea polymorpha*, also high temperatures.

In this study, a metabolic engineering approach was applied to the wild type strain of thermotolerant methylotrophic yeast *O. polymorpha*. Lactate dehydrogenase genes from three different organisms (*Lactiplantibacillus plantarum, Plasmodium falciparum, Bos taurus*) were selected for expression under the control of the strong constitutive promotor of glyceraldehyde-3-phosphate dehydrogenase gene. Yeast transformants that produced sufficient amounts of lactic acid were firstly selected on medium with addition of pH indicator bromophenol blue. Lactate biosynthesis results in decrease of pH that can be visually detected after 24 hours of incubation at 37°C due to the color change of the added pH indicating compound from blue to yellow. Further investigation of the effect of carbon source, agitation and addition of calcium carbonate as a neutralizing agent on lactic acid production was performed with selected *O. polymorpha* transformant strain. Activity of lactate dehydrogenase and alcohol dehydrogenase in engineered yeast strains was studied as well.

THE USE OF FOURIER INFRARED SPECTROSCOPY (FTIR) IN THE STUDY OF THE REACTION OF FOREST HERBACEOUS PLANT SPECIES TO TEMPERATURE INCREASE ON THE EXAMPLE OF YELLOW ARCHANGEL (*GALEOBDOLON LUTEUM*)

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Long-term and even short-term temperature disturbances affect vegetation in many ways, often leading to irreversible changes at various levels of biological organization, from molecules to organized tissues. Fourier infrared spectroscopy was used to detect these changes. Thanks to this technique, it is possible to identify the functional groups of important groups of chemical compounds that are part of the plant's building substances. Comparison of spectra obtained from plants subjected to an environmental factor of different intensity also allows for the detection and recognition of the nature of changes in the biochemical profile of the plant. The recorded changes will reflect disturbances in the functioning of plants under the influence of stressful environmental factors.

In order to analyse the physiological response to increase temperature, the yellow archangel (*Galeobdolon luteum*) was selected as the test organism, which is a forest herbaceous plant that plays an important role in the functioning of the forest ecosystem. Due to its wide and common occurrence and thus the impact on many biological processes taking place in the forest environment, it became important to learn about its reaction to environmental changes. About 200 yellow archangel individuals growing in a breeding room in climatic conditions reflecting the conditions in European forests, i.e. 21˚C day and 13˚C night, with a period of 16 hours of day and 8 hours of night, were used in the experiment, which was the control of the experiment. The next step was to increase the temperature for the above-mentioned day and night at 4˚C. Samples from two experimental setups were collected for the first three days and after a week at 4 predetermined hours during the day at: morning, noon, afternoon and middle of the night. By analysing the material using infrared spectroscopy, changes in the composition of the main plant tissue components were identified. There were clear differences in the absorbance values obtained for the peaks corresponding to the functional groups attributed to proteins (1619 cm^{-1}), carbohydrates (1029 cm^{-1}) and lipids (2926 cm^{-1}) for the test sample after exposure to increase temperature. These results indicate changes in plant metabolism adapting to higher temperature. The observed differences in the biochemical profile were confirmed using one of the classic chemometric method - principal component analysis (PCA).

The obtained results indicate that thanks to the use of infrared spectroscopy, it is possible to identify visible differences between two experimental systems subjected to environmental stress. They also clearly confirm the possibility of using the yellow archangel as a model for analysis of the adverse effect of temperature increase on forest vegetation. However, further targeted analyses, such as the assessment of the content of metabolites and chaperones, are needed to identify in detail the nature of changes in the biochemical profile of the archangel under the influence of thermal stress. To sum up, the obtained research results show the great usefulness of infrared spectroscopy in quick detection and determination of the nature of sudden changes occurring in the plant organism under the influence of temperature change.

BIOSYNTHESIS OF VALUABLE COMPOUNDS WITHIN *PLANKTOCHLORELLA NUREKIS CELLS* UNDER SALICYLATE TREATMENT

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Microalgae are unicellular, photosynthetic organisms found in both freshwater and marine environments. They have the ability to utilize sunlight and carbon dioxide to produce a variety of valuable metabolites, such as phenolic compounds, carotenoids, and polyunsaturated fatty acids. Due to their rapid growth, high biomass productivity, and efficient water utilization, microalgae are an attractive option for the production of bioactive substances. Stress conditions, such as nitrogen depletion, intensive light, and phytohormone treatment, can modify algal cell growth and metabolite biosynthesis.

The aim of our studies was to evaluate the influence of salicylic acid and methyl salicylate (0.1, 1.0, and 10 μM) on the growth and biosynthesis of phenolic compounds, carotenoids, and unsaturated fatty acids within the cells of a recently discovered microalga *Planktochlorella nurekis*, that was modified with colchicine and cytochalasin-B.

The treatment with the highest concentration of salicylic acid resulted in a significant increase in the size of microalgal cells, reaching a diameter of up to 11 μm. In contrast, other cells did not exceed 7 μm in size. The rising concentrations of salicylates caused a gradual increase in the accumulation of carotenoids. The highest levels of carotenoids were found in cells stressed with 10 μm of both salicylates, with more significant growth under methyl salicylate treatment. Generally, the biosynthesis of phenolic compounds was stimulated after the application of methyl salicylate only. PAL (*L*-phenylalanine ammonia-lyase) activity and total phenols levels were significantly increased in the cells treated with 10 μm of both phytohormones. However, the peak PAL activity and concentration of phenolic compounds were observed in *P. nurekis* cells exposed to 10 μm methyl salicylate. The salicylates treatment also caused an increase in the levels of monounsaturated fatty acid (MUFA) fraction.

FLAVOCYTOCHROME *b_2* *OGATAEA POLYMORPHA* AS A TOOL FOR L-LACTATE ANALYSIS AND "GREEN" SYNTHESIS OF NANOMATERIALS

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The yeast L-Lactate-cytochrome *c*-oxidoreductase (EC 1.1.2.3; flavocytochrome b_2, Fcb_2) having absolute specificity for L-Lactate (Lact) is a promising biocatalyst in analytical methods for Lact determination.

In our department the technologies of Fcb_2 isolation, purification and stabilization were developed, several Fcb_2-based analytical methods for Lact determination were proposed, including spectrophotometric (SP) methods and amperometric biosensors (ABS). Additionally, the purified Fcb$_2$ was applied as a tool for “green” synthesis of nanomaterials (NMs).

The aims of our study, based on the usage of Fcb$_2$, are the following: to improve the SP method; to develop the highly sensitive ABSs using red-ox NMs as electroactive mediators; to test the proposed methods for Lact analysis in real samples of biological liquids; to obtain and characterize “green” NMs.

As the results of our research, the enzymatic-chemical SP method was improved and tested on the real samples of yogurts, as well as of mammallian bloods. The positive effect of the red-ox nanomediators on the amplification of electrochemical communication between the immobilized Fcb_2 and the electrode surface in the fabricated ABSs was demonstrated. ABSs exhibited high sensitivities and selectivity to Lact, fast responses, high stabilities and low limits of detection. The applicability of the most sensitive ABS was demonstrated on the samples of commercial yogurts. Thus, the described Fcb_2-based methods for Lact analysis may be useful in the laboratories of food industry and in clinical diagnostics. A number of green-synthesized hexacyanoferrates of transition metals (gHCFs) were obtained and characterized. The main advantages of gNMs are the low cost of their synthesis, lack of toxic chemicals, simplicity of procedure, high adaptability and the presence of functional groups on their surface. We have demonstrated here that the obtained gHCFs are promising platforms for enzymes immobilization during construction of ABSs.

DIFFERENTIAL ACTION OF PYRROLIDINEDIONE-THIAZOLIDINONE HYBRID MOLECULES ON LYMPHOCYTES OF PATIENTS WITH ACUTE AND CHRONIC LYMPHOCYTIC LEUKEMIA

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Human blood cells are a convenient target to assess the potential of anticancer drugs used for the treatment of leukemia. Here we aimed to estimate cytotoxicity of novel pyrrolidinedione-thiazolidinone hybrid molecules Les-6287 and Les-6294 towards mononuclear cells of peripheral blood of patients with acute and chronic leukemia.

The IC_{50} of toxic effect of Les-6287 equaled 0.33-0.74 μM for lymphocytes isolated from blood of patients with acute T-lymphocytic leukemia and 0.73-0.79 μM – for Les-6294 at 24 h treatment. IC_{50} of Les-6287 was 19.03-19.96 μM for lymphocytes of patient with chronic lymphocytic leukemia before chemotherapy. Les-6287 demonstrated IC_{50} of 8.56 μM towards isolated lymphocytes from patient with chronic lymphocytic leukemia after treatment with bendamustine, and similar effect (IC_{50} = 9.36 μM) was found towards isolated lymphocytes from patient with chronic lymphocytic leukemia recurrence after 4 years of remission. Les-6294 demonstrated lower antineoplastic activity towards isolated lymphocytes of patient with chronic lymphocytic leukemia before chemotherapy, after treatment with bendamustine, and in case of chronic lymphocytic leukemia recurrence after 4 years of remission. Lymphocytes isolated from blood of healthy donor were more resistant towards Les-6287 (IC_{50} = 67.1 μM) and Les-6294 (IC_{50} = 36.8 μM). Les-6287 induced mitochondria-dependent pathway of apoptosis and DNA damage in lymphocytes of patient with acute T-lymphocytic leukemia and chronic lymphocytic leukemia before treatment.

All experiments with isolated human lymphocytes were approved by Ethics Committee of the Institute of Cell Biology of National Academy of Sciences of Ukraine (protocols No 2 from January 27, 2019 and No 2 from October 7, 2020) and with written consent of donor.

CAGE AMIDES AND IMIDES AND THEIR ANTIMICROBIAL PROPERTIES

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Infectious diseases have become a major challenge to the global health system as killing millions of people worldwide. Compounds with unusual three-dimensional structures have frequently attracted the attention of chemists as possible synthetic targets, because the shape of chemical structures in drug discovery is a crucial component for modulation of biological activity. VP-4606 bearing 3-azabicyclo[3.2.1]oct-6-ene cage fragment, has strong antimicrobial effect on methicillin-resistant *Staphylococcus aureus* (ATCC 43300). VP-4539 with bicyclo[2.2.2]octene motif demonstrated high activity towards *Cryptococcus neoformans* (ATCC208821). Both compounds were detected by screening which was performed by the Community for Open Antimicrobial Drug Discovery (CO-ADD). Further, we have monitored their biological activity using MTT and CFU assays.

VP-4539 shows antifungal activity towards *Candida albicans* laboratory (ATCC 885-655) and drug resistant (N12) strains. Moreover, the differences in the antifungal activity of VP-4539 compound towards *Candida albicans* laboratory (ATCC 885-655) and resistant (N12) strains indicates the potential of this substance as perspective chemical constructs to overcome fungal multidrug-resistant infections. Both compounds demonstrated low cytotoxicity towards pseudo-normal mammalian cells, namely human keratinocytes of HaCaT line and murine fibroblasts of Balb/c 3T3 line, as well as mitogen-activated lymphocytes from peripheral blood of healthy donor.

Cage amides and imides are perspective synthetic compounds to be used like antimicrobial agents and capable to overcome drug resistance in microorganisms. They possess low toxicity towards pseudo-normal and normal mammalian cells. Motif changing in cage amides and imides provide different biological activity without increasing general toxicity. Their main disadvantage is poor water solubility which could be solved via their immobilization on the amphiphilic polymers

COMPARATIVE STUDIES OF THIOSEMICARBAZONE DERIVATIVES AS POTENTIAL INDUCERS OF IMMUNOGENIC CELL DEATH IN MURINE CARCINOMAS

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Immunogenic cell death (ICD) is a novel mode of action of several anticancer drugs, which is associated with activation of tumor-specific immune responses. It can act in combination with the direct killing functions of chemotherapeutic drugs to enhance their effects, which is important for the long-term success of anticancer therapies. ICD is provoked by various commonly used chemotherapeutics, including adriamycin (Adr) and oxaliplatin. Recently this ability was also claimed for the novel class of metal-binding compounds – thiosemicarbazones (TSCs). The primary aim of the current study was to compare cytotoxic and ICD-inducing properties of previously described TSCs (COTI-2, triapine) and novel COTI-2 derivative (COTI-NMe_2), synthesized by group of Prof. C. Kowol from the University of Vienna.

Methods: cell culture studies *in vitro*, trypan blue assay, tumor cell inoculation *in vivo*, morphophysiological analysis of tumor-bearing animals, haemocytometry. B16F10 and CT26 murine and SW480 human cancer cells were treated with triapine, COTI-2, COTI-NMe_2 and Adr for 24 hours to determine toxicity and LC_{50} using trypan blue assay. TSC-treated (25 μM) and Adr-treated (1 μM) B16F10 and CT26 cells were used for inoculation of C57BL/6 and Balb/C mice, respectively. As a negative control, tumor cells, subjected to multiple freeze-thaw cycles (-196 to +20°C, 3x), were used. For animal immunization studies, C57BL/6 and Balb/C mice were reinoculated with untreated B16F10 and CT26 cells respectively ($5 \cdot 10^5$ cells per animal) 14 days after initial inoculation. Blood samples were collected from surviving animals 30 days after the immunization and analyzed on automatic blood analyzer Dymind DF51 Vet.

Cytotoxic activity of COTI-NMe_2 was 3-fold higher than of COTI-2 towards B16F10 and CT26 cells (LC_{50} of COTI-NMe_2 = 7.2 and 3.7 μM, respectively vs 24.3 μM and 8.7 μM for COTI-2) and comparable to LC_{50} of triapine (9.4, 3.3 μM). However, no difference in activity for all studied TSCs were observed for SW480 cells (LC_{50}(COTI-2) = 6.9 μM, LC_{50}(COTI-NMe_2) = 7.2 μM, LC_{50}(triapine) = 8.5 μM). B16F10 melanoma, despite being the most resistant to TSCs action *in vitro*, was found to be highly susceptible to drug-induced vaccination *in vivo*. We observed a low frequency of secondary tumors (20-40%) during reinoculation by alive melanoma cells 14 days after the vaccination. On the contrary, vaccination studies on CT26 carcinoma cells resulted in high frequency of primary tumors (75-100%) both for TSCs and Adr. These data clearly indicate the crucial role of tumor microenvironment in ICD induction, which is independent from sensitivity of the same tumor cells to anticancer drugs.

Thus, based on these data we can conclude that B16F10 is immunologically “hot” tumor, while CT26 carcinoma is immunologically “cold” and should be replaced with another model from our biobank (e.g. MCA205 mouse fibrosarcoma) in our further studies. COTI-NMe2 is a more potent cytotoxic agent than COTI-2 and comparable to triapine. However, additional data is needed for further understanding of its ICD-inducing potential *in vivo*

ENGINEERING OF *OGATAEA POLYMORPHA* STRAINS WITH ABILITY FOR HIGH-TEMPERATURE ALCOHOLIC FERMENTATION OF CELLOBIOSE

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Plant cell walls are a promising source of sugars for biofuel production. The main complex sugars included in their composition are cellulose (a polymer of glucose) and hemicellulose (a heterogeneous polymer of pentoses, hexoses, and sugar acids). Successful conversion of cellulosic biomass into biofuel will require organisms capable of efficiently utilizing xylose as well as cellodextrins and glucose. *Ogataea polymorpha* is a thermotolerant yeast that naturally metabolizes xylose, making it a good candidate for biofuel production. However, one of the disadvantages of using *O. polymorpha* for producing cellulosic biofuels is its inability to naturally ferment cellodextrins such as cellobiose.

Two cellobiases were selected for the metabolism of cellobiose in the yeast *O. polymorpha*: β-glucosidase (gh1-1) from *Neurospora crassa*, which hydrolyzes cellobiose into two glucose molecules, and cellobiose phosphorylase (CBP) from *Saccharophagus degradans*, which cleaves the disaccharide into glucose and glucose-1-phosphate. Modified cellobiose transporter proteins, CDT-1m and CDT-2m from *N. crassa*, were selected due to their high affinity for cellobiose. Overexpression of *gh1-1*/*CDT-1m*, *CBP*/*CDT-1m*, *gh1-1*/*CDT-2m*, and *CBP*/*CDT-2m* gene combinations in the best ethanol producer (*BEP*/cat8Δ) of the yeast *O. polymorpha*, did not have a significant positive effect on the fermentation of 10% cellobiose. UV mutagenesis of the transformants characterized by the best growth on cellobiose allowed the obtaining of mutant strains with a 4-fold increase in ethanol production from cellobiose, reaching a maximum of 4.2 g ethanol/L during cellobiose alcoholic fermentation at 45°C.

THE MOLECULAR MECHANISMS OF INDUCED RESISTANCE TO ARGININE STARVATION THERAPY

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The effectiveness of arginine starvation therapy (AST) may be hindered by the emergence of resistant cell subpopulations, which often play a significant role in driving malignancy. As a result, there is a critical need to conduct the research of potential molecular targets to overcome the acquired resistance of tumor cells. The aim of our work was to create a resistant to AST cell subline and investigate the molecular mechanisms responsible for the development of induced resistance in tumor cells.

In our study, we examined the impact of arginine limitation on the growth and viability of cancer cells, as well as their cell-functional characteristics and adhesive properties. Additionally, we compared the activation of key signalling pathways associated with the response of tumor cells to arginine deficiency and the development of resistance in both parental SAS and resistant SASR9 cell sublines.

Our data indicate that the SASR9 cell subline exhibits enhanced survival rates in the absence of arginine and displays altered functional properties (clonogenic protential, migration, motility) that contribute to a more aggressive phenotype. Interestingly, the induced resistance observed in SASR9 cells is does not depend on c-myc/HIF1α-mediated *ASS1* gene reexpression. Furthermore, compared to the "parental" SAS line, SASR9 cells exhibit elevated activity in pro-survival signaling pathways such as PI3K/AKT/mTOR, JNK, and MAPK. Additionally, SASR9 demonstrate increased activity in autophagy processes and higher tolerance to ER stress induced by arginine deficiency. It is worth noting that SASR9 exhibited a pronounced epithelial-mesenchymal transition (EMT) phenotype, characterized by elevated expression levels of key markers including *MMP2, vimentin,* and the Snail transcription factor.

In summary, the SASR9 cells, resistant to arginine deprivation therapy, displayed a distinct and more aggressive metastatic phenotype compared to the “parental” SAS cells. This observation led us to hypothesize that the enhanced metastatic behavior of SASR9 cells may be attributed to the induction of EMT progression.

THE *KOMAGATELLA PHAFFII ATG46* (*ACG1*) GENE, ENCODING β-1,6-N-ACETYLGLUCOSAMINYL TRANSFERASE, IS INVOLVED IN THE AUTOPHAGY OF CYTOSOMAL AND PEROXISOMAL PROTEINS

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One of the most efficient producers of recombinant proteins of industrial importance is the methylotrophic yeast *Komagataella phaffii*. Efficient producers should be characterised by minimising the degradation of cytosolic recombinant proteins. The mechanisms of degradation of cytosolic proteins in *K. phaffii* have not yet been elucidated, but there is evidence that they are partially degraded by the autophagic pathway.

To identify factors influencing this process, a system developed for the selection of recombinant *K. phaffii* strains with impaired autophagic degradation of the heterologous model cytosolic protein (yeast β-galactosidase) was used for insertional tagging of the genes involved in cytosolic protein degradation. Over 1000 insertional mutagenesis colonies have been screened. Prospective colonies were analysed for the presence of residual β-galactosidase activity. Three tested transformants showed increased β -galactosidase activity compared to the original strain.

The insertion cassette disrupted the open reading frame of the gene encoding β-1,6-N-acetylglucosaminyltransferase in one of the obtained strains. A recombinant strain with a deletion of this gene has also been obtained. Compared with the parental strain with native β-1,6-N-acetylglucosaminyltransferase, the rate of β-galactosidase enzyme degradation was two times slower in the insertion mutant and 1.5 times slower in the deletion strain. The degradation rate of native *K. phaffii* cytosolic and peroxisomal enzymes, formaldehyde dehydrogenase, formate dehydrogenase and alcohol oxidase showed similar trends to β-galactosidase - slower degradation in the deletion and insertion mutants compared to the wild-type strain, but faster protein degradation compared to the strain completely deficient in autophagy. Our findings suggest that gene designated *ACG1* (or ATG46), encoding β-1,6-N-acetylglucosaminyltransferase, plays a role in the autophagy of cytosolic and peroxisomal proteins in the methylotrophic yeast *K. phaffii*.

HYBRID PYRIDINE-THIAZOLE DERIVATIVE LES-6485

AS POTENTIAL POLY(ADP-RIBOSE) POLYMERASE INHIBITOR

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Targeting DNA repair pathways is an important driver of anticancer chemotherapy. Tumor cells are known to be more susceptible to DNA damage than normal cells and, in order to survive, they rely on specific pathways of functional DNA reparation. Our work was aimed on study of the mechanism of cytotoxic action of new synthetic thiazol compound Les-6485 (4-(2-{1-(2-fluorophenyl)-3-[4-methyl-2-(pyridin-2-ylamino)-thiazol-5-yl]-3 oxopropylsulfanyl}-acetylamino)-benzoic acid ethyl ester), as a potential PARP inhibitor, in tumor and normal cells lines.

The Les-6485 was used to treat 10 cancer cell lines of various tissue genesis. Its IC_{50} ranged from 2.79 μM to 8.05 μM for tumor cells, however, this compound was not harmful to lymphocytes of the peripheral blood of healthy human donors. Preincubation of tumor cells with Fluzaparib (known inhibitor of PARP1) reduced in 3 times the activity of the Les-6485. Les-6485 affected the nativity of DNA and induced morphological changes in the cell nucleus similar to the mitotic catastrophe. It demonstrated red-yellow fluorescence in a region close to the nucleus of MCF-7 cells in time-dependent manner. This compound showed synergistic activity with the O6-Methylguanine-DNA Methyltransferase inhibitor.

Since the preincubation of tumor cells with PARP1 inhibitor reduced their sensitivity to the Les-6485, it was assumed that the mechanism of its action is connected with PAPR1 inhibition. Thus, the action of Les-6485 could be of interest when it is used in combination with DNA repair inhibitors for study of the effects of synthetic lethality or in combination with DNA damage agents.

METABOLIC ENGINEERING OF THE YEAST *CANDIDA FAMATA* FOR IMPROVEMENT OF RIBOFLAVIN PRODUCTION FROM XYLOSE.

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Flavinogenic yeast *Candida famata* are characterized by the ability to grow on unconventional substrates, in particular on xylose. It is important to expand the spectrum of substrates and to increase the yield of riboflavin in particular from xylose-containing lignocellulosic renewable raw materials.

The metabolism of xylose in yeast starts with its reduction by xylose reductase (XR, EC 1.1.1.21) to xylitol, which is further oxidized to xylulose, this reaction is catalyzed by xylitol dehydrogenase (XDH, EC 1.1.1.9). Xylulose, at the next stage, is phosphorylated by xylulokinase (XK, EC2.7.1.17) to xylulose-5-phosphate. This sugar in the non-oxidizing phase of PPP is converted by phosphopentoepimerase to ribulose-5-phosphate which is used as the riboflavin precursor. Strains with constitutively overexpressed *XYL1*, *XYL2* and *XYL3* genes could be characterized with elevated riboflavin production from xylose through the better supplying the non-oxidizing stage of PPP with riboflavin precursor ribulose-5-phosphate.

To achieve this goal *C. famata XYL1* gene under control of strong constitutive *CfTEF1* promoter was cloned into pUC57_CfTEF1pr_DhTEF1tr_pGAL1_Cf_mazF_Ec_tAOX_Pp_NTC (6967 bp) vector. Obtained expression cassette was introduced into *C. famata* VKM Y-9 and BRP#6 strains, and the selection of *NTC* transformants was provided. The ability of resulted strains to biomass accumulation and riboflavin production in xylose medium was studied. *XYL1* overexpression in recombinant BRP#6 strain improved growth kinetics up to 1.3-1.5 times, while the production of riboflavin was about 1,3-2 times higher as compared to the initial strain. Correlation between riboflavin production in recombinant strains with overexpressed *XYL1* gene and activity of its enzyme will be studied.

IN VIVO STUDIES OF ICD-INDUCING PROPERTIES OF THIOSEMICARBAZONES TOWARDS C57BL/6 MICE INOCULATED WITH B1610/WT MELANOMA CELLS

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Immunogenic cell death (ICD)-based ''cancer vaccines'' are a promising area of scientific research to create novel effective cancer treatment schemes. The idea is that immunization of host organism (e.g., mouse) with cancer cells treated with ICD inducers should lead to activation of the immune response towards tumor. So, searching for novel ICD inducers and detailed studies of their mode of action are crucial tasks in current cancer therapy.

The aim of our study was the evaluation of ability of thiosemicarbazones (TSCs) to induce immunogenic cell death in C57BL/6 mice injected with B16F10/wt melanoma cells. Oxaliplatin (OXP) was used as a positive control in this study. Triapine (3-AP) the most prominent representative among α-N-heterocyclic TSCs. COTI-2, DpC and K2550 are novel TSCs.

It was found that KP-2550 is the most active immunogenic cell death inducer among the studied thiosemicarbazone derivatives in vivo. In particular, 60% of the mice injected with KP-2550-treated B16F10/wt melanoma cells had no tumor growth and survived more than 120^{th} days after the first inoculation of tumor cells. On the contrary, traditional ICD inducer OXP led to the survival of only 50% of animals.

Growth of B16F10/wt melanoma was accompanied by a 12-fold increase of the neutrophil to lymphocyte ratio (NLR) in the blood of control mice on the 30^{th} day after tumor inoculation. NLR in mice inoculated with TSCs-treated B16F10/wt cells was significantly lower ($P \leq 0.001$) than in animals injected with untreated melanoma cells (10^6 per mouse) on 120^{th} day of the experiment.

Immunization of mice with TSCs-treated B16F10/wt melanoma cells, besides significant protection of mice towards tumor re-inoculation, also led to normalization of WBC, RBC-, PLT- and HGB indices in their blood up to the levels of healthy animals.