ПРОЄКТНА ПРОПОЗИЦІЯ НА УЧАСТЬ У КОНКУРСІ СПІЛЬНИХ УКРАЇНСЬКО – ЛАТВІЙСЬКИХ НАУКОВО-ДОСЛІДНИХ ПРОЄКТІВ ДЛЯ РЕАЛІЗАЦІЇ У 2023 – 2024 рр.

Program Proposition Form of Ukrainian-Latvian Joint Programme of Scientific and Technological Cooperation Project

Загальна інформація / General Information

Назва проєкту українською мовою / Title of the project in Ukrainian:	**Дослідження ролі глутатіону та білків теплового шоку в стійкості дріжджів до дегідратації-регідратації.**
Назва проєкту англійською мовою / Title of the project in English:	**Investigation of the role of glutathione and heat shock proteins in yeast tolerance to dehydration-rehydration.**
Анотація *(макс. 1000 символів)* **/ Summary** *(max 1000 characters)*	The proposed project is devoted to the construction of yeast *S. cerevisiae* and *O. polymorpha* recombinant strains with increased intracellular glutathione and/or heat shock proteins content and evaluation of their tolerance to dehydration-rehydration. The previously obtained data suggests that glutathione overproduction in *O. polymorpha* positively influence cell survival rate during dehydration-rehydration, so it would be interesting to test if the same is true for *S. cerevisiae* as dehydrated cells of this yeast are of profound industrial importance. Additionally, we plan to perform overexpression of genes *HSP70* and *HSP104* to test how increased levels of the respective heat shock proteins influence yeasts desiccation tolerance. Obtained strains will have a range of practical implementations: production of active dry yeast preparations with increased shelf life and improved activity upon rehydration; biotechnological production of glutathione and Hsp proteins etc.
Відповідність пріоритетній галузі досліджень *(відмітити хрестиком)* **/ Conformity with the priority research area** *(mark with cross mark)*	☐ Енергетика та енергоефективність ☐ Екологія та раціональне природокористування, включаючи морські дослідження, зокрема дослідження забруднення акваторій морів хімічними речовинами та мікропластиком ☐ Нові технології профілактики та лікування найпоширеніших захворювань, дослідження в галузі біотехнології, біоінженерії та генетики ☐ Нові матеріали ☐ Суспільні та гуманітарні науки

Партнери / Partners

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1. Завдання проєкту *(до 0,5 сторінки А4, 12pt) (тут і далі – об'єм вказується для тексту українською мовою; об'єм тексту англійською мовою в залежності від перекладу)* **/ Project objectives** *(up to half A4 size sheet, 12pt)*

The main objective of the project is to study the impact of the increased intracellular level of glutathione and/or heat shock proteins on the dehydration-rehydration tolerance of the cells of baker's yeast *Saccharomyces cerevisiae* and termotolerant methylotrophic yeast *Ogataea polymorpha*. To achieve this goal, we plan to obtain and analyze *S. cerevisiae* and *O. polymorpha* recombinant strains with overexpression of the genes *GSH1* (*GSH2* for *O. polymorpha*) and *MET4* involved in glutathione synthesis and/or genes *HSP70* and *HSP104* encoding heat shock proteins. During analysis, particular attention will be devoted to the recombinant strains tolerance to dehydration-rehydration both under conditions of fast rehydration and slow gradual rehydration by incubation in water vapour. The influence of increased glutathione concentration or heat shock proteins overexpression on the cell wall and plasma membrane of yeast cells will also be studied.

2. Опис поточної ситуації *(до 2 сторінок А4, 12pt)* **/ Description of the current situation** *(up to 2 A4 size sheets, 12pt)*

Yeasts, including industrially important species *Saccharomyces cerevisiae*, *Ogataea polymorpha* and others, are able to survive dessication by entering a state of anhydrobiosis – a temporary reversible suspension of cell metabolism (Beker and Rapoport, 1987; Rapoport, 2017; Rapoport et al., 2019). This property is widely used now for the successful long-term maintenance of viability of microorganisms and large-capacity production of active dry baker's yeast. Active dry yeast preparations is primarily used in winemaking but they also can be used for the biotechnological production of various valuable compounds. Also, the dehydration of yeast has attracted an increasing interest in some rather unexpected biotechnological tasks, for example dried yeasts can be used as efficient biosorbents of different pollutants, including heavy metals (Rapoport et al., 2016).

Ability of the yeast cells to survive during dehydration-rehydration depends on different factors, such as the stability of nucleic acids; the preservation of membrane integrity; the accumulation of significant amounts of intracellular protective substances (trehalose, polyols, ergosterol, glutathione, proline, catalase, and Hsp70) etc (Kulikova-Borovikova et al., 2018). As desiccation is accompanied by oxidative stress (amount of ROS increases with drying), accumulation of antioxidants can improve cellular survival after rehydration. One well-known example of such antioxidants is glutathione.

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is a biologically active substance of a peptide nature that plays an important role in a wide range of cellular reactions. The antioxidant properties of glutathione play a role in maintaining the intracellular redox status. Due to the presence of a thiol group, glutathione acts as an electron donor in cells and ensures the course of the reduction reaction, while it turns into an oxidized form (GSSG). In addition to maintaining the thiol redox status, glutathione is involved in the detoxification of endogenous and exogenous metals and xenobiotics, the deposition and transport of cysteine, the biosynthesis of protein and DNA, the regulation of the cell cycle, etc. Under fasting conditions, glutathione is the main source of nitrogen and sulfur. Glutathione deficiency in humans is associated with a number of medical disorders caused by oxidative stress, poisoning, or a weakened immune system. These disorders include neurodegenerative diseases, cancer, cataracts, cirrhotic diseases, diseases of the lungs, inflammation of the gastrointestinal tract and pancreas, and hemolytic anemia.

This compound has also biotechnological importance as the use of glutathione in various cosmetic products is growing rapidly. Glutathione is used as a component of emulsifiers, oils, and moisturizers, primarily to enhance food-based whitening effects, and to eliminate or prevent acne breakouts. In addition, glutathione is used in sunscreens and as a component of antiaging creams (Posci et al, 2004).

The ability of glutathione to interact with exogenous reactive metals and xenobiotics

allows the use of microbial producers of this tripeptide for detoxification of heavy metals and xenobiotics in wastewater (Penninckx et al, 2002).

In yeast, the synthesis of glutathione occurs in two consecutive reactions catalyzed by γ-glutamylcysteine synthetase (γ-GCC), encoded by the gene *GSH1* in *S. cerevisiae* and γ-glutamylcysteine glycine ligase (glutathione synthetase, GS), encoded by the gene *GSH2* in *S. cerevisiae* (Grant et al. 1997; Griffith et al. 1999).

Ukrainian team has previously constructed a competitive glutathione producer based on the termotolerant methylotrophic yeast *O. polymorpha*. This strain bears overexpression of the *GSH2* gene, encoding γ-glutamylcysteine synthetase, the first enzyme involved in glutathione biosynthesis, and the *MET4* gene coding for central regulator of sulfur metabolism and produces 5 times more glutathione during shake flask cultivation as compared to the wild-type strain (Yurkiv et al., 2018). Latvian team has shown that, unexpectedly, this strain is at least as tolerant to dehydration-rehydration treatment as the wild type strain in contrast to the recombinant strains of various microorganisms that overwhelmingly show high sensitivity to dehydration-rehydration (Kulikova-Borovikova et al., 2018). Unusual tolerance of this recombinant yeast strain to dessication is probably linked with its ability to overproduce glutathione.

Another group of compounds that can play a role in cellular protection during dehydration/rehydration are heat shock proteins. Heat shock proteins are a family of proteins that are produced by cells in response to exposure to stressful conditions. They were first described in relation to heat shock (Ritossa, 1962), but are now known to be expressed also during other stresses including desiccation, exposure to high temperature, cold and osmotic stress, ethanol shock and other (Verghese et al, 2012). Many members of this group perform chaperonic function by stabilizing new proteins to ensure correct folding or by helping to refold proteins that were damaged by the cell stress (Verghese et al, 2012). First results showing the increase of Hsp70 proteins activity during yeast dehydration were already obtained (Guzhova et al, 2008). At the moment we still have no similar information regarding other yeast heat shock proteins. At the same time, it is known that Hsp104 can resolubilize protein aggregates (Lee et al, 2010). Of course, such effect of Hsp104 can be very important at dehydration and especially during rehydration and reactivation of yeast cells after this stress treatment.

Further studies are necessary to understand if any of these protective systems including glutathione and heat shock proteins can work independently or both of them (and probably some others too) are complimentary each to other and are necessary for cells high resistance in conditions of anhydrobiosis.

3. Новизна проєкту *(до 1 сторінки А4, 12pt)* **/ Novelty of the project** *(up to 1 A4 size sheet, 12 pt)*

This project is a continuation of previous collaboration where we plan to study new aspects of the role of glutathione and heat shock proteins in yeast cells resistance to dehydration / rehydration. It was shown that strain with overexpression of the *GSH2* and *MET4* genes is at least as tolerant to dehydration-rehydration treatment as the parental strain and in some special conditions of subsequent rehydration its viability was at least 10–15% higher than that of the parental strain (Kulikova-Borovikova et al., 2018). It is interesting to test if the recombinant strains with overexpression of the *GSH2* or *MET4* gene alone are even more tolerant to dehydration-rehydration as the burden of overexpression of one gene is lighter than for two genes and moderate increase in glutathione production may still be sufficient for the improvement of cells viability during anhydrobiosis. Also it would be interesting to know if additional overexpression of genes encoding Hsp70 or Hsp104 in the mentioned recombinant *O. polymorpha* strains will further improve their tolerance to dessication.

Another novel aspect of the proposed study is investigation of the impact of increase of glutathione production and/or overexpression of the genes encoding heat shock proteins on the tolerance to dehydration-rehydration treatment of the baker's yeast *S. cerevisiae*. The role of glutathione in dehydration-rehydration tolerance in *S. cerevisiae* was established by showing lower level of survival after rehydration for the cells of *S. cerevisiae* mutant with deletion of

GSH1 gene coding for γ-glutamylcysteine synthetase (Espindola et al., 2003). But the impact of the increase of glutathione content in *S. cerevisiae* cells on their dehydration-rehydration tolerance was not tested. We plan to increase glutathione content in *S. cerevisiae* cells by overexpression of genes *GSH1* and/or *MET4* and test the viability of the obtained recombinant strains under the conditions of anhydrobiosis. If these recombinant strains will show higher level of survival in comparison with the parental strain under tested conditions, we will additionally overexpress genes *HSP70* or *HSP104* in these strains to see if it will furter incerease their tolerance to dehydration-rehydration treatment. All mentioned strains and parental strains tolerance to dehydration-rehydration will be tested both under conditions of fast rehydration in distilled water and slow gradual rehydration by incubation in water vapour in a chamber (over distilled water). If the viability of dehydrated *S. cerevisiae* cells with an elevated content of glutathione and/or Hsp proteins will be higher than of the cells of parental strain, these finding will be of high importance as dehydrated cells of this yeast are produced on an industrial scale, and elevated viability of rehydrated cells is an important economic parameter.

4. Очікувані наслідки від результатів проєкту, включаючи продовження співпраці в інших проєктах міжнародного співробітництва *(до 1 сторінки А4, 12pt)* **/**
Expected impacts of project results, including continuation of cooperation in other international cooperation projects *(up to 1 A4 size sheet, 12pt)*

The anticipated scientific results obtained in this study will contribute significantly to understanding if glutathione or heat shock proteins protective systems can work independently or both of them are complimentary each to other and are necessary for cells high resistance during dehydration-rehydration treatment. We are planning to obtain the *O. polymorpha* and *S. cerevisiae* recombinant strains with increased intracellular glutathione content as well as strains overproducing heat shock proteins. Constructed strains will possess higher resistance to desiccation stresses therefore could be used in production of active dry yeast preparations with increased shelf life and improved activity upon rehydration.

Besides that, obtained recombinant strains could be used for the production of respective compounds. Recent studies showed that Hsp70 treatment extended mean and maximum lifespan, improved learning and memory, increased curiosity, decreased anxiety, and helped maintain synaptic structures that degrade with age. These results provide evidence that intranasal administration of Hsp70 could have significant therapeutic potential in preserving brain tissue and memory for middle-age and old individuals and could be applied either as unique self-contained treatment or in combination with other pharmacological therapies (Bobkova et al., 2015). Besides that, the technology of intratumoral injection of pure Hsp70 for anti-cancer immunotherapy passed through preclinical trials and was successfully investigated in clinics (Guzhova, Margulism 2016). It means that the development of efficient recombinant strains for the production of Hsp70 which is planned to reach in our project can have very big importance for pharmacological industry and medicine for the successful treatments of neurodegenerative and some other diseases.

Glutathione is included in rejuvenating, sunscreen and moisturizing creams, primarily as a whitening component, and also has the ability to cleanse the skin and prevent the appearance of acne and wrinkles (Wu et al, 2004). Glutathione has also been used as a component of various food products, including beverages, breakfast cereals, cheeses, condiments, dairy analogues, fats, butter, sauces and meat. Cysteine and glutathione are widely used in high-speed dough production in order to improve the texture of bread, reduce mixing time and reduce energy costs. Also, glutathione may be used in winemaking as it helps preventing wine spoilage through the formation of S-glutathionyl caftaric acid, which is an odorless and colorless chemical compound.

The other possible and till now uninvestigated area of implementation of glutathione-overproducing yeast strains is their usage in dried form for bioremediation of wastewater through sorption of heavy metals and xenobiotics.

Participating in the project will allow young scientists from Ukraine and Latvia to gain additional financial support for their research. The deliverables in the form of scientific reports

on the results of the experimental work will be sent to the Ministries of Education and Science of Ukraine and Latvia as required. The results of this study will be published in at least two joint papers in highly regarded international journals and presented at national and international scientific conferences jointly. It is planned during realization of this project to try to find the topic and consortium in EU Programme Horizon Europe suitable for the research interests and priorities of both countries and our institutions and jointly prepare the application for the project in the frame of this Programme. The possibility to participate jointly also in other suitable international cooperation programmes also will be searched.

5. План роботи (робочі етапи) (далі – ПР) *(до 1 сторінки А4, для кожного ПР, 12pt)* **/ Work plan (work packages) (hereafter - WP)** *(up to 1 A4 size sheet, 12 pt, for each WP)*

ПР № / WP No.: 1
Назва ПР / WP title: Construction and evaluation of *O. polymorpha* and *S. cerevisiae* yeast strain with increased intracellular content of glutathione.
Цілі ПР / WP objectives: This WP will be devoted to the construction of the *O. polymorpha* and *S. cerevisiae* recombinant strains with increased intracellular glutathione content. The obtained recombinant strains will be tested for their tolerance to fast rehydration and slow gradual rehydration. Additionally, we will measure glutathione concentration in the cells and activity of γ-glutamylcysteine synthetase and determine the influence of the increased glutathione content on the state of cell wall and plasma membrane (permeability, temperature of phase transitions of lipids).
Заплановані завдання ПР та їх розподіл серед партнерів проєкту / WP planned tasks and their distribution among the Project Partners:
1.1 Evaluation of the tolerance of the recombinant *O. polymorpha* strains with overexpression of the gene *GSH2* or *MET4* to dehydraqation-rehydration treatment – Latvian side.
1.2 Construction of integrative plasmids containing modules for expression of the genes *GSH1* and *MET4* in *S. cerevisiae* applying standard PCR and cloning techniques – Ukrainian side.
1.3 Transformation of the integrative vectors to the parental *S. cerevisiae* strain – Ukrainian side.
1.4 Evaluation of the glutathione content and γ-glutamylcysteine synthetase activity in the obtained recombinant *S. cerevisiae* strains – Ukrainian side.
1.5 Studies on the tolerance to dehydration-rehydration treatment of the obtained recombinant *S. cerevisiae* strains with elevated glutathione content – Latvian side.
1.6 Studies on the influence of increased glutathione concentration on the cell wall and plasma membrane of yeast cells – Latvian side.

ПР № / WP No.: 2
Назва ПР / WP title: Construction and evaluation of *O. polymorpha* and *S. cerevisiae* yeast strains overproducing heat shock proteins Hsp70 and Hsp104 as well as strains combining increased intracellular content of glutathione with overproduction of heat shock proteins.
Цілі ПР / WP objectives: This WP will be devoted to the construction of the *O. polymorpha* and *S. cerevisiae* recombinant strains overproducing heat shock proteins Hsp70 and Hsp104. Overexpression of corresponding genes *HSP70* or *HSP104* will be performed on the background of wild type or strains with increased intracellular content of glutathione. Constructed strains will be verified by Western blot with anti-Hsp70 –Hsp104 antibodies. The obtained recombinant strains will be tested for their tolerance to fast rehydration and slow gradual rehydration.
Заплановані завдання ПР та їх розподіл серед партнерів проєкту / WP planned tasks and their distribution among the Project Partners:
2.1. Construction of integrative plasmids containing modules for expression of the genes *HSP70* and *HSP104* in *O. polymorpha* and *S. cerevisiae* applying standard PCR and cloning techniques – Ukrainian side.
2.2. Transformation of the integrative vectors to the parental *O. polymorpha* and *S. cerevisiae* strains as well as previously obtained strains with increased intracellular glutathione content –

Ukrainian side.
2.3. Verification of constructed recombinant strains on DNA level by genotyping and on protein level by Western blot – Ukrainian side.
2.4. Studies on the tolerance to dehydration-rehydration treatment of the obtained recombinant strains overproducing heat shock proteins Hsp70 and Hsp104 – Latvian side.
2.5. Studies on the influence of heat shock proteins overexpression on the cell wall and plasma membrane of yeast cells – Latvian side.

6. Графік виконання проєкту / Project implementation time schedule

Відповідне завдання ПР *(має бути ідентичним нумерації завдань ПР, зазначених у пункті 5)* / Corresponding WP task *(must be identical to the numbering of the WP tasks specified in point 5)*	Відповідальний за впровадження (*відмітити хрестиком*) / Responsible for implementation *(cross mark)*		Графік реалізації проєкту (квартали) / Project implementation schedule (quarters)							
	UA	LV	Рік 1 / Year 1				Рік 2 / Year 2			
			1.	2.	3.	4.	1.	2.	3.	4.
1. ПР / WP										
1.1		X								
1.2	X									
1.3	X									
1.4	X									
1.5		X								
1.6		X								
2.ПР / WP										
2.1	X									
2.2	X									
2.3	X									
2.4		X								
2.5		X								

7. Роль/Експертиза партнерів / Role/Expertise of the partners

Український партнер *(до 1,5 сторінки А4, 12pt)* / Ukrainian partner *(up to 1,5 A4 size sheet, 12 pt):*

Ukrainian researchers have profound experience in studying conventional and different non-conventional yeasts, including methylotrophic yeasts *O. polymorpha*, *Pichia pastoris* and flavinogenic yeast *Candida famata*. They performed metabolic engineering of yeast for the construction of yeast strains with specified properties, in particular producers of ethanol, riboflavin (vitamin B2), flavin nucleotides (FMN and FAD), enzymes for the creation of drugs (hepatitis B virus surface antigen, agrin deiminase), a series of bioselective elements that were used to create prototypes of sensors and test systems for determining such important analytes as glucose, ethanol, methanol, formaldehyde, glycerol, lactate, uric acid.

The study of the regulation of glutathione synthesis and degradation in the Department of Molecular Genetics and Biotechnology has been going on for more than 15 years. In particular, a method was developed for the selection of mutants of the methylotrophic yeast *O. polymorpha* capable of producing increased or decreased amounts of glutathione. The method significantly simplifies and speeds up the selection procedure, does not require the involvement of expensive reagents or equipment. Recombinant strains of the methylotrophic yeast *O. polymorpha* with impaired glutathione degradation and transport systems were constructed. Structural (*GSH2*, *GSH1*) and regulatory (*MET4*) genes of glutathione synthesis were cloned and their functions were investigated. The influence of possible regulatory factors on the expression of the *GSH2* gene was studied. Recombinant strains with point substitutions in the structure of the *GSH2* gene were constructed by the method of protein engineering. Mutant strains showed increased

glutathione synthesizing activity. The simultaneous overexpression of *GSH2* and *MET4* genes was performed, which significantly stimulated glutathione synthesis. The cultivation conditions of the engineered recombinant strains were optimized to achieve the maximum titer of the target product. The results of the work carried out were presented in numerous international publications.

In all mentioned publications PI of the proposal (Andriy Sibirny, h-index 34, number of citations 9500) is co-author and in the most of them he is the corresponding author; so he can successfully lead the current proposal. Total number of team members is 4. All participants of the proposal have substantial experience in the field of the yeast genetic engineering and therefore are able to work on the obtaining of recombinant yeast strains. Dr.Sc. Kostyantyn Dmytruk is well-known specialist in cloning and expression of heterologous proteins in *K. phaffii*, *O. polymorpha, S. cerevisiae* and the flavinogenic yeast *Candida famata*. Young scientist Dr. Marta Semkiv has been working on gene cloning in *S. cerevisiae, K. phaffii* and *O. polymorpha* for almost 15 years. Young scientist Anastasiya Zazulya has been working on genetic engineering of *K. phaffii* and *O. polymorpha* for 4 years. Based on the provided information, it could be concluded that the team of the project is highly qualified and prepared for successful work on the current proposal in part of obtaining recombinant *S. cerevisiae* and *O. polymorpha* strains with increased levels of glutathione and heat shock proteins. Ukrainian researchers do not have equipment and experience to be able to analyze yeast tolerance to dehydration-rehydration so this part of the work will be done by Latvian partners.

Department of Molecular Genetics and Biotechnology of the Institute of Cell Biology, NAS of Ukraine, Lviv, possesses modern equipment necessary for successful work on the proposal. Thus, the Department possesses 10 rotary incubator shakers for microorganism cultivation (production of New Brunswick Scientific, USA, and Heidolph, Germany), two fermenters (bioreactors) (Eppendorf, Germany), 2 UV-VIS spectrophotometers (Helios Thermo scientific, UK, and Hoch Lange, Germany), 2 photoelectorcolorimeters (Helios Thermo scientific, UK), 2 fluorometers (Turner, USA), YSI oxygen monitor for respiration assay (USA), electron microscope Philips (The Netherlands), ultramicrotome LKB (Sweden), 5 centrifuges Eppendorf (Germany), one high-speed centrifuge Sorvall (USA), 4 PCR-thermo cycler (Applied Biosystems, USA, Eppendorf, Germany), 2 ultralow temperature freezers, apparatuses for gel electrophoresis, Western hybridization and standard laboratory equipment. Laboratory is also equipped with laminar boxes, thermostat incubators, autoclaves etc. The above listed equipment will be used for project fulfilment.

Основні публікації науковців, які беруть участь у проєкті щодо теми проєкту *(5 публікацій)* / Major publications of the researchers involved in the project on the subject of the project *(specify 5 publications)*:

- Ubiyvovk VM, Nazarko TY, Stasyk OG, Sohn MJ, Kang HA, Sibirny AA. GSH2, a gene encoding gamma-glutamylcysteine synthetase in the methylotrophic yeast Hansenula polymorpha. FEMS Yeast Res 2002; 2:327-32.
- Ubiyvovk VM, Ananin VM, Malyshev AY, Kang HA, Sibirny AA. Optimization of glutathione production in batch and fed-batch cultures by the wild-type and recombinant strains of the methylotrophic yeast Hansenula polymorpha DL-1. BMC Biotechnol 2011; 11:8.
- Grabek-Lejko D, Kurylenko OO, Sibirny VA, Ubiyvovk VM, Penninckx M, Sibirny AA. Alcoholic fermentation by wild-type Hansenula polymorpha and Saccharomyces cerevisiae versus recombinant strains with an elevated level of intracellular glutathione. J Ind Microbiol Biotechnol. 2011; 38(11):1853-9.
- Yurkiv M, Kurylenko O, Vasylyshyn R, Dmytruk K, Fickers P, Sibirny A. Gene of the transcriptional activator MET4 is involved in regulation of glutathione biosynthesis in the methylotrophic yeast Ogataea (Hansenula) polymorpha. FEMS Yeast Res. 2018; 18 foy004.
- Kurylenko O, Rozenfelde L, Khroustalyova G, Vasylyshyn R, Ruchala J, Chang C-R, Daugelavicius R, Sibirny A, Rapoport A. Anhydrobiosis in yeasts: Glutathione synthesis by yeast Ogataea (Hansenula) polymorpha cells after their dehydration-rehydration. J of Biotechnol. 2019; 304:28-30.

Латвійський партнер *(до 1,5 сторінки А4, 12pt)* / Latvian partner *(up to 1,5 A4 size sheet, 12*

pt):
Основні публікації науковців, які беруть участь у проєкті щодо теми проєкту *(5 публікацій)* / Major publications of the researchers involved in the project on the subject of the project *(specify 5 publications)*:

- Guzhova I, Krallish I, Khroustalyova G, Margulis B, Rapoport A. Dehydration of yeast: changes in the intracellular content of Hsp 70 family proteins. Proc Biochem 2008; 43(10): 1138-1141
- Dupont S, Rapoport A, Gervais P, Beney L. The survival kit of Saccharomyces cerevisiae for anhydrobiosis (Review). Appl Microbiol Biotechnol 2014; 98 (21): 8821-8834
- Rapoport A, Turchetti B, Buzzini P. Application of anhydrobiosis and dehydration of yeasts for non-conventional biotechnological goals (Review). World J Microbiol Biotechnol. 2016; 32(6):104
- Rapoport A. Anhydrobiosis and dehydration of yeasts (Review). In: Biotechnology of Yeasts and Filamentous Fungi (Ed. A.Sibirny). Springer International Publishing, 2017, 87-116
- Kulikova-Borovikova D, Khroustalyova G, Chang C-R, Daugelavicius R, Yurkiv M, Ruchala J, Sibirny A, Rapoport A. Anhydrobiosis in yeast: glutathione overproduction improves resistance to dehydration of a recombinant Ogataea (Hansenula) polymorpha strain. Process Biochem. 2018; 71:41–44.

8. Відрядження / Business trips

Відрядження до Латвії / Business trips to Latvia

ПІБ, посада / Name, surname, position	Мета візиту / Purpose of the visit	Рік / Year	Тривалість візиту *(до 30 днів за рік)* / Duration of the visit
Сибірний А.А. Andriy Sibirny	Обмін результатами та обговорення подальших наукових планів Exchange with project results, discussions of further research plans	1-й рік проекту 1st year of Project	4 дні 4 days
Семків М.В. Marta Semkiv	Обмін результатами, стажування у ризькій лабораторії Exchange with project results, training in Riga laboratory	2-й рік проекту 2nd year of Project	4 дні 4 days
Зазуля А.З. Anastasiya Zazulya	Обмін результатами, стажування у ризькій лабораторії Exchange with project results, training in Riga laboratory	2-й рік проекту 2nd year of Project	4 дні 4 days

Відрядження в Україну / Business trips to Ukraine

Ім'я, прізвище, посада / Name, surname, position	Мета візиту / Purpose of the visit	Рік / Year	Тривалість візиту / Duration of the visit
Олександр Рапопорт, Керівник проекту Aleksandrs	Обмін результатами та обговорення подальших наукових планів	1-й рік проекту 1st year of Project	5 днів 5 days

Rapoports, Project leader	Exchange with project results, discussions of further research plans		
Діана Кулікова, дослідник Diana Kulikova, Researcher	Обмін результатами, стажування у львівській лабораторії Exchange with project results, training in Lviv laboratory	1-й рік проекту 1st year of Project	5 днів 5 days
Лінда Розенфельд, дослідник Linda Rozenfelde, Researcher	Обмін результатами, стажування у львівській лабораторії Exchange with project results, training in Lviv laboratory	2-й рік проекту 2nd year of Project	5 днів 5 days
Галина Хрустальова, дослідник Galina Khroustalyova, Researcher	Обмін результатами, стажування у львівській лабораторії Exchange with project results, training in Lviv laboratory	2-й рік проекту 2nd year of Project	5 днів 5 days

9. Витрати на реалізацію проєкту для українського партнера, грн / Project implementation costs for Ukrainian partner, UAH

Витрати	**Рік 1, UAH**	**Рік 2, UAH**
1. Прямі витрати / Direct costs:	110,0 тис.грн.	110,0 тис.грн.
1.1. Витрати на оплату праці, включаючи податки *(макс. 53% від загального обсягу витрат)* / Remuneration of the research staff employed in the project, including Compulsory State Social Insurance Contributions	50,0 тис.грн.	50,0 тис.грн.
1.2. Матеріали, необхідні для виконання робіт, крім спецустаткування (*10-20% від загального обсягу витрат*) / Materials, consumables supplies and similar products	20,0 тис.грн.	20,0 тис.грн.
1.3. Витрати на службові відрядження *(згідно з запланованими відрядженнями) (відповідно до Постанови КМУ від 02.02.2011 №98)* / Travel expenses *(Specify planned business trips)*:	40,0 тис.грн.	40,0 тис.грн.
2. Непрямі витрати *(не більше 30% від загального обсягу витрат*) **/ Indirect costs** *(up to 30% of the*	20,0 тис.грн.	20,0 тис.грн.

total direct costs of the project)		
Разом*, грн / Total, UAH	130,0 тис.грн.	130,0 тис.грн.

* - розрахункова сума на фінансування проєкту залежить від затвердженого бюджету на відповідний рік і орієнтовно становить 130 тис. грн на рік

10. Чи надавалось раніше по темі проєкту державне фінансування? / Has the topic of the project previously received state funding?	☐ Так / Yes ☐ **Ні / No** Якщо «так», то вказати роки / If «Yes», indicate the years
Якщо «Так», то вказати на необхідність та відмінність досліджень, які пропонуються / If "Yes", then indicate necessity and differences of proposed project	*Describe data of previous project(s)* *Indicate necessity and differences of proposed research briefly.*

Інтелектуальна власність: Кожна сторона несе відповідальність за моніторинг захисту інтелектуальної власності, створеної в межах Проєкту відповідно до міжнародних угод, підписаних Сторонами.

Intellectual property: Each party is responsible for monitoring the protection of the intellectual property created under the Project in accordance with international agreements signed by the Parties.

Українська сторона підтверджує, що дослідження за цією тематикою проєкту не фінансуватимуться з державного бюджету протягом 2023-2024 років в межах іншого/-их конкурсів. У разі отримання фінансування цього проєкту з державного бюджету – заявки на проведення досліджень з цієї тематики не будуть подаватися на інші конкурси протягом періоду реалізації проєкту.

The Ukrainian side confirms that research on this topic of the project will not be funded from the state budget during 2023-2024 within the framework of other call/(-s). In case of receiving funding for this project from the state budget – applications for research on this topic will not be submitted to other calls during the project implementation period.

Ми погоджуємось, що Міністерство освіти і науки України (Міністерство) та Латвійська Рада науки (Рада) буде обробляти персональні дані, що містяться в проєкті, шляхом адміністративної оцінки проєкту та публікуватиме проєкти, затверджені для реалізації на вебсайтах Міністерства та Ради.

We agree that the Ministry of Education and Science of Ukraine (Ministry) and the Latvian Council of Science (LZP) will process personal data contained in the project through the administrative evaluation of the project and the publication of the supported projects on the Ministry's and the LZP's website.

Додатки / Attachments:

a) супровідний лист / a cover letter;

b) лист-підтвердження від латвійського партнера-керівника проєкту / a confirmation letter from the Latvian Team Leader of mutual cooperation in English language (scanned copy of the letter is allowed);

c) акт експертизи на відкриту публікацію матеріалів за темою проєкту / an act of expertise for open publication of project materials;

d) CV українського та латвійського наукових керівників проєкту / CV of Ukrainian and Latvian Team Leader.

Латвійський партнер (LV) / Latvian partner (LV):

Науковий керівник / Principal investigator	Prof., Head of Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia *(посада / position)*
ПІБ / Name and surname	Aleksandrs Rapoports (Олександр Рапопорт, Alexander Rapoport)
Дата / Date	*30.08.2022.*
Підпис / Signature	
Керівник установи / Legal representative of the institution	Prof., Vice-Rector for Natural Sciences, Technology and Medicine *(посада / position)*
ПІБ / Name and surname	Valdis Segliņš
Дата / Date	*31.08.2022.*
Підпис / Signature	
Печатка / Stamp	

Український партнер (UA) / Ukrainian Partner (UA):

Науковий керівник / Principal investigator	Prof., Head of the Department of Molecular Genetics and Biotechnology, Institute of Cell Biology NAS of Ukraine *(посада / position)*
ПІБ / Name and surname	Andriy Sibirny (Андрій Сибірний)
Дата / Date	25255758 *30.08.2022.*
Підпис / Signature	

Керівник установи / Legal representative of the institution	Dr. Sci., Deputy Director of Institute of Cell Biology, NAS of Ukraine *(посада / position)*
ПІБ / Name and surname	Kostyantyn Dmytruk (Костянтин Дмитрук)
Дата / Date	*30.08.2022.*
Підпис / Signature	
Печатка / Stamp	