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June 8, 2022

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Lviv

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ABSTRACTS

HEMATOLOGICAL AND SERUM BIOCHEMICAL PROFILE CHANGES IN B16F10/WT MELANOMA-BEARING MICE TREATED WITH LANDOMYCIN A OR ITS WATER-SOLUBLE COMPLEX WITH POLY-2-OXAZONINE POLYMER

Yuliya Kozak, Nadia Skorokhyd, Rostyslav Panchuk, Rostyslav Stoika

Department of Regulation of Cell Proliferation and Apoptosis

The synthesis of new effective compounds for the cancer treatment, design of innovative platforms to improve already studied drugs and development of the innovative highly efficient drug delivery systems are key areas of modern chemotherapy. Low stability in water-based ethanol solutions is the main disadvantage of novel promising experimental antitumor antibiotic **landomycin A (LA)**. **Poly-2-oxazoline (Pox)**-based drug delivery system (Pox) was used to solve this problem. **POx** is safe highly efficient amphiphilic triblock copolymer for the formulation of water-soluble complexes with extremely hydrophobic drugs.

So, the **aim** of this work was to investigate changes of the main hematological and biochemical parameters in mice bearing B1610/wt melanoma under treatment with LA or its complex with POx nanocarrier.

Methods: Hematological and serum biochemical studies were performed using DYMIND DF-51 hematology analyzer and BS-3000M biochemistry analyzer, correspondingly.

Results: Use of LA+POx carrier complex towards mice with B16F10/wt melanoma normalized the number of erythrocytes, platelets, hemoglobin level and neutrophil-to-lymphocyte ratio in their blood to healthy level on 30^{th} and 120^{th} day of the experiment. LA+POx nanocarrier treatment was characterized by massive increase in number of T-lymphocytes, while level of tumor-induced neutrophils was very low at 30^{th} day after tumor inoculation. This indicates on strong immunomodulatory function of POx polymer. The number of white blood cells and lymphocytes was partially reduced in tumor-bearing animals injected with LA+POx polymer on 120^{th} day of the experiment. This can be explained by complete tumor regression in mice of this experimental group. Administration of LA in complex with POx nanocarrier to B16F10/wt melanoma-bearing mice also restored the creatinine level, alkaline phosphatase, aspartate aminotransferase activities and De Ritis ratio in their serum to the healthy level at 30^{th} and 120^{th} day after tumor inoculation.

Conclusion: Immobilization of LA on POx polymer, besides providing its solubility and enhanced stability in aqueous solutions, also leads to normalization of key hematological and biochemical parameters in tumor-bearing mice to the level of healthy animals.

LOW MOLECULAR WEIGHT CHITOSAN DERIVATIVES AS CONVENIENT NANOPLATFORM FOR DELIVERY OF ANTIBIOTICS AND PLASMID DNA

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Department of Regulation of Cell Proliferation and Apoptosis

Chitosan is a polycationic, bio-compatible and biodegradable polysaccharide, however, its application for biomedical purposes is hindered by its high viscosity due to high molecular weight and poor solubility in water solutions at physiological pH. Here, we described preparation of chitosan derivatives which allowed circumventing the above mentioned physico-chemical short-comings of chitosan that block its use in biology and medicine.

We developed a procedure for preparation of low molecular weight (l.m.w.) fractions of chitosan. Their conjugation with branched 25 kDa polyethylenimine (PEI) enhanced significantly chitosan's ability to serve as a platform for delivery of plasmid DNA at transfection of mammalian cells of MCF-7, HeLa, HCT116, and HEK293 lines. The transfection efficiency of such conjugate complexed with plasmid DNA in MCF-7 cells achieved 44.9%. Conjugate of chitosan (l.m.w.) with PEI at concentration range from 1 to 100 μg/mL possessed significantly lower toxicity for these cells, compared to such effect of free PEI. Ampicillin conjugation with l.m.w. chitosan enhanced considerably antibacterial action of this antibiotic towards *Pseudomonas aeruginosa* bacteria that was resistant to ampicillin. Both chitosan and chitosan-ampicillin complex inhibited growth of *Staphylococcus aureus* bacteria.

The conjugate of chitosan (l.m.w.) with PEI was prepared and shown to be efficient platform for transfection of mammalian cells with plasmid DNA. Cytotoxicity of PEI in its conjugate with chitosan was significantly reduced. The conjugate of ampicillin with chitosan (l.m.w.) possessed higher antibacterial activity towards *Pseudomonas aeruginosa* bacteria, compared to free ampicillin. Complexation of ethacridine with chitosan secured gradual dynamics of release of this antibiotic in laboratory rats after injection.

L.m.w. chitosan derivatives were shown to be a perspective nanoplatform for delivery and prolongation of action of antibiotics, and to be a convenient cargo at delivery of plasmid DNA for transfection of mammalian cells.

DEVELOPMENT OF A SYSTEM FOR POSITIVE SELECTION OF YEASTS WITH IMPROVED PARAMETERS OF ALCOHOLIC FERMENTATION BASED ON ETHANOL-INDUCED PROMOTER OF THE *ASPERGILLUS NIDULANS* GENE *ALCA*

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Biotechnological production of fuel ethanol from plant biomass (lignocellulose) is important for energy development and environmental protection. Another promising raw material for the production of fuel ethanol is a by-product of biodiesel industry – crude glycerol. One of the reasons for the unprofitability of ethanol production from such feedstock is the low yield of ethanol due to inefficient microbial fermentation of pentose sugars, mainly xylose, as well as glycerol. The most promising organisms for the production of ethanol from sugars of lignocellulose and glycerol fraction are yeasts, e.g. yeast *Ogataea polymorpha*, which can grow on xylose under elevated temperatures; yeast *Komagataella phaffii*, which can effectively utilize glycerol; and yeast *Saccharomyces cerevisiae*, which produce large amounts of ethanol from glucose.

The aim of our work was to develop a system for selection of *S. cerevisiae*, *O. polymorpha* or *K. phaffii* strains with increased levels of ethanol production from glucose, xylose and glycerol using ethanol-induced AlcR/AlcA gene expression system, which is currently successfully implemented for plants. The proposed system has two essential components: the ethanol-inducible promoter of *Aspergillus nidulans* gene *alcA* and the alcR transcription factor, which binds to and activates the *alcA* promoter in the presence of a co-inducer ethanol. In order to assess the level of induction of the *alcA* promoter, a gene encoding a reporter enzyme or an antibiotic resistance gene can be placed under its control. We used the *LAC4* gene from the yeast *Kluyveromyces lactis*, which encodes the enzyme β-galactosidase, the activity of which can be easily assessed using a chromogenic substrate X-Gal, or the gene *kanMX4* providing resistance to geneticin. We plan that this selection system in combination with UV or insertional mutagenesis will be used to identify genes that affect the efficiency of alcoholic fermentation of glucose, xylose and glycerol in *S. cerevisiae*, *O. polymorpha* and *K. phaffii*.

So far, with the help of this system we identified that gene *HXS1 O. polymorpha* has a profound effect on glucose and xylose utilization and fermentation. Deletion of the gene *HXS1* cause decrease the rate of glucose/xylose consumption and ethanol production, whereas overexpression of this gene has the opposite effect.

APPLICATION OF FLAVOCYTOCHROME B_2 -BASED METHOD FOR DETERMINATION OF L-LACTATE IN YOGHURTS

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Department of Analytical Biotechnology

L-lactate is the final product of anaerobic glycolysis, the last stage of which is the conversion of pyruvate to lactate by the enzyme lactate dehydrogenase. L-lactate plays an important role in many technological processes, it is the main component that affects the taste, pH and emulsifying stability of food.

Determination of L-lactate level is most often used in medicine, as well as in the food industry to assess the quality of food and beverages. Physicochemical, chemical, enzymatic and enzymatic-chemical approaches are widely used for lactate analysis. The standard chemical methods require derivatization of lactate, which makes them very inconvenient to use. So, expanding the range of simple and cost-effective express methods for L-lactate determination is a necessary and urgent task for scientists.

In previous studies, the researchers of our Department have developed the optimal methods for isolating flavocytochrome b_2 (Fc b_2) from the cells of thermotolerant methylotrophic yeast *Ogataea polymorpha 356*, obtained a highly purified target enzyme, investigated its properties and proposed the FCb_2 / Prussian Blue (PB) method for determining L-lactate. This method was successfully tested on the real samples of biological liquids, including rat and human blood, wines and yeast cultures.

The aims of our work were to learn how to perform the technique of determining lactate using FCb_2/PB method and to appreciate its applicability on the real samples of commercial yoghurts.

As a result of research, the conditions for stabilization of FCb_2 isolated from yeast-producing cells, were optimized and FCb_2/PB method for determining lactate was improved. The optimal conditions for the enzymatic reaction were determined, namely: temperature, incubation time and FCb_2 concentration; the inhibitory effect of salts in the reaction on BB formation was demonstrated. Concentrations of L-lactate in the samples of yoghurts from different manufacturers were estimated.

Thus, it was demonstrated, that the analytical FCb_2/PB method is fast, easy to use and cost-effective, as it does not require large costs for reagents and equipment. It may be promising for the implementation in practice.

Implementation into practice :

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.

PRODUCTION OF THE RIBOFLAVIN (VITAMIN B_2) ON WHEY BY THE FLAVINOGENIC YEAST *CANDIDA FAMATA*

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Riboflavin serves as biosynthetic precursor of flavin nucleotides FMN and FAD and is important biotechnological product with annual market around 1 billion US dollars. It is mostly used as component of feed premixes for animals (80%), in food industry as food colorant, in medicine and component of polivitamin mixtures and as drug for treatment of some diseases.

Apart from proteins, fat, microelements and vitamins, whey contains lactose which can be source of carbon for specific *C. famata* strain to produce riboflavin (with addition of ammonium sulfate only). It's a promising discovery to utilize byproduct and receive valuable product. The aim of the current work was to improve the available riboflavin-overproducing strains *C. famata* regarding growth and utilization of lactose.

Results showed that *C. famata* effectively utilizes lactose as sole carbon source and overproduces riboflavin. The synthesis of vitamin B_2 on whey was significantly elevated by expression of homolog of mammal riboflavin efflux protein BCRP and expressing *SEF1* transcription activator under control of *LAC4* promoter induced by lactose. Outcomes so far are very promising. Maximally, 1.2 g of riboflavin per liter was achieved during *C. famata* bioreactor cultivation on whey with ammonium sulfate. Further studies on optimization of dissolved oxygen supply will be done to get even the higher titers of riboflavin.

REDUCTION IN VIABILITY OF HUMAN CERVIX CARCINOMA HELA CELLS UNDER TRANSFER OF *P21* GENE WITH BLOCK POLY(DMAEMA)-BASED BP83-1 CARRIER

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Background. Several cationic polymers have been proposed for application as carriers of genetic materials. The poly(2-dimethylamino)ethyl-methacrylate (poly(DMAEMA)) demonstrated high transfection efficiency and low cytotoxicity when used as gene delivery system. The aim of present study was to evaluate the capability of poly(DMAEMA)-block-poly(N-vinylpyrrolidone)-co-(butyl-acrylate)-co-2-aminoethyl methacrylate carrier BP83-1 to deliver *p21* gene into human cervical carcinoma HeLa cells and to evaluate its effects on the viability of these tumor cells *in vitro*.

Methods used in the research**.** Transfection assay with poly(DMAEMA) carrier and linear polyethyleneimine (PEI), Western-blot analysis, MTT test, DNA comet analysis in alkaline conditions, diphenylamine assay for DNA fragmentation (Barton's assay), FACS analysis of cell cycling.

Results. The BP83-1 polymer effectively transferred pFlag-P21WT plasmid DNA containing *p21* gene into human cervical carcinoma HeLa cells. The level of BP83-1-facilitated delivery of *p21* into HeLa cells was significantly higher than that such level obtained with PEI. A reduction by 26.1 % of the viability of HeLa cells transfected with pDNA/ BP83-1 was observed compared to the non-transfected cells, and by 40 % - of HeLa cells transfected with pDNA/PEI. The reverse dependence between the elevated amount of p21 protein and reduced amount of cyclin-dependent kinase 2 (Cdk2) was determined in the transfected HeLa cells. The number of cells in G1 phase of cell cycle in HeLa cells was increased from 54.9 % to 65.8 % and to 64.9 % after their transfection with pFlag-P21WT/BP83-1 and pFlag-P21WT/PEI polyplexes, correspondingly. In HeLa cells transfected with pDNA/BP83-1 and pDNA/PEI polyplexes, an increased number of single-strand breaks in DNA and content of the fragmented DNA was detected. DNA damaging effects of the BP83-1 carrier and pDNA/BP83-1 polyplex were less pronounced in treated HeLa cells, compared with such effects of the PEI and pDNA/PEI polyplex.

Conclusions. The effective transfer of *p21* gene with BP83-1 poly(DMAEMA) carrier into human cervical carcinoma HeLa cells was demonstrated. The overexpression of the *p21* gene led to inhibition of viability of HeLa cells, DNA damage and blocking of cell cycle progression from G1 phase to S phase via a reduction of the amount of Cdk2 and resulting accumulation of treated cells in G1 phase.

SELECTION OF *OGATAEA POLYMORPHA* STRAINS THAT PRODUCES ETHANOL FROM XYLOSE AND L-ARABINOSE

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Department of Molecular Genetics and Biotechnology

Xylose and L-arabinose are important components of renewable feedstocks (lignocellulose and pectin) for biofuel production, being the second and third sugars by abundance in nature. However, since L-arabinose and xylose are not utilized as carbon sources by most known yeast strains, a significant percentage of total carbon in the production of bioethanol from plant residues is not used. Moreover, despite significant efforts made in the field of alcoholic fermentation of basic sugars from lignocellulose hydrolysates and some achievements in this field (especially on xylose fermentation), yeast strains capable of efficient fermentation of L-arabinose have not been yet identified.

Previously obtained *O. polymorpha* best ethanol producers from xylose (Ruchala et al., 2017; Kurylenko et al., 2018), like the wild-type strain, grow poorly on L-arabinose. In contrast, obtained mutants of *O. polymorpha* by UV mutagenesis robustly grow on L-arabinose, still accumulate half of the biomass compared to the parental strain growing on xylose. However, after analyzing the level of ethanol production during high-temperature alcohol fermentation in an environment with 10% xylose or 5% L-arabinose, it was found that the resulting mutants produced 30% more ethanol from xylose compared to the parent strain. Moreover, the ability to ferment L-arabinose mutants was 6 times higher than the parent strain during high-temperature alcohol fermentation at 45ºC. However, the production of ethanol from L-arabinose remains below 0.2 g/L or 100 times less than that from xylose. In the preliminary experiments, it was found that 6-deoxyglucose inhibits growth of parental strain on xylose whereas mutants growing on L-arabinose are resistant to 6-deoxyglucose. Moreover, mutants able to grow on xylose and one of the compounds: glucosamine, 2-deoxyglucose and 3-bromopyruvate was selected. Such original selection scheme is found to be successful for isolation of *O. polymorpha* mutants accumulating elevated amounts of ethanol from xylose and L-arabinose.

OBTAINING OF RECOMBINANT STRAINS OF YEAST *CANDIDA FAMATA* ABLE TO INCREASED SYNTHESIS OF FLAVINMONONUCLEOTIDE IN MEDIUM SUPPLEMENTED WITH LACTOSE

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Riboflavin (RF) is important nutritional component of animals and people. It is a precursor of flavin mononucleotide (FMN) and flavin adenine dincleotide that participate in many metabolitic processes: redox homeostasis, protein folding, DNA repair, fatty acid β-oxidation, amino acid oxidation, and choline metabolism. It was found that the wild type *Candida famata* is characterized by robust growth on lactose and cheese whey and the engineered strains also overproduce riboflavin and/or FMN on whey which is an industrial waste. Overexpression of the *D. hansenii FMN1* gene (coding for riboflavin kinase) led to the accumulation of FMN by *C. famata* transformants.

The production of FMN still wants to be better, because the yield is low compared to the production of riboflavin. That is why the aim of this work is to obtain transformants of yeast *C. famata* that able to increase synthesis of this nucleotide on lactose and cheese whey. To achieve this goal, previously obtained flavin overproducers of yeast *C. famata* were used: FP (from the original strain AF-4/FMN1/RIB1 that has the *FMN1* gene under the control of the *TEF1* promoter) and # 134 (from the original strain AF4 containing the *SEF1* gene under the control of the *LAC4* promoter).

We obtained the transformants of these strains with *SEF1* gene under the control of the *LAC4* promoter and an additional gene *FMN1*, which encodes RF kinase. Next, we stabilized them and checked on PCR. Then we investigated production of RF and FMN when grown in different medium (YPD, YNB medium with glucose or lactose). The growth and synthesis of RF by recombinants on lactose and whey containing 5% lactose were studied in more detail. One of these transformants – #17 – that contain the *FMN1* gene under the control of the *TEF1* promoter from the original strain #134 produces FMN up to 1.3 times more on whey than on medium with 5% glucose. Another stain – #28 – containing the *SEF1* gene under the control of the *LAC4* promoter from the original strain FP synthesizes FMN up to 2.3 times more on whey than parental strain. Obtained data indicate that the waste whey from milk industry, can be a promising substrate for FMN production by *C. famata.*

L-LACTIC ACID PRODUCTION BY METABOLICALLY ENGINEERED YEAST OGATAEA POLYMORPHA UNDER PH CONTROLLING CONDITIONS

Aksyniia Tsaruk
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Recently demand for L-lactic acid has rapidly increased that resulted in growing interest in improving methods of lactate production. Lactate is widely used in food, medical, pharmaceutical industry as a preservative agent, an additive, and a component of biodegradable polymer materials. Lignocellulosic materials are one of the primary substrates in lactic acid production due to its low cost, abundance and sustainability. Lactate is generally produced by lactic acid bacteria but implementing genetically modified yeast offers a range of benefits such as higher tolerance to stressful conditions and simple growth requirements.

For this study, previously engineered high ethanol producer strain of the thermotolerant methylotrophic yeast *Ogataea polymorpha* (Ruchala et al., 2017) was selected. Lactate dehydrogenase gene derived from Rhizopus oryzae was heterologously expressed under the control of the strong constitutive promotor of glyceraldehyde-3-phosphate dehydrogenase gene. Obtained *O. polymorpha* transformants were investigated for their ability to biosynthesize lactic acid during cultivation on YDP medium containing 0.002% of bromophenol blue. This pH indicator changes color from yellow at pH 3.0 to purple at pH 4.6, thus yeast strains that produce lactic acid develop yellow zone around its colony. One of such recombinant yeast strains with the most distinct yellow zone was selected for fermentation process.

Lactate fermentation under acidic conditions is beneficial to reduce the cost of production, but accumulation of high lactic acid concentration causes cellular toxicity. Therefore, pH of the fermentation medium has to be controlled to prevent strong acidification. In our study, fermentation medium was supplemented with various concentrations of calcium carbonate as a neutralizing agent. It was shown to effectively stabilize the pH and increase lactic acid production by engineered *O. polymorpha* strain.

Ruchala J., Kurylenko O.O., Soontorngun N., Dmytruk K.V., Sibirny A. A. (2017). Microbial Cell Factories, 16(1). doi:10.1186/s12934-017-0652-6

TARGETING CD98-LAT1 AMINO ACID TRANSPORTER UNDER ARGININE DEPRIVATION IN HEAD AND NECK SQUAMOUS CARCINOMA CELLS (HNSCC)

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HNSCC are highly aggressive, heterogeneous tumors with overall 5-year patients' survival rate about 40-50%. Recent published data demonstrated that arginine deprivation therapy (ADT) exhibits some anticancer efficacy *in vitro* against a panel of HNSCC lines. Moreover, CD98 (*SLC3A2*), - the heavy chain of a cell surface transmembrane protein can be a therapeutic target in patients with HPV-negative tumors. CD98 together with LAT1 (*SLC7A5*) protein constitute a heterodimeric transmembrane amino acid transporter LAT1-CD98 that preferentially transports isoleucine, leucine, methionine, valine, histidine, cysteine, tryptophan, and tyrosine.

Therefore, the aim of the study was to elucidate the potential effect of co-targeting of the amino acid transporter LAT1-CD98 together with ADT on HNSCC cells.

First, we performed *in silico* bioinformatics analysis of *SLC3A2* (CD98) and *SLC7A5* (LAT1) gene expression in HNSCC patients in comparison to normal tissues based on the available The Cancer Genome Atlas (TCGA) dataset. We found that both mentioned genes were upregulated in primary HNSCC tissues relative to normal tissues. Moreover, a strong negative correlation between high tumor *SLC3A2* and/or *SLC7A5* levels and clinical outcome (relapse-free and overall survival) is observed in the HNSCC patient cohort (n=500) of TCGA dataset.

In line with our previous findings, ADT alone led to a strict growth arrest in two tested HNSCC cells, SAS and Cal-33. SAS cells were unable to restore their proliferation after 3d of Arg re-supplementation, contrary to Cal-33 cells which were highly resistant to ADT. Also, high levels of CD98 and LAT1 proteins were identified in Cal-33 cell line.

However, CRISPR-Cas9-mediated knockout of *SLC3A2* gene in Cal-33 cells revealed that inhibition of CD98 did not sensitize these cells to the defined individual amino acids deficiency - arginine, leucine or methionine in cell culture medium.

In addition, the effect of CD98 knockout alongside with LAT1selective inhibitor, JPH203, on the growth and survival of Cal-33 cells under ADT conditions *in vitro* was analyzed. Our preliminary studies showed that inhibition of CD98-LAT1 transporter did not play a significant role in the sensitization of Cal-33 carcinoma cells to arginine deficiency as this did not enhance the therapeutic effect of ADT. Further research is needed to clarify the role of CD98-LAT1 molecule in sensitivity of HNSCC cells to individual amino acids starvation as metabolic anticancer therapy.

STRUCTURE *VS* BIOLOGICAL ACTIVITY RELATIONSHIPS OF NOVEL PYRIDINE-THIAZOLE-PYRIDINE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS

Iryna Ivasechko

Department of Regulation of Cell Proliferation and Apoptosis

Study of tumor biology and molecular mechanisms involved in carcinogenesis are under special attention. New ways of molecular design of chemotherapeutic drugs are under development, and novel thiazole-based compounds are synthesized. Recently, thiazole-containing compounds have been successfully developed as potent inhibitors of specific biological targets, including enzyme-linked receptors located on cell membrane, the cell cycle (microtubular) inhibitors and DNA reparation enzyme - PARP1.

Here, we analyzed anticancer activity in vitro of new thiazole derivatives and proposed possible molecular mechanism of action. The compound Les-5303 and its derivatives - Les-6485, Les-6486 - were applied towards >15 cancer cell lines of different tissue origin. Two of them, Les-5303 and Les-6485, showed similar high antiproliferative activity. Change in structure of compound Les-6486 significantly reduced its activity compared to the initial compound. All compounds demonstrated low toxicity towards pseudo-normal and normal cells. The ability of these thiazole derivatives to inhibit the activity of PARP1 enzyme and interact with DNA molecule was determined. It was shown that after preincubation with Fluzaparib - PARP1 inhibitor, Les-5303 and Les-6485 significantly reduced the activity of that enzyme. Besides, these compounds influenced DNA molecule and caused morphological changes in structure of nucleus. Thus, the new tiazol derivatives can cause genetic instability and demonstrate cytotoxic action towards human tumor cells.

ACTIVATION OF THE PHOSPHOKETOLASE PATHWAY OF XYLOSE METABOLISM IN THERMOTOLERANT YEAST *OGATAEA POLYMORPHA*

Anastasiya Zazulya
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One of the most pressing problems of our time is the search, improvement and implementation of new sources of renewable energy. 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. Today, the problem of establishing profitable production of 2nd generation ethanol – ethanol from renewable raw materials such as lignocellulose or pectin is very important. Bioethanol can be mixed with petrol or used alone in specialized cars’ engines.

One of the main obstacles to the efficient production of ethanol from lignocellulose is the lack of yeast strains capable of simultaneous efficient metabolism of the three main components of monosaccharides of this biopolymer: glucose, xylose and L-arabinose. D-xylose is the second most abundant sugar on the planet, and one of the main components of lignocellulosic hydrolysates, accounting for 8 to 40% of its dry weight.

Yeasts convert pentoses to ethanol through non-oxidative part of pentose phosphate pathway, glycolysis and finally ethanol synthesis. However, in some basidiomycete and ascomycete xylose-utilizing yeasts, xylulose-5-phosphate can also be metabolized in the phosphoketolase pathway. Engineering phosphoketolase pathway in *O. polymorpha* looks very promising as it can increase ethanol production from xylose and decrease CO_2 emission during fermentation. Still there is no definite knowledge on existence of the native phosphoketolase in *O. polymorpha* as it could not be excluded that the available old data on phosphoketolase activity in cell-free extracts obtained from *O. polymorpha* cells (Evans and Ratledge, 1984) is artefact. But it is known that gene *PHK1* in filamentous fungus *Aspergillus nidulans* encodes functional phosphoketolase (Panagiotou et al., 2007).

Gene *PHK1* from *A. nidulans* was codon-optimized for expression in *O. polymorpha* and cloned into previously constructed plasmid pUC19-GAPpr-GAPt-natNT2, with a strong constitutive promoter and terminator of the *O. polymorpha GAP1* gene (encoding glyceraldehyde-3-phosphate dehydrogenase) and selective marker *natNT2* providing resistance to the norseotrecin. Obtained vector was used for transformation of *O. polymorpha* strains NCYC495 and BEP/Δcat8 (improved producer of ethanol from xylose, Ruchala et al., 2017). In one of the obtained recombinant strains NCYC/PHK1, ethanol production increased on average 1,9 times compared to the initial strain. In *O. polymorpha* BEP/PHK9, ethanol production increased by an average of 10%.

REVEALING OF THE ROLE OF *SEF1* ORF FROM *PICHIA STIPITIS* IN THE SYNTHESIS OF RIBOFLAVIN BY THE FLAVINOGENIC YEAST *C. FAMATA*

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Vitamin B_2 (RF, or lactoflavin), despite its crucial role in metabolism, is not synthesized in animals and humans. This is one of the reasons for the growing interest in developing methods for synthesizing this compound with the help of microorganisms. For instance, the yeast *Candida famata* is one of the flavinogenic yeasts that is capable to produce elevated amounts of riboflavin under iron deficiency.

Transcription factor Sef1 is one of the positive regulators of riboflavin synthesis in flavinogenic yeast, in particular in *C. famata* (Dmytruk et al., 2006). The corresponding gene is also present in the genomes of non-flavinogenic yeast, however, nothing is known on its role in riboflavin synthesis there (Groom KR 1998). It is not known if *SEF1* gene from non-flavinogenic yeasts could substitute its ortholog from *C. famata* to regulate riboflavin synthesis.

In our previous work, we have shown that riboflavin synthesis in ***sef1Δ*** mutant was restored after introduction of *C. famata SEF1* ORF under control of *SEF1* promoters of flavinogenic yeasts *C. famata* and *C. albicans* but not that from non-flavinogenic yeasts. Here, we decided to study the phenotype of ***sef1Δ*** strains after introduction of *SEF1* ORF from non-flavinogenic yeast *Scheffersomyces stipitis* under the control of the *SEF1* promoter from the flavinogenic yeast *C. famata*. We have found that the resulted transformants are unable to overproduce riboflavin. Since Sef1 factor is regulated by the content of iron ions, we decided to repeat the testing of biochemical characteristics of the obtained strains in conditions of iron deficiency. The synthesis of riboflavin increased in the wild-type strain L20105 and its derivatives, but did not increase in both ***sef1Δ*** mutants and its derivative transformants containing *S. stipitis SEF1* ORF controlled with *C. famata SEF1* promoter. These results suggest that the presence of both the promoter and ORF of the *SEF1* gene of flavinogenic origin are important for the riboflavin oversynthesis.

THE ROLE OF TRANSCRIPTION ACTIVATOR INVOLVED IN CARBOHYDRATE SENSING AZF1 IN XYLOSE AND GLUCOSE FERMENTATION IN THE THERMOTOLERANT YEAST *OGATAEA POLYMORPHA*

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Ogataea polymorpha is one of the most thermotolerant yeasts known. It has the potential to be used for efficient simultaneous saccharification and fermentation (SSF) for its temperature tolerance and ability to ferment xylose to ethanol. However, despite the robust growth on xylose, the ethanol yield and productivity from this sugar in wild-type strains of *O. polymorpha* are very low. At present, the efficacy of xylose alcoholic fermentation of *O. polymorpha* is significantly improved by metabolic engineering. Still, genes involved in the regulation of xylose fermentation are poorly studied.

The *S. cerevisiae AZF1* gene codes for the transcription activator of genes involved in carbon metabolism and energy production on glucose. However, the role of *AZF1* in xylose fermentation in the native xylose-metabolizing yeasts has never been studied. Therefore, the *AZF1* gene overexpressed under the control of *GAP1* promoter on backgrounds of the wild-type strain and of the advanced ethanol producer (strain BEP/Δcat8) to identify the role of alcohol fermentation from xylose and glucose in this research.

The result shows that transcription factor *AZF1* in wild strain and BEP/Δcat8 have a positive effect on xylose and glucose fermentation when overexpression on the background of the advanced ethanol producer from xylose (BEP/Δcat8) resulted in a near 10% increase of ethanol accumulation in glucose and above 30% increase in xylose medium. Moreover, it was found that WT/Δazf1 mutant is characterized by 40 % decrease in growth on xylose whereas growth on glucose was unimpaired the mutant also showed defects in ethanol production on both glucose and xylose media which indicates the potential importance of the corresponding genes for construction of the advanced ethanol producers from the major sugars of lignocellulose.

OPTIMIZATION OF RIBOFLAVIN PRODUCTION FROM WHEY IN BIOREACTOR CULTIVATION

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Whey is a waste by-product of dairy industry which comes from cheese and casein manufacture. Due to problem with disposal of huge amounts of whey it became an environmental problem. Throughout years scientists developed methods of processing whey waste from dairies. However many of them does not ensure microbial conversion of lactose. *Candida famata* belongs to flavinogenic yeasts which overproduce riboflavin (vitamin B2) and secrete it to culture media. Riboflavin is a precursor of flavin coenzymes FMN and FAD which are used in oxidative metabolism and other processes.

Our research is focused on genetic engineered strain *Candida famata* which is able to grow on whey (around 5% of lactose content) with addition of ammonium sulfate only. Best producer so far appeared to be the strain with overexpression of genes *SEF1* (transcription activator), *RIB1* (GTP-cyclohydrolase II), *RIB7* (riboflavin synthase) and RIB6 (3,4-dihydroxy-2-butanone-4-phosphate synthase) (Petrovska et al 2022).

Cultivation conditions are now being optimized in bioreactor (benchtop bioreactor BioFlo 115, New Brunswick). Constant parameters such as temperature 28^{o}C, aeration 1 L/min, pH 5,5±0,5 and content of ammonium sulfate 3 g/l has been established. Further investigations are now focused on dissolved oxygen (DO) content. DO goes together with agitation which compensate oxygen demand during cultivation. Because *C. famata* might be sensitive to shear forces, a perfect solution between DO level and agitation have to be found.

It was found that under optimal conditions, the mentioned strain accumulated 2,2 g/l of riboflavin in batch culture. These results are very promising. Further experiments including fed-batch cultivation are planned in near future.

ROLE OF THE PENTOSE PHOSPHATE PATHWAY IN RIBOFLAVIN OVERSYNTHESIS OF THE FLAVINOGENIC YEAST *CANDIDA FAMATA* (*CANDIDA FLARERI*)

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Riboflavin (vitamin B2) is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are coenzymes of flavoproteins involved in mostly oxidative metabolism in human and animal bodies. Yeast *Candida famata* is able to overproduce riboflavin under iron deficiency.

The ribulose-5-phosphate is a precursor of riboflavin biosynthesis, derived from the pentose phosphate pathway. Genes *ZWF1*, *SOL3*, and *GND1* encode glucose-6-phosphate dehydrogenase, 6-phosphogluconolactonase, and 6-phosphogluconate dehydrogenase in the oxidative phase of the pentose phosphate pathway, respectively.

Therefore, our purpose was to investigate whether overexpression of the mentioned above genes can regulate oversynthesis of riboflavin in strains *C. famata* L20105 (*leu2*), AF4, and AF-4/SEF1/RIB1/RIB7/ARO4 (BRP).

The qRT-PCR analysis of obtained strains containing single gene *ZWF1* or *GND1* showed increased expression levels compared with the parental strains. Moreover, riboflavin production was increased in strains containing *GND1*, but the opposite was seen in strains containing *ZWF1*. Interestingly, although strains AF4/ZWF1/GND1 and BRP/ ZWF1/GND1 expression levels have no obvious increased, the production of riboflavin increased.

Assuming that decrease of flavin production in the strains with *ZWF1* overexpression can be caused by lactonate accumulation in the cell, we decided to reveal if it is avoidable. So our next plan was to overexpress the second gene of the PPP – *SOL3*, which is coding for 6-phosphogluconolactonase. It was hypothesized that this enzyme will change a flux of metabolism of gluconolactonate further through PPP and it will help to avoid acid accumulation in mutant with overexpression *ZWF1* gene.

As a first step in this exploration, we obtained transformants bearing overexpression cassette for *SOL3* gene on the base of *C. famata* L20105, AF-4, and #91 (BRP). It is noticeable that overexpression of *SOL3* in AF-4 gives an increase in the amount of vitamin B_2 in 1.1 - 1.9 times. Only slightly for L20105, but not for 91. Therefore, further experimental work on revealing an impact of *SOL3* gene in strains with already oversynthesised glucose-6-phosphate dehydrogenase seems to be an important task.

HIGHLY POROUS 3D GOLD ENHANCES SENSITIVITY OF AMPEROMETRIC BIOSENSORS BASED ON OXIDASES AND CuCe NANOPARTICLES

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Metallic nanoparticles have wide potential practical applications in various fields of science and industry. In biosensorics, they usually act as carriers, mediators in electron transfer and/or catalysts (artificial enzymes or nanozymes). Nanozymes (NZs) are the newest class of functional nanomaterials; they have enzyme-like activities with different reaction specificities. NZs possess increased stability and greater availability due to their simpler preparation technologies. Most reported NZs are mainly mimetics of oxidoreductases, including peroxidase (PO).

We describe herein the development of amperometric biosensors (ABSs) based on purified oxidases, synthesized nanoparticles of CuCe (nCuCe) and micro/nanoporous gold (pAu), which were electro-deposited on a graphite electrode (GE). As an effective peroxidase (PO)-like NZ, nCuCe was used here as a hydrogen peroxide-sensing platform in ABSs which were based on glucose oxidase, alcohol oxidase, methylamine oxidase and L-arginine oxidase. At the same time, nCuCe is an electroactive mediator and has been used in laccase-based ABSs. As a result, the ABSs we constructed and characterized which were intended for asssay of glucose, methanol, methyl amine and L-arginine. The developed nCuCe-based ABSs exhibited improved analytical characteristics, in comparison with the corresponding PO-based ABSs. Additionally, the presence of pAu with its extremely advanced chemo-sensing surface layer was shown to significantly increase the sensitivities of all constructed ABSs. As an example, the bioelectrodes containing laccase/GE, laccase/nCuCe/GE and laccase/nCuCe/pAu/GE exhibited sensitivities to catechol as 2300, 5055 and 9280 $A \cdot M^{-1} \cdot m^{-2}$, respectively. We demonstrate here that pAu is an effective carrier of electroactive nanomaterials coupled with oxidases, which may be promising in biosensors.

The proposed amperometric biosensor based on alcohol oxidase was used for the quantitative determination of ethanol in human biologic liquids. The obtained results proved to be in a good correlation with the enzymatic reference method. These results highlight the potential of the nCuCe with PO-like activity in bioanalytical application.

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