НАЦІОНАЛЬНА АКАДЕМІЯ НАУК УКРАЇНИ
ІНСТИТУТ БІОЛОГІЇ КЛІТИНИ
РАДА МОЛОДИХ ВЧЕНИХ

КОНФЕРЕНЦІЯ МОЛОДИХ ВЧЕНИХ ІНСТИТУТУ БІОЛОГІЇ КЛІТИНИ

31 травня 2018 року

ЗБІРНИК ТЕЗ КОНФЕРЕНЦІЇ

Львів

NATIONAL ACADEMY OF UKRAINE
INSTITUTE OF CELL BIOLOGY
COUNCIL OF YOUNG SCIENTIST

CONFERENCE OF YOUNG SCIENTISTS OF INSTITUTE OF CELL BIOLOGY

May 31, 2018

ABSTRACTS

Lviv

10:00-10:10	**OPENING OF THE CONFERENCE** **Director of Institute of Cell Biology of NAS of Ukraine, Professor, Member of NAS of Ukraine, Andriy A. Sibirny**	
10:10-10:20	**Marta Semkiv** EFFECT OF TREHALOSE AND GLYCEROL ON THE RESISTANCE OF ENGINEERED *SACCHAROMYCES CEREVISIAE* STRAINS TO DESICCATION, FREEZE-THAW AND OSMOTIC STRESSES	**1**
10:20-10:30	**Orysia Ternavska** IMPROVEMENT OF ETHANOL PRODUCTION FROM CRUDE GLYCEROL BY YEAST *KOMAGATAELLA PHAFFII* WITH OVEREXPRESSION OF THE GENES *ADH1* AND *PDC1*	**2**
10:30-10:40	**Olena Kurylenko** THE ROLE OF TRANSCRIPTION FACTORS IN REGULATION OF GLUCOSE AND XYLOSE METABOLISM AND FERMENTATION IN THE NON-CONVENTIONAL YEAST *OGATAEA POLYMORPHA*	**3**
10:40-10:50	**Mariia Borbuliak** MITOCHONDRIAL TRANSPORTER Tmi1 IS INVOLVED IN REGULATION OF XYLOSE FERMENTATION IN *SCHEFFERSOMYCES STIPITIS*	**4**
10:50-11:00	**Serhii Chornyi** mTORC1 STATUS IN COLORECTAL CANCER AS A PREDICTIVE MARKER FOR ARGININE DEPRIVATION THERAPY	**5**
11:00-11:10	**Yana Petrovska** INTERACTIONS BETWEEN THE PROMOTER REGIONS OF STRUCTURAL GENES OF RIBOFLAVIN SYNTHESIS AND Sef1 TRANSCRIPTION FACTOR IN THE FLAVINOGENIC YEAST *CANDIDA FAMATA*	**6**
11:10-11:20	**Nadiya Shevchuk** ENGINEERING OF YEAST *OGATAEA POLYMORPHA* WITH EXPRESSION OF MODIFIED HEXOSE TRANSPORTERS	**7**
11:20-11:30	**COFFEE BREAK**	
11:30-11:40	**Yuliia Andreieva** IMPLEMENTATION OF CRISPR-CAS9 SYSTEM IN YEASTS *CANDIDA FAMATA* AND *OGATAEA POLYMORPHA*	**8**

11:40-11:50	**Nataliya Finiuk** DMAEM-CONTAINING CARRIERS FOR PLANT TRANSFORMATION POSSESSED LOW PHYTOTOXICITY AND NO MUTAGENIC PROPERTIES	**9**
11:50-12:00	**Olena Kolodii** CLONING AND EXPRESSION OF PUTATIVE RIBOFLAVIN EXCRETASE IN FLAVINOGENIC YEASTS	**10**
12:00-12:10	**Nazar Manko** ANTIMICROBIAL PROPERTIES OF HONEYBEE CHITOSAN DERIVATIVES	**11**
12:10-12:20	**Liubov Dzanaieva** EFFECT OF DELETION OF TRANSCRIPTION FACTORS ON XYLOSE FERMENTATION PERFORMANTS OF ENGINEERED *SACCHAROMYCES CEREVISIAE*	**12**
12:20-12:30	**Yuliya Kozak** IMPACT OF SELENOMETHIONINE ON THE ANTITUMOR ACTIVITY OF DOXORUBICIN AND THEIR COMBINED EFFECT ON THE GLUTATHIONE SYSTEM IN CELL LINES WITH DIFFERENT MECHANISMS OF RESISTANCE TO CHEMOTHERAPY	**13**
	Discussion and closing remarks **AWARDS**	

ABSTRACTS

EFFECT OF TREHALOSE AND GLYCEROL ON THE RESISTANCE OF ENGINEERED *SACCHAROMYCES CEREVISIAE* STRAINS TO DESICCATION, FREEZE-THAW AND OSMOTIC STRESSES

Marta Semkiv
Department of Molecular Genetics and Biotechnology

Baker's yeast *Saccharomyces cerevisiae* is used in the manufacture of bakery products, food and feed supplements, alcoholic fermentation etc. In biotechnological processes, yeast cells are exposed to stress factors (high concentration of sugars and ethanol, elevated temperature, drying or freezing etc.), which negatively affects their viability. Yeast possesses several stress protection systems. Increased accumulation of disaccharide trehalose and the glycerol synthesis are among them.

In the presented study we aimed to construct recombinant strains of *S. cerevisiae* with increased intracellular trehalose level or elevated glycerol production level and estimate resistance of the obtained recombinant strains to different stress factors.

Recombinant strains of *S. cerevisiae* with higher glycerol production (up to 19 g/L) were constructed on the base of BY4742 strain. The industrial ethanol-producing strain Y-563 was used as parental for construction of recombinant strains with up to 3.3-fold increase of the intracellular trehalose level. The resistance of the obtained recombinant strains to different stress factors was evaluated. Strain BY/TPI25/gpd1gpp2f/fps1 with the highest glycerol production level revealed the highest osmotolerance. Strains BY/TPI25/gpd1gpp2f (with increased glycerol production), 563/TSL1, 563/TPS1/2 and 563/TPS1/2/TSL1 (with increased intracellular trehalose content) showed higher viability after freeze-thaw than the corresponding parental strains. Studied recombinant strains didn’t reveal higher resistance to freeze-drying than the parental strains. Recombinant strain 563/TPS1/2/TSL1 with high trehalose content and the corresponding initial strain Y-563 were grown on the industrially used medium in pilot 7 L fermenter and afterwards tested. Recombinant strain cells showed higher activity during fermentation of sugar in sweet dough and longer retained its stability at 35°C.

IMPROVEMENT OF ETHANOL PRODUCTION FROM CRUDE GLYCEROL BY YEAST *KOMAGATAELLA PHAFFII* WITH OVEREXPRESSION OF THE GENES *ADH1* AND *PDC1*

Orysia Ternavska
Department of Molecular Genetics and Biotechnology

A broad development of biodiesel production caused the availability of a large amount of glycerol as by-product. This glycerol, which is also called crude or raw glycerol, contains methanol, heavy metals and other impurities, therefore its purification to food-grade glycerol is cost-prohibitive. Meanwhile crude glycerol is an attractive feedstock for 1,2-propanediol, 1,3-propanediol, 2,3-butanediol (used for synthesis of biodegradable polymers), dihydroxyacetone (used in cosmetic industry), succinate and citrate (used in food industry and as precursors for polymer synthesis and amino acids) production. One of the most important products that could be obtained from crude glycerol is the fuel ethanol. To be able to transform crude glycerol to ethanol, microorganisms have to efficiently utilize glycerol as a Carbon source and be able to withstand harsh conditions imposed by toxic compounds (methanol, heavy metals etc). An example of such a microorganism can be methylotrophic yeast *Komagataella phaffii* (formerly *Pichia pastoris*), which can grow on glycerol and methanol.

In order to improve the ethanol production from crude glycerol, we overexpressed homologous or heterologous *Saccharomyces cerevisiae* genes *PDC1* and *ADH1*, coding for pyruvate decarboxylase and alcohol dehydrogenase respectively, under the control of strong constitutive promoter of the *GAP1* gene (encodes glyceraldehyde-3-phosphate dehydrogenase) in *K. phaffii* cells. All analyzed transformants demonstrated higher activity of studied enzymes compared to the wild-type strain. During alcoholic fermentation wild-type strain of *K. phaffii* produces 2.9 g/L of ethanol in the medium supplemented with 10% of crude glycerol. Overexpression of *ScPDC1* or *ScADH1* resulted in 2.8 or 2 fold increase of ethanol production as compared to wild-type strain, reaching 8.1 or 5.9 g/L, respectively.

We carried out the construction of the plasmid with simultaneous overexpression of the genes *ADH1* AND *PDC1* and we are at the stage of testing the plasmid by restriction analysis.

THE ROLE OF TRANSCRIPTION FACTORS IN REGULATION OF GLUCOSE AND XYLOSE METABOLISM AND FERMENTATION IN THE NON-CONVENTIONAL YEAST *OGATAEA POLYMORPHA*

Olena Kurylenko
Department of Molecular Genetics and Biotechnology

Lignocellulosic biomass represents important renewable feedstock that can be converted to ethanol or other chemicals. The most abundant sugars in lignocellulosic hydrolyzates are glucose and xylose. *Ogataea (Hansenula) polymorpha* is the most thermotolerant xylose-fermenting yeast species with maximal growth temperature of 50 ^{o}C which is compatible with the process of simultaneous saccharification and fermentation. However, the efficiency of xylose fermentation in the *O. polymorpha* wild-type strains is very low. Several metabolic engineering approaches were successfully applied for construction of the advanced ethanol producers from xylose in *O. polymorpha*. Further improvement of high-temperature alcoholic fermentation in *O. polymorpha* depends on identification of bottlenecks in xylose conversion pathway to ethanol.

Recently, the Cat8 was described as the first transcriptional factor involved in regulation of xylose alcoholic fermentation in *O. polymorpha*. Currently, our study is focused on identification of other genes coding for putative transcriptional factors in *O. polymorpha*. Effect of deletion of transcriptional factors Mig1, Mig2, Tup1, Hap4A, Hap4B on metabolism and fermentation of glucose and xylose was studied. Moreover, recombinant strains overexpressing *HAP4A*, *HAP4B*, *ZNF1*, *ASG1*, *SEF1*, *TUP1* genes under control of strong constitutive promoter or native promoter in the frame of the multicopy plasmid were constructed. The *tup1Δ* mutant was characterized by immediate increase of ethanol production during xylose alcoholic fermentation, however, practically was not able to ferment glucose.

MITOCHONDRIAL TRANSPORTER Tmi1 IS INVOLVED IN REGULATION OF XYLOSE FERMENTATION IN *SCHEFFERSOMYCES STIPITIS*

Mariia Borbuliak
Department of Molecular Genetics and Biotechnology

Lignocellulosic biomass represents an abundant renewable energy source and it is considered as an ideal substrate for ethanol production. However, the feasible technology for the bioethanol production from non-starch lignocellulosic materials has not been developed yet. Naturally occurring yeast strain *Scheffersomyces (Pichia) stipitis* are able to ferment both glucose and xylose to ethanol. Moreover, among the xylose-fermenting yeasts, *S. stipitis* seems to be the most promising for industrial application, because it ferments xylose with a high ethanol yield and low xylitol production.

The aim of this work was to investigate the role of the gene *TMI1* (Transport into Mitochondria) in the alcoholic fermentation of glucose and xylose by the yeast *S. stipitis*. The possibility of the *TMI1* gene's influence upon the efficiency of alcoholic fermentation was revealed using the insertional mutagenesis method combined with the positive selection of ethanol overproducers, based on the usage of 3-bromopyruvate as a selection agent. 3-bromopyruvate specifically inhibits key enzymes of glycolysis: hexokinase, pyruvate kinase and pyruvate decarboxylase, therefore the yeast cells resistant to 3-bromopyruvate should have intensified glycolysis, and this may stimulate ethanol production during the alcoholic fermentation.

Among the selected 3-bromopyruvate resistant insertional mutants, strain *#4.6* revealed reproducible increase of ethanol accumulation during glucose or xylose fermentation. In this strain, the insertion was found within the ORF of a gene homologous to *Saccharomyces cerevisiae* gene *YDL119C*, encoding mitochondrial transporter. Confirmation that the observed increased glucose/xylose fermentation performance of strain *#4.6* is a result of insertion cassette integration, rather than the secondary mutation occurring elsewhere in the genome, is an essential part of the study. Wild-type phenotype was restored via complementation of the insertional mutation by the wild type allele of *TMI1* gene, however, deletion of *TMI1* on the background of *Ku80* strain (Maassen et al, 2008) did not improve ethanol production on glucose/xylose containing media.

Obtained results revealed that the gene *TMI1* is involved in the regulation of alcoholic fermentation of glucose and xylose. The mechanism of the enhanced ethanol production in the insertional mutant strain is currently being studied.

mTORC1 STATUS IN COLORECTAL CANCER AS A PREDICTIVE MARKER FOR ARGININE DEPRIVATION THERAPY

Serhii Chornyi
Department of Cell Signaling

Some cancer types are auxotrophic for arginine and rely on delivery of this amino acid from extracellular sources. This cellular inferiority has been used to design selective anticancer therapy based on the enzymatic arginine depletion. For the unknown reason, different colorectal carcinomas cell lines show different level of sensitivity to that therapy. Possible mechanism for such difference could be the inability of some cell lines to inactivate mTORC1 that is a key regulator of the respond of cells on amino acid withdrawal. The goal of the project was to investigate how direct impact on mTORC1 signaling pathways will change the behavior of cancer cells under arginine starvation condition to gain new knowledge on how mTORC1 status in colorectal carcinoma cells affects cells response to arginine deprivation therapy and whether mTOR or TCS2 (inhibitor of mTORC1) may be utilized as predictive markers of sensitivity to this metabolic therapy.

We decided to generate a colorectal carcinoma cell model with hyperactivated mTORC1. For this purpose, we used the technology of gene knocking out (CRISP/Cas9). Specifically, TSC2, an inhibitor of mTOR, was knocked out in the SW 480 cell line. Also, we analyzed the sensitivity of different primary colorectal cell lines (COGA-1, COGA-2, and COGA-3) to the lack of arginine and correlation between the level of mTORC1 activity and sensitivity to arginine deprivation. Cells were cultured in the arginine-supplied (complete) or in the arginine-deplete medium. The sensitivity of this cell lines to the arginine withdrawal was analyzed by Western blot and WST cell viability assay.

As was expected, TSC2 knock out cell lines cannot deactivate mTORC1 under serum withdrawal. Unfortunately, no significant changes in sensitivity to arginine deprivation therapy in the generated cell line compared to the wild-type cell line were detected. Under arginine deprivation (24-72h) TSC2 knock out single cell clones inactivate AKT in opposite to WT with unknown mechanism. We analyzed the level of mTORC1 activity in COGA-1,-2 and -3 primary cell lines, no correlation between mTORC1 activity and sensitivity to arginine deprivation was found.

INTERACTIONS BETWEEN THE PROMOTER REGIONS OF STRUCTURAL GENES OF RIBOFLAVIN SYNTHESIS AND Sef1 TRANSCRIPTION FACTOR IN THE FLAVINOGENIC YEAST *CANDIDA FAMATA*

Yana Petrovska
Department of Molecular Genetics and Biotechnology

Riboflavin (RF) or vitamin B2 - is an important compound for all living organisms. Riboflavin serves as a precursor of flavin coenzymes FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) involved in numerous biochemical processes. Riboflavin biosynthesis pathway is well studied in yeast *Candida famata*, however, its regulation is poorly understood. Several regulatory genes have been identified so far, *SEF1* is among them.

The aim of this work was to study the mechanism of interaction between the promoter regions of structural genes of riboflavin synthesis and Sef1 transcription factor.

The one-hybrid system (Y1H) based on yeast Saccharomyces cerevisiae was used during this work. Strain BY4742 was co-transformed with plasmids, one of which contains *SEF1* gene under the control of the promoter of the gene *GAL1*, induced by galactose, and the other contains a gene *LAC4 Kluyveromyces lactis*, encoding β-galactosidase under the control of promoters of structural genes of riboflavin synthesis *RIB1*, *RIB2*, *RIB3*, *RIB5*, *RIB6*, *RIB7* and *SEF1*. In the case of protein Sef1p binding with the corresponding promoter, increase in β-galactosidase activity was expected. To find the binding site of protein Sef1p, nucleotide sequence of *RIB1* promoter has been modified by removing a potential binding site or by its modification.

As a result, it was revealed that protein Sef1p directly interacts with promoters of most *RIB* genes and its own promoter, providing evidence of autoregulation.

ENGINEERING OF YEAST *OGATAEA POLYMORPHA* WITH EXPRESSION OF MODIFIED HEXOSE TRANSPORTERS

Nadiya Shevchuk
Department of Molecular Genetics and Biotechnology

For the last three decades biofuels produced from renewable feedstocks have received attention because of their potential to replace conventional fossil fuels. A major issue in the conversion of saccharified lignocellulosic biomass into biofuel is the utilization of xylose, since lignocellulosic feedstocks contain a significant amount of this pentose sugar. The ability of the thermotolerant methylotrophic yeast *Ogataea polymorpha* to ferment xylose has made this yeast species a promising organism for high-temperature alcoholic fermentation. D-xylose transporters are competitively inhibited by D-glucose, which is one of the major reasons hampering simultaneous fermentation of D-glucose and D-xylose, two primary sugars present in lignocellulosic biomass.

To increase the specific xylose uptake rate during high-temperature alcoholic fermentation Hxt1 transporter was engineered in *O. polymorpha.* Hxt1 has approximately 60% identity to *Saccharomyces cerevisiae* Hxt3 transporter. The mutation N358 in Hxt3 resulted in activation of xylose uptake rate in *S. cerevisiae* during alcoholic fermentation [Nijland etc., 2014]. The modified versions of Hxt1 of *O. polymorpha* were overexpressed in *hxt1Δ* mutant and the efficiency of xylose and glucose co-utilization during high-temperature fermentation was studied. The mutagenesis of Hxt1 resulted in co-utilization of both sugars during fermentation in obtained recombinant strains. Therefore, the modified versions of Hxt1 were introduced into genome of *O. polymorpha* advanced ethanol producer.

Also in *S. cerevisiae* Gal2 and Hxt7 were determined as transporters with high affinity for D-xylose. Recently, Gal2 and Hxt7 were successfully mutated to yield glucose-insensitive xylose transporters. The Gal2 was modified with the substitution of asparagine to serine at position 376 and the Hxt7 with the substitution of corresponding asparagine to phenylalanine at position 370 [Farwick etc., 2014]. The modified *S. cerevisiae* Gal2 and Hxt7 transporters were introduced into genome of *O. polymorpha* wild- type strain and the best ethanol producer. We expect that generated transporters should contribute to the stimulation of xylose transport and realization of co-fermentation of glucose and xylose in *O. polymorpha*.

IMPLEMENTATION OF CRISPR-CAS9 SYSTEM IN YEASTS *CANDIDA FAMATA* AND *OGATAEA POLYMORPHA*

Yuliia Andreieva
Department of Molecular Genetics and Biotechnology

Recently developed CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system has been successfully applied for modification of genomes from bacteria to human as quick method of rational genome engineering. CRISPR system contains bacterial Cas9 nuclease and a synthetic guide RNA (gRNA) that directs Cas9 to cleave regions in the genome. Homologous genome region (up to 20 bp) hybridizes to guide from the synthetic guide RNA when it is followed by the specific sequence NGG (known as protospacer-associated motif (PAM)). Variations of CRISPR-Cas9 have been developed for numerous groups of organisms; however, the system still was not applied and adapted for biotechnologically important yeast species *Candida famata* and *Ogatea polymorpha.* The aim of this work is development CRISPR-Cas system as a genome editing tool for yeast species *C. famata* and *O. polymorpha.* Gene *ADE2* was used as a target locus to assess the efficiency of the system for these yeasts. Disruption of the gene leads to accumulation of red intermediate to confer easily detectible phenotype of the *ade2* mutants.

It was found that CRISPR-Cas9 system optimized for *Candida albicans*, revealed low efficiency in flavinogenic yeast *C. famata*. Only 5% of selected transformants showed the mutant red colonies phenotype. Obtained result could be explained by inefficient expression of genes encoding components of the system. In *O. polymorpha*, an endogenous gene promoter *GAP1*, encoding glyceraldehyde-3-phosphatedehydrogenase was cloned in front of *CAS9* gene to ensure effective expression Cas9 nuclease. Increased gene expression *CAS9* led to an increase in the frequency of mutations in the gene *ADE2*. Among the selected transformants ≈ 72% colonies accumulated red substance.

To optimize gRNA expression in CRISPR-Cas9 of *C. famata*, several parameters were varied, including the promoter *TEF1* (translational elongation factor EF-1 alpha) or *MAL2* (alpha-glucosidase) used to drive *CAS9* transcription. The gRNA was flanked by the 5’ $tRNA^{Ala}$ and 3’ self-cleaving hepatitis delta virus (HDV) ribozyme and driven by the strong *TEF1* promoter. We anticipate, the modifications will improve gRNA maturation and expression leading to increase efficiency of CRISPR-Cas mutagenesis in *C. famata*.

DMAEM-CONTAINING CARRIERS FOR PLANT TRANSFORMATION POSSESSED LOW PHYTOTOXICITY AND NO MUTAGENIC PROPERTIES

Nataliya Finiuk

Department of Regulation of Cell Proliferation and Apoptosis

Nanocomposites are widely used in science, manufacturing and biomedicine, including a DNA transfer into the target cells. However, naked genes are rapidly degraded by nucleases and show a poor cellular uptake. Under these circumstances the development of safe and efficient gene carriers become one of the prerequisites for the successful targeted gene transfer.

The aim of present study was to study gene transfer efficiency of novel poly(2-dimethylamino)ethyl methacrylate (DMAEM)-based polymers (BGA-21, BGA-22, BGA-22(2ph) and PEG-containing BG-24, BG-25 and BG-26) and evaluate a level of their phytotoxicity and mutagenic potential.

Formation of complexes of plasmid DNA with DMAEM-based polymers was determined at 0.01-0.03 % of the carriers. Moss *Ceratodon purpureus* (Hedw.) Brid. was a plant model used to establish the transformation efficiency. The application of classical PEG-based transformation method did not enable us to obtain moss transformants. Application of novel DMAEM-based polymers as pDNA carriers at 0.0025 % allowed obtaining both transient and stable transformants of *C. purpureus*. As a result of stable transformation of *C. purpureus* protoplasts, 3 clones were picked up using BGA-22 carrier, and 2 clones – when using BGA-21 carrier, and 1 clone – when using BGA-22(2ph), BG-24, BG-25, BG-26 polymers.

PEG-containing DMAEM-based polymers (BG-24, BG-25, BG-26) possessed more pronounced phytotoxicity (inhibition of onion seed germination and root growth) in the *Allium cepa* test. Studied polymeric carriers have higher impact on catalase activity if compare with superoxide dismutase activity and changes of malondialdehyde level. PEG at 0.0025 and 0.025 % significantly enhanced the CAT, SOD activities and increased the MDA level.

The results of ana-telophase test in *A. cepa* didn’t pointed to genotoxic activity of the polymeric carriers BGA-21, BGA-22, BGA-22(2ph), BG-24, BG-25 and BG-26 used in 0.0025 %. The micronuclei frequency wasn’t affected under the examined polymers action. No mutagenic potential was revealed for all examined DMAEM-based polymeric carriers in the Ames test on *Salmonella typhimurium* strains TA100 and TA98 without S9 and in the presence of S9.

The low phytotoxicity and absence of mutagenic potential of the DMAEM-based polymers polymeric carriers allows them to be a promising carriers for the gene delivery into plant cells.

CLONING AND EXPRESSION OF PUTATIVE RIBOFLAVIN EXCRETASE IN FLAVINOGENIC YEASTS

Olena Kolodii
Department of Molecular Genetics and Biotechnology

Riboflavin (RF) is the precursor of two coenzymes: FMN and FAD, which are involved in a wide range of biochemical processes, mostly of oxidative metabolism. Flavins are manufactured for use as vitamins in human and animal nutrition, as pharmaceuticals and as a food colorant. Currently, vitamin B_2 is produced on a large scale microbial synthesis. However, microbial RF producers require to increase productivity and genetic stability. The mechanisms of RF efflux in microorganisms remain elusive. Early, the transport system for efflux of RF from the cells was described for yeast *Pichia guilliermondii*. However, the gene encoding the excretase has not been cloned. It can be assumed that its overexpression can enhance RF excretion from the cells and possibly leading to an increase in RF synthesis and its accumulation in the culture medium.

Earlier, the human protein BCRP (breast cancer resistance protein) was shown to be responsible for RF pumping into milk. Eight genes coding for similar proteins were founded in *P. guilliermondii* genome by homology search. Deletion of gene PGUG_04776.1 did not affect phenotype of *P. guilliermondii* RF-transporting strain R93. Recombinant transformants of R93 cells bearing additional copy of the native gene PGUG_05894.1 possessed 2-6 folds increased RF excretase activity relative to the parental strain. Obtained results could suggest that gene PGUG_05894.1 encodes a transporter involved in excretion of RF by *P. guilliermondii.*

Candida famata represents the yeast with highest flavinogenic potential. Very stable RF and FMN-overproducing strains of this yeast were constructed. The RF excretase genes of *C. famata* are not identified due to the lack of genome sequencing data. Thus, we used transporter homologous to BCRP identified in the genetically close *Debaryomyces hansenii* yeast species. Yeast integrative plasmid containing *D. hansenii* homolog of *BCRP* gene under the control of *TEF1* promoter of the same species was constructed. Introduction of *BCRP* gene into genome of RF overproducing strains *C. famata* AF4 and 91 resulted in an increase of RF excretase activity and total RF production.

ANTIMICROBIAL PROPERTIES OF HONEYBEE CHITOSAN DERIVATIVES

Nazar Manko
Department of Regulation of Cell Proliferation

Chitosan is natural polysaccharides possessing antimicrobial properties. This aspect has been studied for chitosan isolated from marine organisms. Preparations of chitosan from honeybees and their antimicrobial properties are studied fragmentary and not completely enough. We isolated chitosan from honeybee corpses in laboratory and studied their antimicrobial effect.

The purpose of the work was to study the antimicrobial properties of chitosan-melanin complex and its fragmentation form, and influence of mol.mass on antimicrobial activity.

Antibacterial activity of chitosan-melanin complex was studied on bacteria - *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa* using the MTT assay which was slightly modified. Chitosan-melanin complex sample powder or solution was added to the Eppendorf tube, 100 µl of bacterial suspension in Saburo medium, pH 7.2 was added to the tube. The mixture was incubated for 4 hours at 37°C. Control was performed in two samples, one in the start, and one in the end of incubation. Then MTT (10µl, 5mg/ml) was added to the tube and incubated for 1 hours at 37 ° C. The tube was precipitated on 3000 rpm, 10 minutes, the supernatant was removed, 1ml DMSO was added and wait 1 hour. Measuring of formazan is carried out on ULAB102UV, at 570nm. Inhibition of growth expressed in percentage relative to the control sample with unbroken growth of bacteria. Also, we performed an antifungal activity of soluble chitosan with different mol.mass.

It was revealed that chitosan-melanin complex in fragmented and non fragmented form exhibits antibacterial activity. Honeybee chitosan-melanin complex showed antibacterial effect in dose 1mg/ml against - *E.coli* (21% of alive bacteria's), *P.aeruginosa* (79% of alive bacteria's) *S.aureus* (82% of alive bacteria's) also it show strong antifungal activity against *Candida albicans*, in particular, high molecular weight chitosan has a strong antifungal effect, in contrast to low molecular weight chitosan. A microscopic examination of the *Candida albicans* under the action of chitosan was also carried out, we observed aggregation of yeast cells, staining with DAPI, confirm that the cells are dead.

Thus, it has been established that chitosan-melanin complex is shown strong antimicrobial activity against *C.albicans*, it was also confirmed that aggregated yeast cells are dead.

EFFECT OF DELETION OF TRANSCRIPTION FACTORS ON XYLOSE FERMENTATION PERFORMANTS OF ENGINEERED *SACCHAROMYCES CEREVISIAE*

Liubov Dzanaieva
Department of Molecular Genetics and Biotechnology

Today biofuel industry primarily produces ethanol from a corn or sugarcane. However, this so-called first generation ethanol produced from starch and sugar, is in competition with a food and animal feed industry. In contrast, lignocellulosic biomass (crop wastes, agricultural and forestry residues, and municipal waste) offers a high potential as feedstock for biofuels, because it is the most abundant sustainable raw material worldwide and occurs as byproduct without competing uses. Studies on conversion of lignocellulosics to ethanol focused on the searching for natural microbial strains and construction of recombinants able to ferment efficiently all sugars of lignocellulosic hydrolysates. Effective alcoholic fermentation of xylose, the second abundant after glucose sugar of lignocellulose hydrolysates (consists approx. 30% of hydrolyzate sugars) is one of the main unresolved problems.

Although there are many bacterial and yeast strains capable of naturally utilizing xylose, *Saccharomyces cerevisiae* has advantages over the innate xylose-utilizing microorganisms regarding robustness against various stresses in industrial environments, such as low pH, high osmotic pressure, high alcohol concentration, and phage contamination. *S. cerevisiae* cannot naturally utilize xylose. Metabolic engineering approaches for introducing heterologous xylose utilization pathways and optimizing internal metabolisms have been undertaken to develop efficient xylose-fermenting *S. cerevisiae* strains. Despite of intensive engineering of *S. cerevisiae* strains, xylose fermentation rate still remains lower than for glucose. Study the regulation of xylose catabolism in engineered *S. cerevisiae* can facilitate xylose fermentation performance.

Effect of deletion of transcription factors Adr1, Asg1, Cat8, Hap4, Sip4, Tup1 and Znf1 on xylose fermentation of engineered *S. cerevisiaen* was studied. In this study, we report the isolation of ***adr1Δ, asg1Δ, cat8Δ***, *hap4Δ, sip4Δ, tup1Δ* and *znf1Δ* mutants on the background of xylose-utilizing *S. cerevisiae* strain. Constructed ***cat8Δ*** strain possessed 20% increase in xylose fermentation rate when compared to initial strain.

IMPACT OF SELENOMETHIONINE ON THE ANTITUMOR ACTIVITY OF DOXORUBICIN AND THEIR COMBINED EFFECT ON THE GLUTATHIONE SYSTEM IN CELL LINES WITH DIFFERENT MECHANISMS OF RESISTANCE TO CHEMOTHERAPY

Yuliya Kozak
Department of Regulation of Cell Proliferation and Apoptosis

Chemotherapy is one of the most effective methods of treating cancer. However, its use is limited to the development of severe side effects in patients, and rapid emergence of multiple drug resistance (MDR) of cancer cells. A possible solution to these problems is the use antioxidant compounds in combination with antitumor drugs.

Basically, MDR develops due to overexpression of transporter proteins (ATP Binding Cassette transporters) namely P-gp (P-glycoprotein), MRP-1 (multidrug-resistance associated protein) and BCRP (brest cancer resistance protein). Therefore, for our work we used human promyelocytic leukemia cells of different subline such as HL-60/wt, HL-60/vinc (P-gp overexpression), HL-60/Adr (MRP-1 overexpression).

To investigate the impact of SeMet (selenomethionine) on the cytotoxic activity of Dx (doxorubicin) and on the glutathione system under action of this antitumor drug on cells with different mechanisms of resistance.

Trypan blue assay, determination of the level of total (tGSH) and oxidized glutathione (GSSG), measurement of the activity of glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST).

We have shown that HL-60/wt cells are sensitive to Dx (LC_{50}=0.5 μM). SeMet exhibits a protective effect on the action of this antibiotic. This is evidenced by the increase in the number of cells (by 18%) under the action of Dx in combination with SeMet compared with the action of Dx alone. In contrast, cells of HL-60/vinc and HL-60/Adr sublines exhibit 5 and 10-fold (respectively) increase to Dx resistance. Interestingly, SeMet enhances the activity of Dx towards resistant cells.

We have shown that Dx induced a 1.5-fold increase in the level of GSSG in wild-type cells and 2-fold increase of this level in cells of HL-60/vinc subline. While the effect of Dx on HL-60/Adr cells caused a 2.5-fold decrease in the level of GSSG and did not affect the level of tGSH. It was shown that SeMet in combination with Dx led to 2-fold decrease GSSG and tGSH levels compared to this levels under the action of Dx alone.

Investigating the activity of the key enzymes of the glutathione system (GR, GPx, GST) has shown the important role of GST in providing resistance of cancer cells to chemotherapy, which is mediated by overexpression of P-glyprotein. SeMet effectively inhibited this enzyme during the combined action of this antioxidant with Dx.

КОНФЕРЕНЦІЮ ОРГАНІЗУВАЛИ ТА ПРОВЕЛИ:
Рада молодих вчених спільно з Адміністрацією Інституту біології клітини НАН України

ORGANIZERS OF THE CONFERENCE:
Council of Young Scientists together with Administration of Institute of Cell Biology of NAS Ukraine

BEST PRESENTATION COMMITTEE:
Member of NASU, Dr. Sc. Sybirny A.A. (head),
Corr. Member of NASU, Dr. Sc. Stoika R.S.,
Dr. Sc. Gonchar M.V., Dr. Sc. Lutsyk M.D.,
Dr. Sc. Fedorovych D.V., Dr. Sc. Kit Yu.Ya,
Dr. Sc. Antonyuk V.O., Dr. Sc. Dmytruk K.V.,
PhD Stasyk O.V., PhD Barska M.L.