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ABSTRACTS

Lviv

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ABSTRACTS

ANTI-GLIOMA ACTION OF NOVEL THIAZOLE DERIVATIVE

Nataliya Finiuk
Department of Regulation of Cell Proliferation and Apoptosis

Gliomas are the most aggressive primary brain tumors that represent up to 80% of brain malignancies. Treatment of glioma patients includes a combination of chemotherapy, radiation therapy, and surgery.

The aim of present study was to investigate the anti-proliferative and pro-apoptotic effects of N-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide derivative (compound 5) in human glioblastoma cells. This thiazole derivative was synthesized at the Department of Organic Chemistry of Ivan Franko National University of Lviv. Traditional anti-glioma medicine Temozolomide (TMZ) and Doxorubicin (Dox) were applied as positive controls. The anti-proliferative action of the compound 5, TMZ and Dox towards human glioblastoma U251 and human glioblastoma multiform T98G cells was measured by the MTT test. Western-blot analysis, fluorescent microscopy, DNA retardation assay in the agarose gel, FACS analysis and DNA comet assay in the alkaline conditions were applied to study the effect of compound 5 towards glioma U251 cells.

The compound 5 showed the growth inhibition action towards U251 and T98G cells. This compound demonstrated approximately 20 times higher cytotoxicity for these cells compared to the effects of TMZ and approximately 2 times higher cytotoxicity than the Dox. It induced chromatin condensation and/or nucleus fragmentation, as well as plasma membrane blebbing in treated glioma cells. Apoptosis in the glioma U251 cells under the action of compound 5 involved the mechanisms of PARP1 and caspase 3 cleavage, as well as an increase in the level of Bax and Bim pro-apoptotic proteins and a decrease in the level of phosho-ERK1/2 kinase. The cytotoxic action of the compound 5 in U251 and T98G glioma cells was accompanied by an increase in production of the hydrogen peroxide and formation of DNA single-strand breaks. This compound did not intercalate into DNA molecule.

Thus, the novel N-(5-benzyl-1,3-thiazol-2-yl)-3,5-dimethyl-1-benzofuran-2-carboxamide derivative (compound 5) is a potential anti-glioma medicine that demonstrates much higher cytotoxic action towards human glioblastoma cells than the TMZ and Dox. Its cytotoxicity is associated with apoptosis induction, production of the reactive oxygen species (ROS), and formation of DNA single-strand breaks via mechanisms that are not dependent directly on binding or intercalating DNA molecule.

IMPROVEMENT OF THE ALCOHOLIC FERMENTATION EFFICACY IN THE INDUSTRIAL STRAINS OF YEAST *SACCHAROMYCES CEREVISIAE* BY POSITIVE SELECTION AND ADAPTIVE EVOLUTION

Marta Semkiv
Department of Molecular Genetics and Biotechnology

Global warming, environmental pollution and the threat of exhaustion of world resources of fossil fuels are the pressing concerns that had arisen in XX century and followed humanity into the new millennium. Search for “green” energy resources that can be used instead of fossil fuels brought to humankind attention among others such options as biogas, bioethanol and biodiesel. These biofuels are produced from renewable material and after combustion generate an acceptable level of emissions gases. Bioethanol is a liquid biofuel obtained from carbohydrate feedstock. Bioethanol can be mixed with petrol or used alone in specialized cars’ engines.

Bioethanol is predominantly produced by baker’s yeast *Saccharomyces cerevisiae*. Even a slight improvement of ethanol production by *S. cerevisiae* can yield several additional millions litters of ethanol to worldwide ethanol production annually. Important characteristic of the industrial ethanol-producing *S. cerevisiae* strains is their robustness under the stressful conditions of the industrial alcoholic fermentation.

We’ve analysed ethanol production by a range of industrial *S. cerevisiae* strains in different conditions, in particular in a mineral medium with a high glucose concentration; wheat hydrolysate; corn hydrolysate; wheat and corn hydrolysates with the additional glucose. In most of these conditions, the highest level of ethanol production was demonstrated by the strain AS400-510-42, but it lost its superiority to another industrial *S. cerevisiae* strain PE-2 in conditions with the highest glucose concentration. Strain AS400-510-42 was obtained by a positive selection of the industrial ethanol producer AS400 on glyoxylic acid and glucosamine and produced 3-3.5% more ethanol compared to the starting strain.

Strains AS400-510-42 and PE-2 were subjected to the adaptive evolution. The aim of this process was to adapt mentioned strains to the cultivation in the medium with both high glucose and ethanol concentrations, which had been shown to be more toxic than the medium with high glucose or high ethanol concentration. Strain AS400-510-42 was less robust in this conditions than PE-2, but during the course of adaptation it increased its growth capacity more. Adapted strains-derivatives from the strain AS400-510-42 revealed substantial (up to 10%) increase in ethanol production in comparison with the initial strain, whereas for the derivatives of the strain PE-2 the results were not that promising. Therefore, adaptive evolution is more efficient when the conditions are more stressful to the evolving strain.

BIOSYNTHESIS OF RIBOFLAVIN BY *CANDIDA FAMATA* STRAINS WITH OVEREXPRESSED *BCRP* GENE

Khrystyna Pavliukh
Department of Molecular Genetics and Biotechnology

Riboflavin (vitamin B2, RF) is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are coenzymes of flavoproteins involved in oxidative metabolism and other processes in cells. RF is an obligatory component of human and animal diets, because it is synthesized only by plants and most microorganisms (Abbas and Sibirny, 2011). The yeast *Candida famata* is one of the best producents of RF. There are a lot of different approaches to increase RF production, including an enhancement of a RF excretion from the cells. Flavinogenic yeasts *C. famata, Pichia guilliermondii* and *Debaryomyces hansenii* contain genes homologous to the mammal BCRP (Breast Cancer Resistant Protein) gene coding the protein responsible for secretion of RF from the cells. *BCRP* gene was isolated from *D. hansenii* and expressed under the own *TEF1* promoter in flavinogenic strain of the yeast *C. famata.* Resulted transformants produced elevated in 1.5 times amounts of RF.

The aim of this work was to optimize a composition of medium and growing conditions for increasing RF production by *C. famata* strains with the overexpressed *BCRP* gene.

We used two recombinant strains (#47 and #48) obtained from RF overproducer strain *C. famata* #91 with overexpressed the RF excretase gene *BCRP*. For cultivation we used the medium with different carbon and nitrogen sources. We were looking for the industrial substrates besides, and cultivated *C. famata* strains using molasses, beer wort, hydrolysate of bagasse, raw glycerol and milk whey. Analysis of consumption the carbon sources during the fermentation was performed using HPLC (PerkinElmer, Series 2000, USA) by ion exchange column Aminex HPX-87H (Bio-Rad, Hercules, USA). We also analyzed optimal pH and aeration conditions for better yeast`s growth and RF production. The RF concentration in culture medium was measured using Turner® Quantech™ Digital Filter Fluorometer.

It was found the optimal conditions (carbon and nitrogen sources, industrial substrates, pH, aeration) for growth and RF production by flavinogenic *C. famata* strains. Stability of the mutant *C. famata* strains was the same as in the recipient *C. famata* #91 strain. The developed scheme of cultivation and further improvement of RF overproducers are prerequisites to their industrial application. There is a big potential for searching *C. famata* mutants with increased RF production growing on industrial substrates.

INVESTIGATION OF THE ROLE OF PEROXISOMAL ENZYMES IN XYLOSE METABOLISM AND ALCOHOLIC FERMENTATION IN THE YEAST *OGATAEA POLYMORPHA*

Dmytro Bratiichuk
Department of Molecular Genetics and Biotechnology

Ogataea (Hansenula) polymorpha is one of the most thermotolerant methylotrophic yeast species with ability to utilize xylose and ferment this sugar to ethanol. Methylotrophic yeasts possess well-developed peroxisomes which could occupy up to 80% of cellular volume during growth on methanol. The genes coding for peroxisomal enzymes are strongly induced by methanol as sole carbon source, repressed during growth on glucose and partly derepressed in the presence of xylose.

The key peroxisomal enzyme is alcohol oxidase. Overexpression of *AOX1* gene coding for alcohol oxidase in *O. polymorpha* resulted in increased ethanol production from xylose relative to the wild type strain. Moreover, overexpression of *AOX1* gene led to increase in the expression of *DAS1* and *TAL2* coding for peroxisomal transketolase (known also as dihydroxyacetone synthase) and transaldolase, respectively. It was shown that peroxisomal transketolase and transaldolase in *O. polymorpha* are required for xylose alcoholic fermentation but not for growth on this pentose. Mutants with knock out of *DAS1* and *TAL2* normally grew on xylose but were defective in its conversion to ethanol. Separate overexpression or co-overexpression of *DAS1* and *TAL2* in the wild type strain increased ethanol production from xylose 2-4 times with no effect on glucose alcoholic fermentation. For comparison, we also overexpressed the genes, *TKL1* and *TAL1*, coding for cytosolic transketolase and transaldolase, respectively. We found that overexpression of these genes also stimulated ethanol production from xylose during fermentation.

In the current study, genes coding for peroxisomal enzymes (*AOX1*, *DAS1*, *TAL2*) were overexpressed together with genes for cytosolic enzymes (*TKL1* and *TAL1*) in the genome of the previously isolated *O. polymorpha* advanced ethanol producer from xylose and obtained recombinant strains were studied regarding their ethanol production during high-temperature xylose alcoholic fermentation.

IDENTIFICATION OF TRANSCRIPTIONAL FACTORS INVOLVED IN REGULATION OF XYLOSE METABOLISM AND FERMENTATION IN THE YEAST *OGATAEA POLYMORPHA*

Olena Kurylenko
Department of Molecular Genetics and Biotechnology

Ogataea polymorpha belongs to the most thermotolerant xylose-fermenting yeast species. However, regulation of xylose metabolism in these yeasts is poorly understood. Therefore, identification of specific regulatory genes involved in xylose-dependent regulation of gene expression and in xylose alcoholic fermentation are of great importance.

The transcriptional regulator Hap4 is known to be involved in the balance between fermentation and respiration in *Saccharomyces cerevisiae*. Two putative orthologs of Hap4 protein were identified in the genome of *O. polymorpha*, named Hap4A and Hap4B. The activation of xylose alcoholic fermentation was observed in *hap4Δ* mutant whereas overexpression of *HAP4A* gene led to decreased ethanol production from xylose. The deletion or overexpression of *HAP4B* gene did not result in significant change in the amount of accumulated ethanol from xylose as compared to the wild type strain. The Tup1-Cyc8 (Ssn6) corepressor complex is required for repression of transcription in several regulatory pathways in yeast cells, including glucose repression. The knock out of *TUP1* gene in *O. polymorpha* resulted in immediate increase of ethanol production during xylose alcoholic fermentation and total inability to ferment glucose. The overexpression of *TUP1* gene decreased ethanol production from xylose. The putative targets of the Tup1 in the genome of *O. polymorpha* are under investigation.

IMPACT OF SELENOMETHIONINE AND D-PANTETHINE ON THE FUNCTIONAL STATUS OF THE GLUTATHIONE SYSTEM IN TUMOR CELLS WITH DIFFERENT MECHANISM OF DRUG RESISTANCE UNDER DOXORUBICIN ACTION

Yuliya Kozak
Department of Regulation of Cell Proliferation and Apoptosis

One of the main problem of modern chemotherapy is rapid development of multiple drug resistance (MDR) of tumor cells leading to ineffective treatment. In our previous studies we have shown that antioxidants selenomethionine (SeMet) and D-Pantethine (D-Pt) enhanced cytotoxic effect of doxorubicin (Dx) by 20%-30% toward drug-resistant malignant cells ($P \leq 0.05$). BUT the molecular mechanisms underlying such features of SeMet and D-Pt remain unexplored. To investigate the impact of SeMet and D-Pt on the level of reduced and oxidized glutathione and the activity of key enzymes of the glutathione system in human leukemia cells with different mechanism of drug resistance under Dx action.

Determination of the level of reduced (GSH) and oxidized (GSSG) glutathione, measurement of the activity of glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferase (GST). In this work we used human leukemia cells of different lines: HL-60/wt (wild type cells), HL-60/vinc (vincristine-resistant cells, P-gp+), HL-60/adr (adriamycin-resistant cells, MRP-1+).

Cells of each of the studied cell lines are characterized by different basal levels of both oxidized and reduced glutathione, leading to different GSH/GSSG ratios – 0.34 in HL-60/wt, 2.2 in HL-60/vinc and 0.06 in HL-60/adr. Treatment of cells with Dx led to the versatile action of this drug on the glutathione level in each of the studied cell lines. HL-60/wt cells were characterized by 8-fold lower GSH level under Dx treatment compared to control, while in HL-60/vinc and HL-60/adr cells GSH level was increased 2.2- and 8.2-fold (compared to untreated cells), correspondingly. We have shown that a combined effect of SeMet or D-Pt on the background of the cytotoxic action of doxorubicin on HL-60/vinc cells is accompanied by a 2-fold decrease in both oxidized and reduced glutathione levels. Investigating the activity of the key enzymes of the glutathione system (GR, GPx, GST) has shown the important role of GR and GST in providing resistance of cancer cells to chemotherapy, which is mediated by overexpression of P-glyprotein. SeMet and D-Pt effectively reduce GST activity. This correlates positively with a decrease in the level of reduced glutathione and a decrease in glutathione reductase activity in these cells (HL-60/vinc). Such an effect of SeMet or D-Pt on the functioning of the glutathione system increases the sensitivity of tumor cells with P-gp overexpression to doxorubicin action.

DELETION OF *VMA1*, ENCODING VACUOLAR ATPASE, INCREASES RIBOFLAVIN PRODUCTION IN THE FLAVINOGENIC YEAST *CANDIDA FAMATA*

Yuliia Andreieva
Department of Molecular Genetics and Biotechnology

Yeast *Candida famata* belongs to so-called flavinogenic yeast able to riboflavin (vitamin B2) oversynthesis under iron starvation. It is known that iron ions repress the synthesis of enzymes involved in conversion of GTP and ribulose-5-phosphate to riboflavin. Earlier studies defined that the disruption of *VMA1*, encoding vacuolar ATPase, which supported active RF transport from the cytoplasm to the vacuole, resulted in complete excretion of synthesized RF into the medium and increased the total production of RF in the filamentous fungus *Ashbya gossypii*. In contrast, *P. guilliermondii* does not accumulate the synthesized riboflavin in vacuoles. Therefore, regulatory impact of Vma1 in flavinogenic yeasts remains elusive. It was decided to isolate vma1Δ strain of *C. famata* and study its properties. For that reason deletion cassette, containing selectable marker gene *ble* conferring resistance to phleomycin flanked with noncoding regions of *VMA1* gene was constructed. Deletion was obtained by gene replacement. Among 46 analyzed transformants selected on phleomycin containing medium, one was found with the deletion of the *VMA1* gene. Proper deletion of target gene was verified by PCR. It was shown that the vma1Δ mutant possessed 16-fold increase in riboflavin accumulation, as compared to that of the parental wild-type strain on the medium supplemented with iron. Growth of the mutant in iron-deficient medium, as well as in the higher temperature conditions (34°C) was retarded. Isolation of the mutants with deletion of *VMA1* gene in riboflavin overproducing strains *C. famata* AF-4 and #91 is under way.

A NEW APPROACH TO THE STUDY OF PROTEIN DEGRADATION IN YEAST *KOMAGATELLA PHAFFII* BASED ON THE FUSION PROTEIN COMPRISING β-GALACTOSIDASE AND GFP

Anastasya Zazulya
Department of Molecular Genetics and Biotechnology

The methylotrophic yeast *Komagataella phaffii* (formerly *Pichia pastoris*) as well as *Escherichia coli* and *Saccharomyces cerevisiae* has become an important host organism for recombinant protein production. *K. phaffii* has very strong and tightly regulated promoters of the genes of the methanol utilization pathway such as the alcohol oxidase, formaldehyde dehydrogenase gene etc., which are very convenient for directed expression of heterologous proteins. Proteolytic degradation has been a perpetual problem when yeasts are employed to express recombinant proteins. The maximum reduction in the level of degradation of the target recombinant protein in the cytosol is one of the prerequisites for successful overproduction of the proteins of industrial significance. To reduce the level of degradation of the protein in cytosol, it is necessary to understand the mechanism by which it occurs.

There are no convenient methods for the selection of *K. phaffii* mutants with damaged pathways of proteins degradation. The new model has been developed to detect mutants and analyze the way in which they degrade the proteins. This model involves constructing a vector for expression of β-galactosidase gene *LAC4* from *Kluyveromyces lactis* fused with fluorescent tag green fluorescent protein (GFP). This fusion protein was cloned under the control of the methanol-regulated promoter of the gene *FLD1* (encoding formaldehyde dehydrogenase). Obtained vector was used for the transformation of *K. phaffii* wild type strain GS200, as well as the strain SMD1163 with damaged autophagy. Methanol-induced gene expression will result in the formation of a chimeric protein that will retain β-galactosidase activity and, at the same time, will be noticeable in the cells under a fluorescence microscope due to the GFP presence.

Two types of transformants have been obtained and tested for the presence of integrated vector at the moment. Subsequent stages of the study involve cultivation on methanol to activate β-galactosidase and GFP gene expression. It is planned to observe the cellular localization of β-galactosidase-GFP fusion after it has been produced and during degradation. Obtained recombinant strains will be further used for the identification of insertional mutants with impaired protein degradation, which will possibly allow as to identify genes involved in protein degradation in *K. phaffii*.

EFFECT OF FLAVONOID DERIVATIVES ON TUMOR AND PSEUDONORMAL MAMMALIAN CELLS

Olena Bahniuk
Department of Regulation of Cell Proliferation and Apoptosis

Many flavonoids possess properties of both free radical scavengers and antitumor agents.

The purpose of this work was to analyze in vitro a survival and death of cells with different level of neoplastic transformation under the action of flavonoid derivatives (flavanones and chalkons) present in plant material, as well as in products of bio-transformation.

Research tasks:

1) Investigation of cell survival and calculation of 50% inhibiting concentration (IC_{50}) of flavonoid derivatives targeting leukocytes, epithelial, mesenchymal neoplastic and pseudonormal lines of mammalian cells.

2) Study of the level of expression of pro- and anti-apoptosis regulatory proteins in target cells treated with specific derivatives of flavonoids.

3) Examination of the interaction of flavonoid derivatives with 1,1-dyphenyl-2-picryl-hydrazyl (dPPH) depending on the reaction time.

Human myeloid leukemia HL60 cells, murine J774.2 macrophages, epithelial cells of MDA-MB-231 and MCF-7 lines of mammary carcinoma, human embryonic kidney HEK293 cells, mesenchymal glioma cells of U251 and U373 lines, and pseudo-normal murine NIH3T3 fibroblasts were treated cells with flavonoid derivatives (flavanones and chalcons) and the MTT assay was applied. The IC_{50} of flavonoids derivatives was calculated.

Compounds # 3, 7, 10, 13, 15, 17, 18 demonstrated cytotoxicity towards treated cancer cells. Two compounds 3'-brom-flavonon (#13) and 2'-hydroxy-3- brom-chalkon (#15) were selected for future study of the mechanisms of their actions.

Western blot analysis revealed a decrease in the level of the Bcl-2 protein (anti-apoptotic) and an increase in the level of cleaved caspase-3 (pro-apoptotic).

The reaction of interaction of 1,1-diphenyl-2-pyrilhydrazil (dPPH) was used to examine the pro- and antioxidant activity of the compounds #13 and #15. The antioxidant action of these compounds was detected.

PRE-CONCENTRATED SILVER CATIONS ENHANCE THE ANTINEOPLASTIC EFFECTS OF DOPED CLINOPTILOLITE PARTICLES

Olga Pylypyak
Department of Regulation of Cell Proliferation and Apoptosis

Silver (Ag) refers to the physiologically active components and pre-concentration, removal and/or separation of this element should be carried out at analysis of trace amounts of Ag in water, technological solutions and biological substrates. Samples containing a significant amount of Cl-, is difficult because of the formation of insoluble AgCl. Here we investigated sorption properties of Transcarpathian clinoptilolite (silicate compound) with respect to formation of complex anion $Ag(NH_3)_2^+$.

It was found that at high concentrations of Cl-, clinoptilolite absorbs up to 96% of trace amounts of $Ag(NH_3)_2^+$ in the medium, and the most effective sorption of $Ag(NH_3)_2^+$ was observed for partially dehydrated clinoptilolite samples obtained by previous calcination of natural zeolite at 150°C. A method of preliminary Ag concentration in the presence of high content of Cl- at preparing water for the atomic absorption analysis was proposed.
The antibacterial properties of silver cations were detected. Here we studied the effect of sorption of Ag+ cation and $Ag(NH_3)_2^+$ complex cation on cytotoxic effects of the Transcarpathian clinoptilolite (particles <1 micrometer) enriched with silver towards *Candida* yeast cells, as well as cultured pseudonormal human embryonic kidney HEK293 cells and tumor cells of various tissue origin (breast cancer cells of MCF-7 line, larynx cancer cells of KB 3-1 line, pancreatic cancer cells of Capan 1 line). The viability (MTT test) of these mammalian cells was measured, and the IC_{50} was calculated. The amount of silver cation in the applied clinoptilolite-Ag complex equaled to the IC_{50} of “pure” Ag cation.

The most prominent negative effect was observed at treatment of the pancreatic cancer cells of Capan 1 line. Morphological changes were also examined using fluorescence and light microscopy in cells treated with a clinoptilolite-Ag composite and its pro-apoptotic effect was detected.

Silver-enriched clinoptilolites could be used for creating sorption and purification systems for the biomedical application (cartridges for devices for the artificial purification of biological fluids).

ANTIFUNGAL AND CYTOTOXIC ACTIVITY OF CHITOSAN-MELANIN FRAGMENTS EXTRACTED FROM HONEYBEE CORPSES

Nazar Manko
Department of Regulation of Cell Proliferation and Apoptosis

Antimicrobial effect of marine crustaceans chitosan and its derivatives is well characterized, and investigations in this direction are conducted in sense of basic and applied science [1,2]. Such property of chitosans derived from insects, in particular honeybees, are much less developed, although they might be of practical interest. Recently, we have isolated a fragment of chitosan-melanin complex (CMC) from the honeybee that exhibits a cytotoxic action toward *Candida albicans* fungi, and show no toxic effects for human normal, pseudonormal and tumor cells. The aim of this work was to study of the ability of CMC fragments to suppress growth of *Candida albicans* yeast and cytotoxic effects against mammalian cells.

Fragment of the CMC was obtained by a limited hydrolysis of honeybee CMC and chromatography on Toyopearl HW-60, as described [3]. Water solution (2 mg/ml, pH 5.6) was used as a stock for introduction into culture medium. The objects for testing were: *Candida albicans* C88 (conventional), *C. albicans* N12 (MDR), as well as the mammalian cell lines – human breast carcinoma cells of MCF-7 line, murine macrophages of J774.2 line, NIH3T3 murine fibroblasts, human embryonic kidney cells of HEK293 line, PHA-activated lymphocytes of human peripheral blood. The microbial cells were cultured in Saburo medium, pH 5.6 for Candida sp. and pH 7.2 - for bacteria. Cell viability was evaluated using MTT-test for bacterial and mammalian cells and using a method of the colony-forming units (CFU) for Candida fungi. The apoptotic changes in cells were detected by fluorescent microscopy after their staining with Hoechst 33342, DAPI and Ethidium bromide dyes.

CMC fragment showed the highest cytotoxic effect toward *C. albicans* with LD_{50} achieved at 7.0±1.0 μg/ml concentration. That activity was comparable to the activity of a known anti-fungi medicine, the Nystatin (LD_{50} 9±2 μg/ml). We compared the sensitivity to fragment of clotrimazol sensitive C. albicabs C88 strain (clotrimazole LD_{50} 12 μg/ml) and clotrimazole resistant strain C. albicans N12 (clotrimazole LD_{50} 70 μg/ml). There was revealed no significant difference between those strains in their sensitivity to fragment being on the level of 7.0±1.0 μg/ml. CMC fragment did not exhibit a harmful (pro-apoptotic) effect on normal, pseudonormal and tumor mammalian cells in doses up to 200 μg/ml at 72 h incubation.

CMC exhibits high damaging effect toward *Candida albicans* in concentrations comparable with the action of classic antifungal antibiotics and synthetic agents, thus, being perspective for further investigation as the antifungal medicine.

THE ROLE OF PEROXISOMES IN XYLOSE ALCOHOLIC FERMENTATION IN THE ENGINEERED *SACCHAROMYCES CEREVISIAE*

Liubov Dzanaieva
Department of Molecular Genetics and Biotechnology

Peroxisomes are membrane-enclosed organelles occurring in the cytoplasm of most eukaryotic cells. Peroxisomes contain more than 50 different enzymes, which are involved in a variety of biochemical pathways. In yeasts, peroxisomes are involved in fatty acid beta-oxidation, glyoxylic acid cycle, catabolism of unusual carbon sources like purines, methanol etc. Peroxisomes reside many oxidases producing hydrogen peroxide and catalase, which decomposes this toxic compound. Very few are known on the role of peroxisomes on the metabolism of carbohydrates.

Wild type strains of *Saccharomyces cerevisiae* are not able to grow on xylose or ferment this pentose to ethanol. In this work, we have studied role of peroxisomes in xylose alcoholic fermentation in the engineered xylose-utilizing strain of *S. cerevisiae* (Scalcinati et al., 2012). Peroxisome-deficient *pex3Δ* mutant was constructed on the background xylose-utilizing strain of *S. cerevisiae*. The *pex3Δ* revealed slight decrease in ethanol production from xylose, while glucose alcoholic fermentation remained unimpaired. The Pex34 belongs to the peroxisome integral membrane proteins, which are responsible for constitutive peroxisome division (Zhou et al., 2016). The *PEX34* gene was overexpressed under control of strong constitutive *TEF1* promotor aiming to increase peroxisome proliferation. Constructed strain possessed slight increase of ethanol production from xylose relative to that of parental strain. Obtained results let as to suppose that peroxisomes somehow involved in xylose alcoholic fermentation in xylose-utilizing strain of *S. cerevisiae*.

Scalcinati G.et al., FEMS Yeast Res. 2012, 12(5):582-97.

Zhou Y.J. et al., J Am Chem Soc. 2016, 138(47):15368-15377.

DELETION OF GENE *SFU1* 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 involved in this process, particularly, the gene *SEF1* coding for transcription activator, have been identified. It has been shown that in flavinogenic yeast *C. albicans,* Sfu1 (GATA-type transcription factor) represses *SEF1* (Chen C, Pande K, French SD, Tuch BB, Noble SM. An iron homeostasis regulatory circuit with reciprocal roles in Candida albicans commensalism and pathogenesis. *Cell Host Microbe*. 2011;10(2):118–135. doi:10.1016/j.chom.2011.07.005).

The aim of this work was to study the influence of gene *SFU1* on the suppression of the riboflavin synthesis in *C. famata*.

Gene inactivation by deletion cassette in flavinogenic yeast *C. famata* was used during this work. Strains L20105, AF4 and #91 were transformed with plasmid, which contains the deletion cassette of the *SFU1* gene and selective marker *ble* gene conferring resistance to antibiotic phleomycin. The deletion of *SFU1* was confirmed in *C. famata* L20105. The rise in the level of riboflavin production in *sfu1Δ* was noticed.

The effect of deletion in the *SFU1* gene on the riboflavin synthesis is currently under investigation.

PRACTICAL APPLICATION OF AN ENZYMATIC-CHEMICAL METHOD FOR DETERMINATION OF L-LACTATE BASED ON FLAVOCITOCHROME b_2 WITH THE FORMATION OF BERLIN BLUE

Mariia Pidluzhna
Department of Analytical Biotechnology

To date, studies that are relevant to the development of selective, sensitive and, at the same time, inexpensive methods for analyzing biomarkers of the most common diseases and quality indicators for pharmaceuticals and food products, in particular for L-lactate (L-Lact), are important. An enzymatic-chemical method for the determination of L-lactate is based on using flavocytochrome b_2 (EC 1.1.2.3; FC b_2) isolated from the yeast *Ogataea polymorpha 356.* This method, developed earlier (Gonchar et al. 2009) in the Department of Analytical Biotechnology of the Institute of Cell Biology, NAS of Ukraine, is selective, sensitive and inexpensive. The principle of the method is based on FC b_2-dependent enzymatic transformation of L-Lact coupled with nonenzymatic generation of dissolved form of Prussian/Berlin Blue (BB) at the presence of specific solubilizer. The formation of a colloidal solution of BB indicates the presence of L-Lact, and the brightness of the color of BB correlates with concentration of L-Lact. During our work we have optimized the method of L-Lact determination, in particular, the optimal conditions of the enzymatic-chemical reaction, the stability of the enzyme and of the chemical product during storage, influence of salt concentration and temperature on the reaction. As a result, the practical applicability of the method for L-Lact analysis in the real samples of biological liquids was demonstrated. A series of measurements of the L-Lact concentration in several human blood samples was carried out. A high correlation between the data, obtained by this method comparing with the results of reference methods (chemical and LDH) was demonstrated.

Reference: Gonchar, M., Smutok, O., Os’mak, H. 2009. Flavocytochrome b_2-based enzymatic composition, method and kit for L-lactate, US Patent Application PCT/US2008/069637, Pub. No WO/2009/009656.

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