Summary of Professional Accomplishments

Justyna Magdalena Ruchała, PhD.

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Rzeszów 2021

1. Name and Surname: **Justyna Magdalena Ruchała**

2. Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation:

2011	master of science; biology; University of Rzeszow; title of master thesis: „The content of antioxidants in the exhaled air in the subsequent phases of the woman's monthly cycle"; supervisor: prof. dr hab. Grzegorz Bartosz
2015	doctor of philosophy in biology; doctoral thesis: "The construction of strains of the yeast *Hansenula polymorpha* with improved characteristics of alcoholic fermentation of xylose"; supervisor: prof. dr hab. Andriy Sybirnyy

3. Information on employment in research institutes or faculties/departments or school of arts

2015 – 2016	Department of Biotechnology and Microbiology, Faculty of Biology and Agriculture, University of Rzeszow, position: research assistant
2016 – 2018	Department of Biotechnology and Microbiology, Faculty of Biology and Agriculture, University of Rzeszow, position: adjunct lecturer
2018 – 2019	Department of Microbiology and Biotechnology, Faculty of Biotechnology, University of Rzeszow, position: adjunct lecturer
2019 – 2021	Department of Microbiology and Biotechnology, Institute of Biology and Biotechnology, College of Natural Sciences, University of Rzeszow, position: adjunct lecturer

2021 – present Department of Biology, Institute of Biology and Biotechnology, College of Natural Sciences, University of Rzeszow, position: adjunct lecturer

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act

A scientific achievement under Art. 219 paragraph. 1 point 2 of the Act of 20 July 2018 Law on Higher Education and Science is a series of 6 thematically related scientific publications on the fermentation and metabolism of pentoses in the non-conventional yeast *Ogataea polymorpha*. These works were published in 2017-2021. These publications are the results of the scientific collaboration with research institution from abroad.

The total impact factor of the works that make up the main scientific achievement is **34.291**. The total value of Ministry of Education and Science points that make up the main scientific achievement is **510** (according to the date of publication).

Title of the Main Scientific Achievement:

Identification of genes involved in regulation of xylose metabolism and fermentation in the thermotolerant yeast *Ogataea polymorpha* and construction of the efficient ethanol producers from this pentose

A.) Publications included in the Main Scientific Achievement (in the order of discussion):

P1. Ruchala J, Kurylenko OO, Soontorngun N, Dmytruk KV, Sibirny AA. Transcriptional activator Cat8 is involved in regulation of xylose alcoholic fermentation in the thermotolerant yeast *Ogataea (Hansenula) polymorpha*. Microb Cell Fact. 2017; 16(1):36. doi: 10.1186/s12934-017-0652-6. (**IF_{2017} 3.831; MES_{2017}= 35**).

P2. Kurylenko O[#], **Ruchala J[#],** Kruk B., Vasylyshyn R, Szczepaniak J, Dmytruk K, Sibirny A. The role of Mig1, Mig2, Tup1 and Hap4 transcription factors in regulation of xylose and glucose fermentation in the thermotolerant yeast

Ogataea polymorpha. FEMS Yeast Res. 2021; 21(4): foab029. doi: 10.1093/femsyr/foab029. (**IF$_{2019}$ 2.796; MES$_{2020}$= 100**).

# Equal contribution

P3. Dmytruk KV, **Ruchala J**, Grabek-Lejko D, Puchalski C, Bulbotka NV, Sibirny AA. Autophagy-related gene *ATG13* is involved in control of xylose alcoholic fermentation in the thermotolerant methylotrophic yeast *Ogataea polymorpha*. FEMS Yeast Res. 2018; 18(2): foy010. doi: 10.1093/femsyr/foy010. (**IF$_{2018}$ 2.458; MES$_{2017}$= 30**).

P4. Kurylenko OO#, **Ruchala J#**, Vasylyshyn RV, Stasyk OV, Dmytruk OV, Dmytruk KV, Sibirny AA. Peroxisomes and peroxisomal transketolase and transaldolase enzymes are essential for xylose alcoholic fermentation by the methylotrophic thermotolerant yeast, *Ogataea (Hansenula) polymorpha*. Biotechnol Biofuels. 2018; 11:197. doi: 10.1186/s13068-018-1203-z. (**IF$_{2018}$ 5.452; MES= 45**).

# Equal contribution

P5. Ruchala J, Kurylenko OO, Dmytruk KV, Sibirny AA. Construction of advanced producers of first- and second-generation ethanol in *Saccharomyces cerevisiae* and selected species of non-conventional yeasts (*Scheffersomyces stipitis*, *Ogataea polymorpha*). J Ind Microbiol Biotechnol. 2020; 47(1):109-132. doi: 10.1007/s10295-019-02242-x. (**IF$_{2020}$ 3.346; MES$_{2020}$= 100**).

P6. Ruchala J, Sibirny AA. Pentose metabolism and conversion to biofuels and high-value chemicals in yeasts. FEMS Microbiol Rev. 2020:fuaa069. doi: 10.1093/femsre/fuaa069. (**IF$_{2019}$ 16.408; MES$_{2020}$= 200**).

B.) Description of the scientific purpose of the above- mentioned works and the results achieved, together with a discussion of their applications:

Introduction:

Global energy demand and environmental concerns have recently intensified research efforts to produce biofuels from renewable sources around the world. Especially this relates to the transport sector, which consumed 31.8% of the oil produced in 2017 (https://www.iea.org/weo/weo2018/secure/, was responsible for 41.5% of global CO2 emissions in 2016 (https: //www.iea.org/statistics/co2emissions/). An additional element stimulating this branch of science is political movement to energy independence from global oil suppliers. As a result, the number of countries applying renewable energy policies in the transport sector increased from 56 in 2012 to 66 in 2015. (Sawin et al., 2016). The above-mentioned problems have contributed to the exponential growth of world ethanol production over the last decade, reaching 120 billion liters in 2018, of which 100 billion liters is bioethanol (also known as fuel ethanol) (https://knect365.com/energy/article/c07f7fba-48fa-464f-9f21-12f913fc67f7/world-ethanol-production-to-expand-steadily-in-2019).

Bioethanol is a renewable liquid transport fuel widely used mainly in the United States and Brazil and may become the dominant renewable biofuel in the transport sector in the future. Currently, most of ethanol is produced from corn (USA) or sugar cane (Brazil), but future supplies may come from cellulosic and hemicellulosic sugars found in green plant biomass and residues of agriculture and wood industry (Jansen et al., 2017). Bioethanol can be blended with gasoline or used as a pure alcohol in dedicated engines; it has a higher-octane number and a higher evaporation temperature relative to gasoline. Moreover, it is considered an excellent fuel for advanced flex-fuel hybrid vehicles (Lopes et al., 2016).

Currently, bioethanol produced from conventional raw materials such as glucose (derived from maize starch), and sucrose (cane or beet sugar) is known as a first-generation ethanol (1G). However, there is a lot of controversy around the use of this type of raw material including ethical concerns as mentioned feedstocks can be used for the production of food and animal feed, and at the same time their resources are limited. For these reasons, the possibility of bioethanol producing from inedible, renewable raw materials such as lignocellulose, called second-generation ethanol (2G), is of particular interest. Currently, limited amounts of 2G ethanol are produced in a number of pilot and demonstration plants

around the world, but due to the higher cost of 2G ethanol production on a large scale, the process is not yet profitable. Lignocellulose contains about 25% lignin and 75% polysaccharides, cellulose (glucose homopolymer) and hemicelluloses (a heteropolymers that mainly contain pentose sugars, xylose and L-arabinose) (Baig, 2020).

In terms of availability, xylose is the second most abundant sugar of lignocellulose after glucose, therefore efficient xylose conversion by microorganisms is an important prerequisite for development of an economically viable second-generation ethanol production technology (produced mainly from lignocellulose). (Rosalen-Calderon, et al., 2019). Wild-type strains of thermotolerant methylotrophic yeast *Ogataea polymorpha* grow well on xylose and ferment it to ethanol under limited aeration, however, the amount of the accumulated ethanol during fermentation is 200 times lower than that from glucose. The reasons for this difference are poorly understood and we hope that molecular genetics methods can help to better understand and overcome this phenomenon. These studies could be promising as the genome sequence of this species is already known (http://genome.jgi-psf.org/Hanpo2/Hanpo2.home.html). Therefore, *O. polymorpha* is now considered a promising organism that could be used in the future to produce ethanol from xylose. Additionally, *O. polymorpha*, being thermotolerant organism, can be applicable for simultaneous saccharification and fermentation process (SSF) which significantly reduces the cost of lignocellulose conversion into ethanol (Olofsson et al., 2007; Abdel Banat et al., 2010).

It should be underlined that *O. polymorpha* is a species with several unique characteristics:

- is the most thermotolerant yeast known with the highest maximal temperature of growth at 50°C, which cannot be achieved by mesophilic yeasts like *Saccharomyces cerevisiae*, and also naturally xylose fermenting species *Scheffersomyces stipitis*, *Pachysolen tannophilus* etc. (**P6**), (Ishchuk et al., 2009)
- it grows and ferments xylose (Ryabova et al., 2003) and converts glycerol to ethanol (Suwannarangsee et al., 2010)

- the low efficiency of ethanol production by wild type strains can be improved using advanced metabolic engineering techniques (Kata et al., 2016; **P1**)

As noted above, despite the almost identical robust growth of *O. polymorpha* on both on glucose and xylose, the level of ethanol produced from xylose by the wild-type strain is up to 200 times lower than that from glucose. The reasons of this phenomenon had not been sufficiently investigated by the time the research reported as a Main Scientific Achievement started. My interest to this problem has been developed during the preparation of the doctoral thesis. Scientific work in this field contributed to the identification of several bottlenecks in the process of xylose converting to ethanol, which resulted in construction of the *O. polymorpha* strains producing 40-50 times more ethanol from xylose as compared to the wild-type strain. In the course of my doctoral thesis, I constructed strains with a 25-30-fold increased ethanol production from xylose as compared to the wild type strain. For this purpose, the protein engineering of the first xylose catabolism enzyme, xylose reductase (XR, EC 1.1.1.21) was carried out and 3 genes of the first steps of the sugar metabolism *XYL1m*, *XYL2*, *XYL3* were overexpressed; besides, for the first time the positive selection of ethanol overproducers was used to search for resistant mutants to the glycolysis inhibitor, 3-bromopyruvate (Kurylenko et al., 2014). Additionally, for this purpose, the *CAT8* gene coding for the transcription factor was deleted. However, the efficiency of the strains constructed during my dissertation was still too low, and the knowledge on the factors limiting the effective production of ethanol from renewable raw materials was still insufficient.

Summarizing, the reasons that prompted me to undertake the research were:

- too slow metabolism of pentose sugars (xylose, L-arabinose), including their conversion to ethanol (alcoholic fermentation)
- lack of knowledge about the possible role of peroxisomes and autophagy in xylose metabolism and fermentation
- inefficient transport of pentose sugars to the cell
- the occurrence of the so-called catabolite repression caused by glucose, which limits the possibility of simultaneous conversion of the sugar mixture and favoring glucose metabolism

- insufficient knowledge about the role of transcriptional factors in the alcoholic fermentation

and many others.

The above-mentioned aspects prompted me to study the following problems, which were recognized as a Main Scientific Achievement:

- elucidate the role of transcription factors in xylose metabolism
- investigate the role of autophagy in xylose fermentation
- explain the role of peroxisomes and peroxisomal enzymes in xylose metabolism in methylotrophic yeast
- and finally, to construct the strains with much more efficient alcoholic fermentation of xylose at elevated temperature.

My research reported as a Main Scientific Achievement allowed me to find new bottlenecks limiting the pathway of xylose conversion to ethanol, and on the other hand, I constructed mutants with increasing capacity to convert xylose to ethanol at elevated temperature (45°C). My work showed for the first time the role of transcriptional factors (Cat8, Hap4-A, Mig1) in alcoholic fermentation of xylose (**P1**; **P2**). Additionally, I have observed for the first time the role of the peroxisomes in the fermentation of xylose (but not glucose), and the importance of peroxisomal enzymes (transketolase, another name, dihydroxyacetone synthase, EC 2.2.1.3, and transaldolase, EC: 2.2.1.2) in this process (**P4**; **P5**). Furthermore, I discovered the important role of autophagy, namely, the *ATG13* gene, in alcoholic fermentation of xylose (**P3**).

During my studies, I have used modern methods of metabolic engineering and original methods of classical selection. Among the metabolic engineering methods, I successfully used the methods of deletion and/or overexpression of genes encoding transcriptional factors *CAT8*, *HAP4A*, *MIG1* and autophagy-related gene *ATG13* (**P2, P3**), deletion and overexpression of the genes *DAS1* and *TAL2* encoding peroxisomal transketolase and transaldolase, respectively, as well as *TKL1*, coding for cytosolic transketolase and *TAL1*, those for transaldolase (**P4**; **P5**). I also used fluorescent microscopy to prove peroxisomal localization of transaldolase Tal2 (**P4**). In addition, using protein engineering approaches, I modified the *O. polymorpha* hexose transporter Hxt1 that it can actively transport xylose into the cell and its activity is not inhibited by glucose. In the

same work, I also used methods of fluorescent microscopy to localize transporting proteins in the cell (Vasylyshyn et al., 2020). I started to investigate the role of the Cat8 transcriptional factor during work on my PhD studies, which was defended in 2015, but in the frame of current Main Scientific Achievement I explored the role of this factor in much more details. In particular, strains overexpressing the *CAT8* gene have been obtained and effect of such genetic change on alcoholic fermentation of xylose has been studied; deletion and overexpression of this gene were confirmed by qRT-PCR; in addition, the specific activities of the xylose metabolic enzymes and accumulation of byproducts, including that of xylitol in transformants, were determined (**P1**). Continuing this area of research, I paid attention to a number of other transcription factors involved in the regulation of carbon catabolism in yeast and investigated the role of Hap4-A and Hap4-B transcription activators as well as Tup1, Mig1 and Mig2 transcription repressors in the regulation of alcoholic fermentation of xylose in *O. polymorpha* (**P2**). For the first time, it was found the important role of autophagy, namely, the *ATG13* gene (**P3**) as well as peroxisomes, peroxisomal and cytosolic transketolases and transaldolases (**P4**) in the regulation of alcoholic xylose fermentation.

Based on an analysis of the literature in the field of xylose fermentation, including my own results, I have hypothesized that it is possible to further improve the characteristics of xylose alcoholic fermentation in *O. polymorpha* by selecting mutants capable of robust growth on another pentose, L-arabinose, as the sole carbon and energy source. (**P6**). This hypothesis has already been confirmed by preliminary experimental data and will be verified in further investigations. This new selection method resulted in mutants accumulating 20 g ethanol / L from xylose at 45°C, which is 50 times higher than accumulated by the wild-type strain of *O. polymorpha* (0.4 g / L).

Study of xylose conversion to ethanol also requires a careful characterization of the mechanisms responsible for the control of fermentation, including the cellular localization of enzymes associated with them.

The main research data of six articles presented as a Main Scientific Achievement describe the factors involved in regulation of xylose metabolism and fermentation in the non-conventional thermotolerant yeast *O. polymorpha* and the developed by me methods to increase ethanol production efficiency.

Briefly speaking, the described research work reported as a Main Scientific Achievement allowed the identification of previously unknown genes involved in xylose metabolism and fermentation and the use of the obtained data of basic research to construct improved xylose ethanol producers of the thermotolerant yeast *O. polymorpha*.

Below I present a detailed description of six scientific papers included in the series of articles constituting Main Scientific Achievement:

Publication no. 1 (P1):

Ruchala J, Kurylenko OO, Soontorngun N, Dmytruk KV, Sibirny AA. Transcriptional activator Cat8 is involved in regulation of xylose alcoholic fermentation in the thermotolerant yeast *Ogataea (Hansenula) polymorpha*. Microb Cell Fact. 2017; 16(1):36. doi: 10.1186/s12934-017-0652-6. (**IF_{2017} 3.831; MES_{2017}= 35**).

The research conducted under publication number 1 was carried out in collaboration with foreign research centers - the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine) and King Mongkut's University of Technology Thonburi, Bangkok (Thailand). In addition, it was supported by two research projects (Research and Training Grants, European Federation of Microbiological Societies FEMS, project no.: FEMS-RG-2015-0096 - project manager and Opus, National Science Center, project no.: 2012/05 / B / NZ1 / 01657 – my role as main investigator).

Part of the research was carried out by me during my research internships at King Mongkut's University of Technology Thonburi, Bangkok (Thailand) (1 month), as well as at the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine) (3 months). The aim of this work was to investigate the role of the transcriptional factor *CAT8* of the non-conventional yeast *O. polymorpha* in metabolism and alcoholic fermentation of glucose and xylose. The *CAT8* gene encodes a zinc finger cluster-containing transcription activator necessary for expression of genes involved in gluconeogenesis, respiration, the glyoxylate cycle and ethanol utilization. The described functions of the Cat8 transcriptional factor (encoded by the *CAT8* gene) in the activation of many

metabolic processes in *S. cerevisiae*, mainly gluconeogenesis and ethanol utilization, led me to hypothesis that it could also be involved in the regulation of xylose metabolism in *O. polymorpha*. One of the reasons why *CAT8* was chosen among many genes encoding transcription factors involved in carbon metabolism was based on data that knock-out of the *CAT8* gene leads to the activation of alcoholic fermentation of glucose in *S. cerevisiae* (Watanabe et al. 2010) and in the non-conventional yeast *Pichia guilliermondii* (Qi et al., 2014). As part of this work, I isolated the deletion mutants of the *CAT8* gene on the genetic background of the wild-type strain and the best ethanol producer obtained during previous research (BEP = Best Ethanol Producer). The deletion of the *CAT8* gene in *O. polymorpha* did not cause any significant changes in ethanol production from glucose, while a two-fold increase in xylose fermentation in the wild type strain and a 25% increase in ethanol production by the BEP *cat8Δ* deletion strain was observed. The reasons for this difference are not fully understood, but an important difference between glucose and xylose could be the involvement of gluconeogenesis enzymes in the production of hexoses during growth on xylose (not on glucose). Therefore, it is very possible that inhibition of gluconeogenesis in *cat8Δ* mutants redirects more carbon from xylose towards catabolism and fermentation, while when grown on glucose, gluconeogenesis does not play a major role. It is strange, however, that in *cat8Δ* mutants of the yeasts *S. cerevisiae* and *P. guilliermondii*, a slight increase in ethanol production from glucose was observed (Watanabe et al. 2010; Qi et al., 2014). It should also be emphasized that respiration of *cat8Δ* mutants on xylose was damaged to a greater extent than on glucose as a substrate. It is also possible that in the *cat8Δ* mutant xylose metabolism is redirected from respiration (Krebs cycle, electron transport chain, oxidative phosphorylation) to fermentation, which leads to an increase in ethanol production. The reason of the increase in ethanol production from xylose by *cat8Δ* deletion strains may be also dependent on the observed by us activation of xylulokinase, alcohol dehydrogenase and ribose-5-phosphate epimerase (EC 5.1.3.1), which could be limiting factors during xylose fermentation. Interestingly, overexpression of the *CAT8* gene significantly decreased ethanol production from xylose. It should be also noted that isolated by me *cat8Δ* mutants were totally avoided from xylitol

accumulation, an undesirable byproduct during xylose alcoholic fermentation so common for *S. cerevisiae*, *Kluyveromyces marxianus* and other organisms.

In conclusion, in this work, for the first time in the yeast naturally metabolizing and fermenting xylose, *O. polymorpha*, a transcription factor was identified that specifically participates in the regulation of alcoholic fermentation of this pentose.

The most important achievements of publication no. 1:

- I constructed deletion mutants of the *CAT8* gene on the background of the wild- type strain and the best ethanol producer previously isolated
- I constructed strains overexpressing the *CAT8* gene
- I examined the physiological (growth, respiration, fermentation), genetic (expression of a number of genes) and biochemical (specific activities of xylose metabolizing enzymes, by-product accumulation) characteristics of the mutants with deletion and overexpression of the *CAT8* gene
- I proved the specific role of the Cat8 transcriptional activator in xylose fermentation in the methylotrophic naturally xylose metabolizing yeast *O. polymorpha*.

Publication no. 2 (P2):

Kurylenko O#, **Ruchala J#,** Kruk B, Vasylyshyn R, Szczepaniak J, Dmytruk K, Sibirny A. The role of Mig1, Mig2, Tup1 and Hap4 transcription factors in regulation of xylose and glucose fermentation in the thermotolerant yeast *Ogataea polymorpha*. FEMS Yeast Res. 2021; 21(4): foab029. doi: 10.1093/femsyr/foab029. (**IF_{2019} 2.796; MES_{2020}= 100**).

# equal contribution

The part of research of publication 2 was also carried out at the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine), which is also emphasized by the affiliation of this institution next to my name. The research presented in this publication was supported by National Science Center - Opus project no. 2016/21 / B / NZ1 / 00280, where I was the main investigator.

In the previous publication, I showed the important role of the Cat8 transcription factor in the xylose alcoholic fermentation in *O. polymorpha*, which prompted me to further research in this field. There are many (more than 100) transcription regulators (activators and repressors) identified in yeast, mainly in *S. cerevisiae* (Turcotte et al., 2010). Therefore, I decided to work with the transcription factors that are presumably involved in the metabolism of sugars in *O. polymorpha*. They include two orthologs of *S. cerevisiae* transcription activator Hap4 (Hap4-A and Hap4-B) (Sybirna et al., 2005, 2010) and the Mig1, Mig2 and Tup1 transcriptional repressors (Stasyk et al., 2007) described earlier by our group. The main aim of this study was to analyze the role of *MIG1*, *MIG2*, *TUP1* and *HAP4* genes in *O. polymorpha* in xylose and glucose metabolism and fermentation. A search of the *O. polymorpha* genome database revealed the presence of two putative *S. cerevisiae* Mig1 transcriptional repressor orthologs coding for the C2H2 zinc finger protein (Stasyk et al. 2007). It was found that in *O. polymorpha* Mig1, Mig2, and Tup1 practically do not participate in catabolite repression of the localized in peroxisomes alcohol oxidase (Oliveira et al., 2003; Stasyk et al. 2007). At the same time, Tup1 deficiency leads to complete macropexophagy defect induced by glucose or ethanol, in contrast to Mig1 and Mig2 which only partially are involved in pexophagy (Leao-Helder et al., 2004; Stasyk et al.2007).

During work on this publication, I did construction of the strains with overexpression of the genes encoding the mentioned transcriptional factors, as previously only deletion mutants have been constructed. The studies of the available and newly isolated strains allowed to prove for the first time the role of the above-mentioned transcription factors in the metabolism of xylose and glucose in the naturally xylose fermenting yeast *O. polymorpha*. I compared the effect of deletion and overexpression of *MIG1*, *MIG2*, *TUP1*, *HAP4-A* and *HAP4- B* genes of *O. polymorpha* on glucose and xylose metabolism and fermentation. The obtained results suggest that the role of Mig1 is not crucial for the regulation of glucose metabolism in *O. polymorpha*, but the simultaneous deletion of *MIG1* and *MIG2* resulted in a significant reduction of glucose utilization and ethanol production from glucose. I also observed a decrease in ethanol production from xylose in the double *mig1Δ mig2Δ* mutant, but to

a lesser extent than that from glucose. Moreover, it was proved in the work that the level of *HAP4-A* expression is more important for xylose metabolism and fermentation than for glucose. I observed that deletion of the *HAP4-A* gene increased, and its overexpression decreased ethanol production from xylose. The deletion of the *TUP1* gene caused activation of xylose alcoholic fermentation, but interestingly, reduced the production of ethanol from glucose. Nevertheless, the high level of *TUP1* expression negatively affected both glucose and xylose fermentation. As the results on my and our team work the differences in the roles of Mig1, Hap4-A Tup1 transcription factors in the regulation of glucose and xylose metabolism and fermentation in *O. polymorpha* was observed. It is important to emphasize that in the course of work on the above publication, the role of the Tup1 transcriptional activator in the negative regulation of metabolism and xylose fermentation in *O. polymorpha* was shown for the first time, although the mechanism of its action is still unknown, and its elucidation needs further research.

The most important achievements of publication no. 2:

- I proved that the level of *Hap4-A* gene expression is more important for xylose metabolism and fermentation than that for glucose
- I proved the key role of Mig1 in the regulation of glucose metabolism in *O. polymorpha*
- I proved the role of the Tup1 transcriptional activator as a negative regulator of metabolism and fermentation *in O. polymorpha*
- I proposed new method of construction of the improved ethanol producers from xylose by deletion the genes of the transcription factors *HAP4-A* and *TUP1*.

Publication no. 3 (P3):

Dmytruk KV, **Ruchala J**, Grabek-Lejko D, Puchalski C, Bulbotka NV, Sibirny AA. Autophagy-related gene *ATG13* is involved in control of xylose alcoholic fermentation in the thermotolerant methylotrophic yeast *Ogataea polymorpha*. FEMS Yeast Res. 2018; 18(2):foy010. doi: 10.1093/femsyr/foy010. (**IF_{2018} 2.458; MES_{2017}= 30**).

The research described in publication number 3 was carried out in collaboration with the foreign research center - the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine). The research was carried out with support of the grant financed by the National Science Center, Opus, no. 2016/21 / B / NZ1 / 00280, where I was the main investigator.

As a result of insertion mutagenesis and selection of the wild type strains *O. polymorpha* resistant to 3-bromopyruvate (3-BrPA), the strain (# 63) was isolated, which was characterized by a 50% increase in ethanol production from xylose as compared to the wild type strain NCYC495 *leu1-1*, but not from glucose. Sequencing of the flanking regions revealed that the insertion cassette changed the ORF sequence of the gene homologous to the *S. cerevisiae ATG13* gene. This gene encodes the regulatory subunit of the Atg1-Atg13 signaling complex, stimulating the activity of the Atg1 kinase, which is required for vesicle formation during autophagy and the Cvt pathway of the cytoplasm to the vacuole targeting. It was shown that the insertion cassette was inserted into the *ATG13* gene at position +1272 bp from the starting ATG codon. The *ATG13* gene in *S. cerevisiae* is involved in the initiation of autophagy, while the *atg13Δ* mutant of this yeast species showed autophagy defects (Alers et al., 2014).

Therefore, it was also decided to construct a deletion strain of the *ATG13* gene. Interestingly, I have found that the *atg13Δ* mutant produced increased quantities of ethanol from xylose, similarly to the insertion mutant, with no effects on glucose alcoholic fermentation. Analysis of the expression level of the selected genes related to xylose metabolism showed that the deletion of the *ATG13* gene significantly increased the expression of *PDC1*, and especially of *DAS1* and *AOX1*, which suggests a possible role of Atg13 in the negative regulation of the expression of the mentioned genes. I also assayed the specific activities of the enzymes involved in the initial reactions of xylose metabolism pathway - xylose reductase (XR, EC 1.1.1.21), xylitol dehydrogenase (XDH, EC 1.1.1.9) and alcohol dehydrogenase (ADH, EC 1.1.1.1) in the insertion #63 and deletion *atg13Δ* strains. XR activity was slightly decreased, while XDH and ADH activity showed a slight increase as compared to the wild-type strain. The obtained results may explain the reasons for the increase in alcoholic fermentation of xylose in strains #63 and *atg13Δ*, because previously we found that overexpression of *XYL2*,

PDC1, *ADH1* and *DAS1* on the genetic background of the wild-type strain led to increased ethanol production from various substrates (Kurylenko et al., 2014, 2016).

Summarizing, here it was for the first time shown the potential biotechnological importance of autophagy, especially the *ATG13* gene due to activation of xylose fermentation due to its impairment or deletion.

The most important achievements of publication no. 3:

- I described the new function of Atg13 of *O. polymorpha* as a negative regulator of alcoholic fermentation of xylose
- For the first time, I demonstrated the role of the *ATG13* gene in autophagy in *O. polymorpha*
- I showed that the deletion of the *ATG13* of *O. polymorpha,* similar to the insertion into this gene, leads to increased production of ethanol from xylose, and at the same time is responsible for derepression of several genes involved in xylose catabolism and alcohol fermentation

Publication no. 4 (P4):

Kurylenko OO#, **Ruchala J#**, Vasylyshyn RV, Stasyk OV, Dmytruk OV, Dmytruk KV, Sibirny AA. Peroxisomes and peroxisomal transketolase and transaldolase enzymes are essential for xylose alcoholic fermentation by the methylotrophic thermotolerant yeast, *Ogataea (Hansenula) polymorpha*. Biotechnol Biofuels. 2018; 11:197. doi: 10.1186/s13068-018-1203-z. (**IF_{2018} 5.452; MES_{2017}= 45**).

# Equal contribution

The research carried out under publication number 4 was carried out in collaboration with a foreign research center - the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine). The research was supported by grant financed by the National Science Center - Opus, no. 2016/21 / B / NZ1 / 00280, where I was the main investigator.

PDC1, *ADH1* and *DAS1* on the genetic background of the wild-type strain led to increased ethanol production from various substrates (Kurylenko et al., 2014, 2016).

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- I showed that the deletion of the *ATG13* of *O. polymorpha,* similar to the insertion into this gene, leads to increased production of ethanol from xylose, and at the same time is responsible for derepression of several genes involved in xylose catabolism and alcohol fermentation

Publication no. 4 (P4):

Kurylenko OO#, **Ruchala J#**, Vasylyshyn RV, Stasyk OV, Dmytruk OV, Dmytruk KV, Sibirny AA. Peroxisomes and peroxisomal transketolase and transaldolase enzymes are essential for xylose alcoholic fermentation by the methylotrophic thermotolerant yeast, *Ogataea (Hansenula) polymorpha*. Biotechnol Biofuels. 2018; 11:197. doi: 10.1186/s13068-018-1203-z. (**IF_{2018} 5.452; MES_{2017}= 45**).

# Equal contribution

The research carried out under publication number 4 was carried out in collaboration with a foreign research center - the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine). The research was supported by grant financed by the National Science Center - Opus, no. 2016/21 / B / NZ1 / 00280, where I was the main investigator.

The main research goal of the publication number 4 was to explain the role of peroxisomes and several peroxisomal enzymes in xylose alcoholic fermentation in the methylotrophic yeast *O. polymorpha*. Peroxisomes are organelles that typically contain enzymes producing hydrogen peroxide and also catalase. Most peroxisomes in various organisms also contain enzymes involved in β-oxidation of fatty acids. However, peroxisomes are metabolically versatile organelles containing enzymes that catalyze numerous catabolic reactions and some biosynthetic reactions, for example of penicillin biosynthesis. In yeast, peroxisomes are involved in the catabolism of many carbon and nitrogen sources, such as methanol, n-alkanes, purines, D-amino acids, methylamine, ethylamine, pipecolic acid, sarcosine, glycolate, and spermidine. Studies have shown that some of the glycolytic enzymes in the *Cryptococcus neoformans* and *Ustilago maydis* have a dual localization in both cytosol and peroxisomes. Importantly, defects in peroxisomal localization of glycolytic proteins or insufficient peroxisome biogenesis impaired growth of these organisms on glucose. *Candida albicans* and related species are characterized by cytosolic and peroxisomal localization of enzymes of the oxidative branch of the pentose phosphate pathway, namely glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6- phosphogluconate dehydrogenase (EC 1.1.1.43) (Sibirny, 2016). In the current work, I have studied the ability to grow and ferment xylose by the mutants with defective peroxisome biogenesis by deletion of the *PEX3* gene in *O. polymorpha* and, for comparison, in the non- methylotrophic species *Scheffersomyces stipitis*. I have found that the *pex3Δ* mutant of *O. polymorpha* showed defect in xylose fermentation (though growth on this pentose was unimpaired), while *pex3Δ* mutant of S. *stipitis* normally fermented this pentose. The main hypothesis raised during this work was that the peroxisomal enzymes of the pentose phosphate pathway could be important not only for methanol metabolism, but also for xylose fermentation to ethanol. The gene designated by us as *TAL2* was found in the *O. polymorpha* genome database, which contains the PTS1 signal sequence and encodes a peroxisomal transaldolase (Tal2). I proved that this protein is located in peroxisomes when grown on glucose, xylose or methanol as carbon sources. Using isolated by us mutants with deletions of genes coding for peroxisomal and cytosolic transketolases and transaldolases, it was observed that both peroxisomal transketolase Das1 and transladolase Tal2 are not essential

for growth on xylose as sole carbon and energy source, in contrast to their cytosolic counterparts, Tkl1 and Tal1, but are required for fermentation of xylose to ethanol. On the other hand, knock-out of genes encoding cytosolic transketolase *TKL1,* and transaldolase *TAL1,* resulted in complete absence of growth on xylose, while fermentation of this sugar was only partially impaired. It should be also stated that peroxisomal transaldolase is not involved in methanol metabolism in *O. polymorpha.*, whereas the role of peroxisomal transketolase in the metabolism of methanol in methylotrophic yeasts was established many years ago (Veenhuis et al., 1983; Rußmayer et al., 2015). I also proved that overexpression of *DAS1* and / or *TAL2* increased ethanol production from xylose (similarly to the effect of overexpression of *TKL1* and *TAL1*). In addition, I overexpressed both *DAS1* and *TAL2* genes in the available that time the best ethanol producer with deletion of *CAT8* (*cat8Δ*) (**P1**). It allowed to obtain the strains accumulating about 16 g of ethanol per liter, which is 40 times higher than the ethanol level synthesized during xylose fermentation by the wild-type strain (0.4 g / L). It is worth emphasizing that the overexpression of *DAS1* and / or *TAL2* influenced xylose but not glucose fermentation. It can be hypothesized that overexpression of the *DAS1* and *TAL2* genes increased production of pentose phosphates and thus activated xylose metabolism in *O. polymorpha*. However, the specific mechanisms of Das1 and Tal2 involvement in *XYL1* regulation remain to be elucidated. We suggest that peroxisomes are required for fermentation of xylose to ethanol due to localization of the enzymes Das1 and Tal2 in these organelles.

In summary, this was for the first time that the role of peroxisomes and peroxisomal enzymes in alcoholic fermentation of xylose was proved.

The most important achievements of publication no. 4:

- I proved that alcoholic fermentation of xylose in the methylotrophic thermotolerant yeast *O. polymorpha* depends on functional peroxisomal transketolase (Das1) and transaldolase (Tal2), while their cytosolic counterparts (Tkl1 and Tal1) are necessary for growth on this pentose

- I showed that a defect in peroxisome biogenesis due to *pex3Δ* deletion in *O. polymorpha* strongly damaged alcoholic fermentation of xylose, showing no effect on this process in the non-methylotrophic yeast *S. stipitis*
- I found that deletions in genes *DAS1* and *TAL2* coding for peroxisomal transketolase and transaldolase, respectively, strongly suppressed alcoholic fermentation of xylose
- I found that alcoholic fermentation of glucose does not depend on peroxisomes and cytosolic transketolases and transaldolases
- I showed that the simultaneous overexpression of peroxisomal transketolase and transaldolase in a previously isolated advanced xylose ethanol producer further increased ethanol accumulation to 16.1 g / L at 45 ° C.

Publication no. 5 (P5):

Ruchala J, Kurylenko OO, Dmytruk KV, Sibirny AA. Construction of advanced producers of first- and second-generation ethanol in *Saccharomyces cerevisiae* and selected species of non-conventional yeasts (*Scheffersomyces stipitis*, *Ogataea polymorpha*). J Ind Microbiol Biotechnol. 2020; 47(1):109-132. doi: 10.1007/s10295-019-02242-x. (**IF_{2020} 3.346; MES_{2020}= 100**).

The results obtained in the course of the experimental research on the construction of the non-conventional yeast for production of bioethanol from xylose prompted me to write a review paper that would summarize the current state of knowledge in this area. This work was created in collaboration with scientists from the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine) and supported by grant, where I was the main investigator, financed by the National Science Centre - Opus, project no. 2016/21/B/NZ1/00280, as well as The Subcarpathian Centre for Innovation, project no. 06/UR/1/DG/PCI/2019, in which I was principal investigator.

This review discusses the main methods of construction of improved ethanol producers from xylose in the conventional yeast *S. cerevisiae* (naturally incapable of metabolizing xylose) and non-conventional yeasts *S. stipitis* and *O. polymorpha* naturally capable of xylose metabolism and fermentation. The

review illustrates the ways applied for construction of *S. cerevisiae* strains capable of xylose metabolism and fermentation, and methods for improving these capabilities primarily through metabolic engineering methods. *S. stipitis* is one of the most efficient natural xylose-fermenting ethanol producers and can be considered as "conventional" and the best studied among all yeasts naturally capable of xylose fermentation. It is emphasized that the best engineered strains of *S. cerevisiae* are still inferior to the wild-type strains of *S. stipitis* in terms of ethanol production efficiency. However, *S. stipitis* has own disadvantages, for example, low tolerance to ethanol and inhibitors found in lignocellulose hydrolysates. In this publication, I compared the results of my own experimental studies on *O. polymorpha* with those obtained on S. *cerevisiae* and *S. stipitis*. I also emphasized the advantages of my own research object, *O. polymorpha*, including its thermotolerance, well-developed methods of molecular genetics, natural ability to metabolize of xylose, GRAS status and relatively high resistance to ethanol. In this article, I also discuss my own experimental data obtained on all three reviewed species, including data on the role of the transcriptional factor Cat8 and peroxisomal and cytosolic transketolases Das1 and Tkl1 and transaldolases Tal2 and Tal1 in alcoholic fermentation of xylose in *O. polymorpha*. I also described new methods of positive selection of *S. cerevisiae* mutants producing increased amounts of ethanol and insertion mutants of *S. stipitis* with increased production of ethanol from xylose and glucose, especially the mutant with damage to the *HEM25* gene. The article ends with a conclusion on which yeast characteristics should be considered to obtain competing producers of ethanol from lignocellulosic sugars, including xylose.

The most important achievements of publication no. 5:

- I summarized the latest knowledge in the field of construction of the best producers of ethanol from xylose in the yeasts *S. cerevisiae*, *S. stipitis* and *O. polymorpha*
- I summarized the most important own achievements, including proposed and approved new methods of the isolation of the efficient ethanol producers from xylose.

Publication no. 6 (P6):

Ruchala J, Sibirny AA. Pentose metabolism and conversion to biofuels and high-value chemicals in yeasts. FEMS Microbiol Rev. 2020: fuaa069. doi: 10.1093/femsre/fuaa069. (**IF_{2019} 16.408; MES_{2020}= 200**).

This publication was created in cooperation with scientists from the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv (Ukraine) and supported by the grant of the National Science Centre - Opus, project no.2020/37/ B/NZ1/02232. This review summarizes the latest information on the metabolic pathways in several yeast species during conversion of all natural pentose sugars, and not only xylose and L-arabinose which are often discussed in the literature. The article also describes the issues related to metabolism of ribose, 2-deoxyribose, D-arabinose and lyxose.

This work is particularly important because earlier, there was no scientific articles that so broadly describe metabolism of all natural pentose sugars. The review describes many species of yeast, including *S. cerevisiae, S. stipitis, Scheffersomyces shehatae, Pachysolen tannophilus, Spathaspora passalidarum, Kluyveromyces marxianus, O. polymorpha, Candida intermedia, Candida tenuis, Meyerozyma guilliermondii, Yarrowia lipolytica, Komagataella phaffii*, most of which are naturally capable of utilizing xylose, while three of them (*S. cerevisiae, K. phaffii, Y. lipolytica*) naturally do not metabolize this pentose, though they acquired this ability as a result of metabolic engineering. The review includes a description of the different pentose metabolic pathways, the genetics of each species, their biotechnological potential, not only for the production of ethanol, but also of other valuable substances, like lactic acid, xylitol, isobutanol, lipids and fatty acids. This article also summarizes research on the simultaneous metabolism of glucose, xylose and L-arabinose, and on available methods of alleviation or complete removal of catabolic repression that prevents simultaneous utilization of glucose and pentose sugars. This article also discusses on yeast use for the industrial production of ribose – the sugar with significant demand in various sectors of the economy, especially in pharmacy. The article ends with a summary of the current data obtained through the analysis of over 600 literature sources, but also indicates possible directions for the development of future research. Such directions should include the development of new methods of strain development with improved efficiency of the ethanol and other substance synthesis from xylose and lignocellulose

hydrolysates, increased tolerance of yeasts to inhibitors found in these hydrolysates, more effective co-utilization of glucose and pentoses, construction of yeast strains capable of hydrolyzing polysaccharides of lignocellulose sequentially with fermentation or conversion of the hydrolysis products to high-value commodities. In conclusion, I also suggested to potential readers / researchers to pay special attention to the role of transcriptional factors in the regulation of xylose (and other pentoses) metabolism and fermentation. The review also includes some important hitherto unpublished own data, especially about a new successful method of positive selection of the improved ethanol producers from xylose by obtaining mutants showing robust growth on another pentose - L-arabinose.

The most important achievements of publication no. 6:

- I collected the latest data on the metabolism of pentose sugars, not only xylose and L-arabinose, but also ribose, 2-deoxyribose, D-arabinose and lyxose
- I described in detail the possibilities of converting pentose sugars to ethanol, other biotechnologically valuable products (lactic acid, xylitol, isobutanol, lipids and fatty acids) and the biotechnological potential of the appropriate yeast species and strains

Summary of the Main Scientific Achievement:

The series of publications which present the main scientific achievement, allows to understand the mechanisms related to the regulation of sugar metabolism in lignocellulose polymers (mainly xylose). Undoubtedly, my most important achievement is proving for the first time the important regulatory role of transcription factors in alcoholic fermentation of xylose in yeasts that naturally metabolize this sugar, using *O. polymorpha*, namely the transcriptional activators Cat8, Hap4-A and Hap4-B, and repressors Tup1, Mig1 and Mig2. The importance of transcription factors in the regulation of alcoholic fermentation, especially in yeasts naturally capable of pentose metabolism, has hitherto been overlooked or denied, but the results of my work have prompted many research groups around the world to pay attention to this phenomenon (Wei et al., 2018;

Martinez et al., 2019; Xie et al., 2020; Dzanaeva et al., 2021; Li et al., 2021). My results in this field, being of basic character, were used for construction an improved ethanol producer from xylose by gene deletion of one of the transcriptional activators, *CAT8*, in the genetic background of the advanced ethanol producer from xylose, previously isolated by another methods. I also found that autophagy (the degradation of cellular material that occurs in the vacuoles) is involved in the regulation of xylose fermentation. This conclusion is supported by data on an increase in ethanol production from xylose as a result of disruption or deletion of the *ATG13* gene, which product, together with the product of the *ATG1* gene, initiates autophagy. My research also allowed to prove the role of peroxisomes in xylose alcoholic fermentation in *O. polymorpha*. Moreover, I proved that alcoholic fermentation of xylose in the methylotrophic thermotolerant yeast *O. polymorpha* depends on functional peroxisomal transketolase (Das1) and transaldolase (Tal2), while their cytosolic counterparts (Tkl1 and Tal1) are necessary for growth on this pentose. This knowledge allowed to construct the strain characterized by an increased accumulation of ethanol up to 16.1 g / L at 45 ° C (40 times more as compared to the wild-type strains) in the genetic background of an earlier isolated advanced strain by simultaneous overexpression of peroxisomal transketolase and transaldolase. It is worth noting that the enzymes mentioned (transketolase, transaldolase) are not involved in the regulation of alcoholic fermentation of glucose. It is important to emphasize that the use of the new method of positive selection consisting in isolating mutants forming large colonies on L-arabinose, led to isolation of the strains accumulating 20 g ethanol / L from xylose, which is 50 times more relative the level of ethanol produced by the wild-type strain. These results allow to plan the future implementation of the strains I have isolated which show the absolute record of ethanol production from xylose at so high temperature (45°C), as they are close to the levels obtained for the best mesophilic yeast strains at 30°C. If to compare accumulation of ethanol from xylose by engineered strains of another species of thermotolerant yeast *Kluyveromyces marxianus*, the last one shows maximum ethanol production capacity at 40-42°C, while at 45°C its ethanol production capacity is substantially reduced. In addition, all known strains of *K. marxianus* accumulate high concentrations of the undesirable xylitol, while the strains constructed in the course of main scientific achievement

do not accumulate visible amounts of this by-product of alcoholic fermentation (Zhang et al., 2015; Suzuki et al., 2019, **P1**). In fact, the published in literature selection methods for the improved ethanol producers from xylose mainly consisted of protein engineering the first or the second enzymes of xylose catabolism and overexpression of the first three genes of this pathway *XYL1*, *XYL2*, *XYL3*. I was the first who implemented the deletion of transcriptional factor genes (on the example of *CAT8*) and overexpression of the genes coding for peroxisomal enzymes, transketolase and transladolase, into the practice of selection of the improved ethanol producers in non-conventional yeasts (**P1**; **P4**). Also, for the first time, I introduced two novel methods of isolating improved ethanol producers by selection of the mutants resistant to the anticancer factor, 3-bromopyruvate, and selection of the mutants capable of robust growth on another pentose, L-arabinose. (**P6**). At the same time, it is worth emphasizing that my work was performed with the use of the modern methods of molecular genetics and microbial biochemistry, including molecular cloning, activation (overexpression) or inactivation (deletion) of cloned genes, multi-copy integration of introduced genes, protein engineering, analysis of gene expression, determining the specific activities of enzymes and concentrations of metabolites as well as methods of fluorescent microscopy.

Future Prospects

During my further work, I plan to identify new regulatory genes involved in xylose alcoholic fermentation in the yeast *O. polymorpha*. In fact, I already developed several new original methods for positive selection of regulatory mutants. One, already mentioned, method consists of selection of the mutants that produce large colonies on L-arabinose, while the second is based on the selection of *O. polymorpha* mutants resistant to galactose (non-metabolized sugar in *O. polymorpha*) inhibition growth on xylose. The mutants isolated in the first method, synthesize more ethanol from xylose, while those isolated by the second method (i.e. galactose-resistant mutants) do not ferment xylose at all. After identifying the relevant genes, I plan to overexpress and delete them in order to construct a more efficient thermotolerant ethanol producers from xylose that would accumulate at least 30 g of ethanol / L from this pentose at an elevated temperature of 45°C (currently my best strains accumulate 20 g / L, while

the wild type strain produces only 0.4 g ethanol / L). Particular attention will be paid to construction of the *O. polymorpha* strains that simultaneously use and ferment glucose and xylose. This goal will be achieved by modification of the genes responsible for sugar transport in *O. polymorpha*, which increase the affinity of the transporters for xylose and reduce it for glucose. Another goal will be to study metabolism and alcoholic fermentation of the third most abundant sugar in the biosphere after glucose and xylose, L-arabinose. I found that the mentioned above the *O. polymorpha* mutants that grow on L-arabinose produce some amounts of ethanol from this pentose, though still very low (the method of obtaining such mutants allowed for a patent application currently pending (the Polish Patent Office), **P.435340**. Methods of obtaining yeast ethanol producers from xylose from thermotolerant yeast *Ogataea polymorpha*, registration date 15/09/2020, reporting entity University of Rzeszów, The Subcarpathian Centre for Innovation Ltd., project no. 06/UR / 1 / DG / PCI / 2019. Moreover, currently I am under developing a new strategy that will enable the isolation of *O. polymorpha* strains capable of effective simultaneous fermentation of all major sugars of lignocellulose hydrolysates, glucose, xylose and L-arabinose at elevated temperatures. It will be an important step in the construction of a modern, efficient thermotolerant yeast producer of second-generation bioethanol from a renewable raw material - lignocellulose.

The research results of the habilitation cycle have been presented at numerous conferences, both domestic and foreign, including: Gdańsk, Wrocław, Rzeszów, Lviv (Ukraine), Kyiv (Ukraine), Nova Gorica (Slovenia), Levico Terme (Italy).

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I realized the Main Scientific Achievement not only at my *Alma Mater*, but also as part of scientific collaboration at:

- Institute of Cell Biology of the National Academy of Sciences of Ukraine, Lviv, Ukraine

Part of the research presented in the context of Main Scientific Achievements was performed in scientific institutions abroad in the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv during my multiple stays in this institution. The results in numerous scientific publications were obtained in the framework of this collaboration. Here it is worth mentioning, that I carried out the construction of strains and determination of metabolites using HPLC as part of the publication no. **P2** with collaboration with Drs Kostyantyn Dmytruk and Olena Kurylenko in the Institute of Cell Biology of the National Academy of Sciences of Ukraine in Lviv during my stay there. For this reason, I affiliate the publication not only with the address of my home institution - the University of Rzeszów, but also the Institute of Cell Biology of the National Academy of Science of Ukraine in Lviv. Interestingly, due to my continuous scientific interest to pentose metabolism and experience in this field, numerous conversations with my colleagues from the above-mentioned institute in Lviv and the exchange of views on this subject, the review was created (**P6**). Just for this reason, the mentioned article also contains the affiliation of Ukrainian Institute next to my name. At the same time, I would like to emphasize that the collaboration is continued.

The works contain the results obtained during the implementation of the following research projects:

- Federation of European Microbiological Societies, FEMS-RG-2015-0096.R1 (2016), title: „Studying the role of *CAT8* transcriptional activator in the yeast xylose alcoholic fermentation" – principal investigator

- National Science Centre, 2016/21/B/NZ1/00280 (2017 – 2020), title: „The role of transcription factors in regulation of glucose and xylose metabolism and fermentation in the non-conventional yeast *Ogataea polymorpha*" – investigator, co-author of the project
- National Science Centre, 2020/37/B/NZ1/02232 (2021 – 2024), title: „Genetic control of pentose (D-xylose, L-arabinose) metabolism and alcoholic fermentation in the thermotolerant yeast *Ogataea polymorpha*" – investigator, co-author of the project
- The Precarpathian Centre for Innovation, 06/UR/1/DG/PCI/2019 (2020), title: „The construction of yeast strains capable of efficient high-temperature fermentation of glucose and xylose" – principal investigator
- The Precarpathian Centre for Innovation, 13/UR/1/DG/PCI/2019 (2020), title: „Obtaining the riboflavin (vitamin B2) on whey with *Candida famata* yeast" – investigator, co-author of the project
- National Science Centre, 2018/29/B/NZ1/01497 (2019 – 2022), title: „Regulatory mechanism involved in riboflavin overproduction in the flavinogenic yeast *Candida famata*" – investigator, co-author of the project

5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions:

A.) Scientific work

Since the beginning of my academic activity, my interests have been focused on biotechnology of non-conventional yeast, in my research I have taken up various issues on this topic.

In my scientific work I used the methods of classical microbiology, as well as the modern techniques of genetic engineering and biochemistry. My research models were yeasts, with particular emphasis on conventional (*Saccharomyces cerevisiae*) and non-conventional yeast (*Ogataea polymorpha, Komagataella pastoris, Scheffersomyces (Pichia) stipitis*), as well as bacterium *Streptomyces davaonensis*. These studies resulted in scientific articles, chapters

in monographs, national and international collaboration (specific achievements are presented in Appendix 4).

My total output includes (scoring according to the year of publication):

Total *Impact Factor* (MES points)	**Considering main scientific achievement**	**Without taking into account the articles constituting the Main Scientific achievement**	**Including articles constituting the Main Scientific Achievement**
Total	34.291 (510)	69.185 (1150)	103.476 (1660)
Before a doctoral degree	0 (0)	4.221 (40)	4.221 (40)
After a doctoral degree	34.291 (510)	64.964 (1110)	99.255 (1620)

My scientific achievements can be divided according to the following research topics:

5.1. The construction of yeasts actively producing of biofuels

My main research topic is the construction of yeasts actively producing biofuels, which is confirmed by numerous scientific publications that were not included in the main scientific achievement. With the help of protein engineering and overexpression of the sugar transporter genes, it was possible to construct *O. polymorpha* mutants capable of simultaneous metabolism and fermentation of xylose and glucose (Vasylyshyn et al., 2020). I am also interested in the role of transcription factors and regulatory genes related to the alcoholic fermentation of sugars in conventional yeasts, hence I was the advisor of the PhD student from the King Mongkut's University of Technology Thonburi (Bangkok, Thailand) Ms. Pattanan Songdech conducting research on transcription factors

in my laboratory (Songdech et al., 2020). I participated in research on the role of peroxisomes, peroxisomal enzymes and transcription factors Znf1, Sip4, Adr1, Tup1, and Hap4 in xylose catabolism in *S. cerevisiae* (Dzanaeva et al., 2020; 2021). It should be pointed out that these works have been started after my publications on the role of transcription factor Cat8 and peroxisomal enzymes in xylose alcoholic fermentation in *O. polymorpha* (**P1, P4).** I participated in the study of insertion mutants of another species of naturally fermenting xylose yeast, *S. stipitis*, which resulted in the identification of the *HEM25* gene involved in the regulation of alcoholic fermentation of xylose and glucose (Berezka et al., 2021). As mentioned earlier, I was the principal investigator of a scientific project financed by the Subcarpathian Centre for Innovation, concerning the further improvement of the current ethanol producer from xylose. During the project implementation, a new method of positive selection of *O. polymorpha* mutants producing increased amounts of ethanol from xylose was developed and a patent application was filed (**P.435340**. Methods of obtaining yeast ethanol producers from xylose from thermotolerant yeast *Ogataea polymorpha* (see above). It is worth mentioning that I am interested in studying the synthesis of higher alcohols by non-conventional yeast. I have observed that multinuclear yeast with giant cells, *Magnusiomyces magnusii*, produces large amounts of isobutanol, about 20-30 times more than the known *S. cerevisiae* baker's yeast (Kurylenko et al., 2020). I was also involved in the study of glycerol synthesis and its bioconversion to ethanol in yeast (Kata et al., 2016; Semkiv et al, 2020).

<u>After obtaining a doctoral degree:</u>

1. Dzanaeva L, Kruk B, **Ruchala J**, Sibirny A, Dmytruk K. The impact of transcription factors Znf1, Sip4, Adr1, Tup1, and Hap4 on xylose alcoholic fermentation in the engineered yeast *Saccharomyces cerevisiae*. Antonie Van Leeuwenhoek. 2021;25. doi: 10.1007/s10482-021-01607-6. **IF 2,271; MES: 70**
2. Berezka K, Semkiv M, Borbuliak M, Blomqvist J, Linder T, **Ruchała J**, Dmytruk K, Passoth V, Sibirny A. Insertional tagging of the *Scheffersomyces stipitis* gene *HEM25* involved in regulation of glucose and xylose alcoholic fermentation. Cell Biol Int. 2021; 45(3):507-517. doi: 10.1002/cbin.11284. **IF 3.612; MES: 70**

3. Vasylyshyn R, Kurylenko O, **Ruchala J**, Shevchuk N, Kuliesiene N, Khroustalyova G, Rapoport A, Daugelavicius R, Dmytruk K, Sibirny A. Engineering of sugar transporters for improvement of xylose utilization during high-temperature alcoholic fermentation in *Ogataea polymorpha* yeast. Microb Cell Fact. 2020; 19(1):96. doi: 10.21203/rs.2.22909/v1. **IF 5.328; MEiN: 100**
4. Dzanaeva L, Kruk B, **Ruchala J**, Nielsen J, Sibirny A, Dmytruk K. The role of peroxisomes in xylose alcoholic fermentation in the engineered *Saccharomyces cerevisiae*. Cell Biol Int. 2020; 44(8):1606-1615. doi: 10.1002/cbin.11353. **IF 3.612; MES: 70**
5. Dzanaeva L, Ruchala J, Sibirny A, Dmytruk K. The impact of transcriptional factors Znf1 and Sip4 on xylose alcoholic fermentation in recombinant strains of yeast *Saccharomyces cerevisiae*. Cytol Genet. 2020; 54(5): 386-392. doi: 10.3103/S0095452720050035. **IF 0.579; MES: 20**
6. Kurylenko OO, **Ruchala J**, Dmytruk KV, Abbas CA, Sibirny AA. Multinuclear yeast *Magnusiomyces (Dipodascus, Endomyces) magnusii* is a promising isobutanol producer. Biotechnol J. 2020; 15(7):e1900490. doi: 10.1002/biot.201900490. **IF 4.677; MES: 100**
7. Semkiv MV, **Ruchala J**, Dmytruk KV, Sibirny AA. 100 Years Later, What Is New in Glycerol Bioproduction? Trends Biotechnol. 2020; 38(8):907-916. doi: 10.1016/j.tibtech.2020.02.001. **IF 19.536; MES: 200**
8. Songdech P, **Ruchala J**, Semkiv MV, Jensen LT, Sibirny A, Ratanakhanokchai K, Soontorngun N. Overexpression of transcription factor *ZNF1* of glycolysis improves bioethanol productivity under high glucose concentration and enhances acetic acid tolerance of *Saccharomyces cerevisiae*. Biotechnol J. 2020; 15(7):e1900492. doi: 10.1002/biot.201900492. **IF 4.677; MES: 100**
9. Kurylenko O, Semkiv M, **Ruchala J**, Hryniv O, Kshanovska B, Abbas C, Dmytruk K, Sibirny A. New approaches for improving the production of the 1st and 2nd generation ethanol by yeast. Acta Biochim Pol. 2016; 63(1):31-38. doi: 10.18388/abp.2015_1156. **IF 1.159; MES: 15**
10. Kata I, Semkiv MV, **Ruchala J**, Dmytruk KV, Sibirny AA. Overexpression of the genes *PDC1* and *ADH1* activates glycerol conversion to ethanol in

the thermotolerant yeast *Ogataea (Hansenula) polymorpha*. Yeast. 2016; 33(8):471-8. doi: 10.1002/yea.3175. **IF 2.259; MES: 25**

Chapters in monograph:

1. Dmytruk KV, Kurylenko OO, **Ruchala J**, Abbas CA, Sibirny AA. Genetic improvement of conventional and nonconventional yeasts for the production of first- and second-generation ethanol. In: Sibirny A. (eds) Biotechnology of Yeasts and Filamentous Fungi. Springer, Cham. 2017. doi: 10.1007/978-3-319-58829-2_1. **MEiN$_{2017}$= 20**
2. Dmytruk K., Kurylenko O., **Ruchala J.**, Ishchuk O., Sibirny A. Development of the thermotolerant methylotrophic yeast *Hansenula polymorpha* as efficient ethanol producer. In: Satyanarayana T, Kunze G (eds) Yeast Diversity in Human Welfare. Springer, Singapore. 2017. doi: 10.1007/978-981-10-2621-8_11. **MEiN$_{2017}$= 20**

Before doctoral degree:

1. Kurylenko OO, **Ruchala J**, Hryniv OB, Abbas CA, Dmytruk KV, Sibirny AA. Metabolic engineering and classical selection of the methylotrophic thermotolerant yeast *Hansenula polymorpha* for improvement of high- temperature xylose alcoholic fermentation. Microb Cell Fact. 2014; 13:122. doi: 10.1186/s12934-014-0122-3. **IF 4.221; MES: 40**

Chapters in monograph:

1. Semkiv M, Kurylenko O, **Ruchala J**, Hryniv O, Kshanovska B, Dmytruk K, Sibirny A. Yeast alcoholic fermentation: achievements and challenges. In: Novikov V (eds) Modern directions in chemistry, biology, pharmacy and biotechnology. Lviv Politechnic National University, Lviv, Ukraine. 2015, 235-248, ISBN 978-617-607-824-1.
2. Semkiv MV, Kurylenko OO, **Ruchala J**, Hryniv OB, Dmytruk KV, Sibirny AA. Yeast metabolic engineering for construction of the advanced bioethanol producers from renewable feedstocks In: Sibirny A, Fedorovych D, Gonchar M, Grabek-Lejko D (eds) Living organisms and bioanalytical approaches for detoxification and monitoring of toxic

compounds. University of Rzeszow in corporation with Institute of Cell Biology National Academy of Sciences of Ukraine, Rzeszow. 2015, 333- 341, ISBN 978-83-7667-203-8.

3. Kurylenko O, **Ruchala J**, Dmytruk K, Sibirny A. New targets for improvement of xylose alcoholic fermentation in the methylotrophic yeast *Hansenula polymorpha*. In: Sibirny A, Fedorovych D, Gonchar M, Grabek-Lejko D (eds) Living organisms and bioanalytical approaches for detoxification and monitoring of toxic compounds. University of Rzeszow in corporation with Institute of Cell Biology National Academy of Sciences of Ukraine, Rzeszow. 2015, 247-257, ISBN 978-83-7667-203-8.

5.2. Genetic control of riboflavin production in flavinogenic yeasts *Candida famata*

My next scientific interest deals with the synthesis of riboflavin (vitamin B_2) in the flavinogenic yeast *Candida famata*. As part of this research cycle, I proved the important role of riboflavin excretion and purine nucleotide biosynthesis in the overproduction of riboflavin. These observations allowed to construct more efficient producers of vitamin B_2 (Tsyrulnyk el al., 2020; Dmytruk et al., 2020). It has been proved that overexpression of the yeast gene homologous to the mammal BCRP gene coding the protein transporting riboflavin from the mammary gland to the milk leads to overproduction of riboflavin in *Candida famata* (Tsyrulnyk et al., 2020). With the help of protein engineering of the gene products of *PRS3* and *ADE4* involved in purine nucleotide biosynthesis *de novo* and their overexpression, it was possible to improve the existing stable overproducer of riboflavin, because the purine nucleotide GTP acts as precursor in the biosynthesis of vitamin B_2 (Dmytruk et al., 2020). I also found that constructed by us the *C. famata* strain produces an increased amounts of riboflavin in whey medium (a byproduct of the dairy industry) and I hope that this discovery will be implemented into practice so that the byproduct (whey) will be converted into a high-value product (vitamin B_2). I developed this idea as an investigator of a scientific project financed by The Subcarpathian Centre for Innovation (project contract number: 13 / UR / 1 / DG / PCI / 2019), as a result of which a patent application currently under consideration was filed

(Patent Office of the Republic of Poland), **P.435341**. A new strain of yeast *Candida famata* capable of overproducing riboflavin, the use of the yeast *Candida famata* BCRP strain to produce riboflavin and a method of producing riboflavin" 15/09/2020 (registration date), entity reporting the University of Rzeszów, The Subcarpathian Centre for Innovation Ltd., Rzeszów, Poland. Recently, work on riboflavin has also been continued in a new dimension: I have constructed strains of the non-conventional yeast *Komagataella phaffii (Pichia pastoris)*, which produces the bacterial flavin antibiotic - aminoriboflavin. It is very important that it is the first such example to engineer yeast producer of the antibiotic naturally synthesized by bacteria. Aminoriboflavin isolated from recombinant yeast appeared to be active against pathogenic bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* (I worked on this as part of the Polish-Austrian exchange project at University of Natural Resources and Life Sciences, BOKU, Vienna, Austria). In conclusion, my experience confirms the importance of cell factories of various species of non-conventional yeasts for production of biofuels, glycerol, vitamins and antibiotics.

<u>After obtaining a doctoral degree:</u>

1. Tsyrulnyk AO, Andreieva YA, **Ruchala J,** Fayura LR, Dmytruk KV, Fedorovych DV, Sibirny AA. Expression of yeast homolog of the mammal *BCRP* gene coding for riboflavin efflux protein activates vitamin B2 production in the flavinogenic yeast *Candida famata*. Yeast. 2020;37(9-10):467-473. doi: 10.1002/yea.3470. **IF 3.239; MES: 70**
2. Dmytruk KV, **Ruchala J,** Fedorovych DV, Ostapiv RD, Sibirny AA. Modulation of the purine pathway for riboflavin production in flavinogenic recombinant strain of the yeast *Candida famata*. Biotechnol J. 2020; 15(7):e1900468. doi: 10.1002/biot.201900468. **IF 4.677; MES: 100**

5.3. <u>Glutathione production by yeasts</u>

The next series of publications deals with glutathione (L-γ-glutamyl-L-cysteinyl-glycine) biosynthesis. Glutathione is the main thiol of eukaryotic cell. This tripeptide is the most important intracellular redox buffer. Glutathione is involved in the detoxification of heavy metals, xenobiotics, oxygen radicals, is involved in DNA synthesis and other reactions. Glutathione is the major

product of the biotechnology used in medicine as a drug, especially for the treatment of a number of tumors and as a cryoprotector and imunomodulator, as well as in food and cosmetic industries to remove harmful oxidative processes and detoxify toxic substances (Ruchala et al., 2015). The estimated annual production of glutathione worldwide is around 1,000 tons, for a total of around US $ 200 million (Marz 2014). Yeasts, and in particular methylotrophic yeast *O. polymorpha* are among the most efficient producers of glutathione (Ubiyvovk et al. 2011). I conducted research in which it was showed that dried cells of O. *polymorpha* producing increased amounts of glutathione maintain good viability during their storage. The engineered strain with increased glutathione production is more stable when stored dry relative to the parent strain. This can be explained by better protection of intracellular membranes during cell dehydration by higher amounts of glutathione in these cells. A special procedure to pre-hydrate dry cells with water vapor restored vitality of almost 100% of the dehydrated cells. At the same time, we proved that dehydration - rehydration of cells does not affect their ability to produce glutathione, which allows the use of dry, active *O. polymorpha* preparations in biotechnological processes related to the production of glutathione (Kurylenko et al., 2019).

<u>After obtaining a doctoral degree:</u>

1. Kurylenko O, Rozenfelde L, Khroustalyova G, Vasylyshyn R, **Ruchala J**, Chang CR, Daugelavicius R, Sibirny A, Rapoport A. Anhydrobiosis in yeasts: Glutathione synthesis by yeast *Ogataea (Hansenula) polymorpha* cells after their dehydration-rehydration. J Biotechnol. 2019; 304:28-30. doi: 10.1016/j.jbiotec.2019.08.005. **IF 3.503; MES: 70**
2. Muter O, Khroustalyova G, Rimkus A, Kalderis D, **Ruchala J**, Sibirny A, Rapoport A. Evaluation of the enhanced resistance of *Ogataea* (*Hansenula*) *polymorpha* to benzalkonium chloride as a resource for bioremediation technologies. Process Biochem. 2019; 87:157-163. doi: 10.1016/j.procbio.2019.08.026. **IF 2.952; MES: 70**
3. Kulikova-Borovikova D, Khroustalyova G, Chang CR, 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. doi: 10.1016/j.procbio.2018.05.016. **IF 2.883; MES: 70**

Before obtaining a doctoral degree:

Chapters in monograph:

1. **Ruchala J**, Kurylenko OO, Sibirny AA. Production of glutathione by yeasts and the role of this thiol in methylotrophic metabolism and xenobiotic detoxification. In: Sibirny A, Fedorovych D, Gonchar M, Grabek-Lejko D (eds) Living organisms and bioanalytical approaches for detoxification and monitoring of toxic compounds. University of Rzeszow in corporation with Institute of Cell Biology National Academy of Sciences of Ukraine, Rzeszow. 2015, 227-236, ISBN 978-83-7667-203-8.

B.) Collaborations

International collaborations:

In addition to active participation in numerous scientific conferences, I also carry out international cooperation with several researchers. This results in the implementation of certain research data in joint scientific publications and ventures. I run a close scientific cooperation with **Drs Kostyantyn Dmytruk and Olena Kurylenko** (Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine), which results in numerous scientific publications mentioned in the Summary of Professional Accomplishments. In addition, I had 3-month internship, carrying out my research project in the laboratory of Dr. Kostyantyn Dmytruk financed by the Federation of European Microbiological Societies (FEMS). The result of this stay is a scientific publication included in the Main Scientific Achievement Ruchala et al., 2017 (**P1**).

As part of the construction of yeast strains capable of increased glutathione production, I started cooperation with **prof. Alexander Rapoport** (University of Latvia; Riga, Latvia) and **prof. Rimantas Daugelavičius** (Vytautas Magnus

University; Kaunas, Lithuania). As part of the cooperation, in addition to scientific publications, together with prof. Alexander Rapoport, as Guest Editors, we are under preparation the special issue of the *Fermentation* (IF: 3.975; MDPI).

During my doctoral studies, I had a pleasure to work with **prof. Volkmar Passoth** (SLU Swedish University of Agricultural Sciences; Uppsala; Sweden) and with already passed away **prof. Jure Piskur** (Lund University; Lund; Sweden). Thanks to the Visby project (project no: 00577/2010), in 2013 I was on a 6-month research internship in the laboratory of prof. Jure Piskur participating in the research task entitled "Disruption of genes of *Dekkera bruxellensis* yeast involved in pathways of amino acid biosynthesis". On the other hand, the scientific interests of **prof. Volkmar Passoth** were directed to the alcoholic fermentation of *S. stipitis*, and the result of our cooperation was resulted in the joint recent scientific publication - Berezka et al., 2021.

Due to the spreading global problem of antibiotic resistance, my interest is also focused on the search for new effective antimicrobial drugs that would be effective against many pathogenic bacteria resistant to antibiotics. According to the scientific literature, the antibiotic roseoflavin - natural analog of riboflavin (vitamin B_2) produced by actinomycetes *S. davaonensis* and *Streptomyces cinnabarinus* effectively inhibits the growth of gram-positive bacteria such as *S. aureus* and *L. monocytogenes*, whereas its chemical derivatives display anticancer properties. However, due to the fact that natural producers accumulate quite low amounts of antibiotic, we decided to express the genes related to the synthesis of roseoflavin in one of the best yeast expression systems, the yeast *Komagataella phaffii*. For this reason, we started collaboration with one of the best research groups working with this species in Europe, headed by **prof. Diethard Mattanovich** (University of Natural Resources and Life Sciences, BOKU, Vienna, Austria). The result of this collaboration is a Polish-Austrian personal exchange project financed from the Polish side by the Ministry of Science and Higher Education (now this function has been taken over by NAWA) entitled: "Cloning the genes involved in biosynthesis of the antibiotic roseoflavin from the natural producer *Streptomyces davawensis* in riboflavin-overproducing strains of the yeasts *Candida famata* and *Pichia pastoris*", project

no. DWM.ZWB.183.218.2015. As part of this project, I was twice in the laboratory of prof. Diethard Mattanovich (1 month in 2016; 2 months in 2017) carrying out the project task. The results were presented by me at scientific conferences, including the 8th International Weigl Conference (2019), Łódź, Poland, as well as being an invited speaker at the 35th International Specialized Symposium on Yeasts (2019), Antalya, Turkey. The corresponding joint scientific publication is in preparation.

In addition, I am also a participant in the international program COST (*European Cooperation in Science and Technology*) on yeast, YEAST4BIO. The title of the program is: "Non-conventional yeasts for the production of bioproducts", so it concerns the possible biotechnological use of non-conventional yeast, I am a member of the group WG4: *Bioproducts generation from the sugars platform by non-conventional yeasts*. The program aims to create a network of researchers across Europe, including finding partners to submit EU research projects.

I was a winner of numerous scholarships allowing me to participate in scientific conferences organized or co-financed by FEMS, including:

- FEMS Meeting Grant to participate in The 8th Congress of European Microbiologist (FEMS 2019); Glasgow, Scotland, 2019
- FEMS Meeting Grant to participate in The 33rd International Specialized Symposium on Yeast (ISSY33), Cork, Ireland, 2017
- FEMS Meeting Grant to participate in the The 6th Congress of European Microbiologists (FEMS 2015), Maastricht, the Netherlands, 2015

In 2018, I was the head of the secretariat of the international scientific conference on non-conventional yeasts "Non-conventional Yeasts: from Basic Research to Application" organized in Rzeszów, Poland, which gathered 148 participants, including 117 foreign participants representing 36 countries.

In 2020, I was the head of the secretariat and a member of the scientific committee of the 1st Polish Yeast Conference (which, due to the COVID-19 epidemic situation, was moved to 2022), and I also received the appropriate

FEMS grant to organize this conference (FEMS Meeting Organizer Grant, decision ID: FEMS-GO-2019-569).

Additionally, since 2013 I have been a member of the Polish Society of Microbiologists and since 2021 I am the member of the board of the Rzeszów branch of this society.

Editorial reviews for scientific journals:

I was a reviewer of 5 publications in international scientific journals as well as 7 research projects financed by the National Research Foundation of Ukraine, NRFU.

Article reviews for JCR journals:

I have reviewed scientific articles for Microbial Cell Factories (4) and Current Genetics (1).

I am the Guest Editor of a special issue of the MPDI journal "Fermentation" (IF: 3.975)

C.) Summary

My scientific achievements include:

22 original papers and review articles published in journals with an impact factor
6 original papers and review articles published in monographs
3 patent applications
56 conference reports presented at international (**54**) and national (**2**) conferences, **13** of which are oral presentations
Total impact factor according to the Journal Citation Report consistent to the year of publication: **103.476**
The total score of MES for all publication, according to the year of publication: **1680**
Number of citations of publications according to the Web of Science: **125** (data from 02.09.2021)
Number of citations of publications (without self-citations) according to the Web of Science: **95** (data from 02.09.2021)

Hirsch index according to the Web of Science: **6** (data from 02.09.2021) and **7** according to the Scopus (data from 11.06.2021)

6. Presentation of teaching and organizational achievements as well as achievements in popularization of science or art:

A.) Achievements in teaching and in popularizing science:

Since the time of preparing my doctoral thesis, I have been actively involved in teaching as part of didactic duties. At the University of Rzeszów, I conduct laboratory classes and lectures in subjects such as: Microbiology (Biology, 2nd year, first-cycle studies); Basics of Biotechnology (Biology, 3rd year, first-cycle studies) Molecular Biology (Biology, 1st year, second-cycle studies); Modern Techniques of Genetic Engineering (Biology, 1st year, second-cycle studies); Microbiological Technologies (Biotechnology, 2nd year, first-cycle studies); Genetic Engineering of Microorganisms (Biotechnology, 3rd year, first-cycle studies); Food Microbiology, (Food Technology and Human Nutrition, 1st year, first-cycle studies); Microbiology (Agriculture, 2nd year, first-cycle studies); Agrobiotechnology (Agriculture, 1st year, second-cycle studies). I also conducted classes - both lectures and laboratory classes for ERASMUS+ students in the subjects: Genetic Engineering and Molecular biology.

In addition, from 30.10.2019 I am a member of the Biology Program Team at the University of Rzeszów, the author of the educational programme (curriculum, learning outcomes) for biology field of study.

B.) Scientific supervision of students

I was the supervisor of **6** bachelor's theses (2 experimental) and **9** master's theses in the fields of Food Technology and Human nutrition and Biology Currently, I am a supervisor of **5** students preparing master's theses, **2** student preparing a bachelor's thesis in the fields of Biology and Biotechnology.

C.) Activity towards raising professional qualifications

As part of improving my professional qualifications, I was a participant in the International Summer School for Microbiology teachers jointly organized by the

European Federation of Microbiological Societies (FEMS) and the University of Rzeszów in 2019.

D.) Publications for general public

I was a participant in the 4th Art & Science "Art of Origin of Life", organized by the Nencki Institute of the Polish Academy of Sciences and the University of Rzeszów, as well as the Marceli Nencki Foundation for Supporting Biological Sciences and the Nencki Art Collection, under which a general public article and patent application were published:

1. **Ruchala J**. Microbial masterpieces – pigment production by microbes. In: Iskra-Paczkowska A, Wnuk M, Szewczyk A, Fabczak H (eds) 4th Art & Science Projects, The Art of the Origin of Life. University of Rzeszow and Nencki Institute of Experimental Biology, Polish Academy of Science, 2021, 150- 156, ISBN 978-83-7996-845-9.
2. **P. 436255. Ruchała J**, Nikiel A, Wnuk M. Otrzymanie i zastosowanie wyrobu pigmentowego na bazie ekstraktu *Serratia marcescens* jako materiału plastyczno-artystycznego; 12.04.2020 (registration date). Entity submitting: University of Rzeszów.

7. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.

In the years 2018-2021, I was the head of the Department (2018-2019, Department of Microbiology and Biotechnology, Faculty of Biotechnology, University of Rzeszów; 2019-2021, Department of Microbiology and Molecular Genetics, Institute of Biology and Biotechnology, College of Life Sciences, University of Rzeszów. The Department was dissolved due to the reorganization of the structure of the Institute of Biology and Biotechnology of the University of Rzeszów, as a result of which several departments were merged, resulting in the establishment of the Department of Biology.

Justyna Ruchała