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RNDr. Martina BORČINOVÁ (née PLAČKOVÁ)

OSOBNÍ ÚDAJE

Datum narození: 15. 03. 1986
Národnost: ČR

VZDĚLÁNÍ

2011 - dosud **PhD. studium Mikrobiologie a Molekulární Biologie**

Univerzita: Univerzita Karlova v Praze, ČR

Školitel: RNDr. Pavel Kyslík, CSc., Laboratoř Enzymových Technologií, Mikrobiologický ústav v.v.i., Česká Akademie věd, Praha, ČR.

Projekt: Integrated development of a bioprocess: From the soil enzyme to the yeast production platform

2014 **Státní rigorózní zkouška z Mikrobiologie**

Univerzita: Univerzita Karlova v Praze, ČR

Udělený titul: RNDr.

2008 – 2011 **Magisterské studium Mikrobiologie**

Univerzita: Univerzita Karlova v Praze, ČR

Školitel: Mgr. Kateřina Svobodová, PhD. Laboratoř environmentální biotechnologie, Mikrobiologický ústav v.v.i., Česká Akademie věd, Praha, ČR.

Projekt: Laccase activity profiling in *Trametes versicolor* cultures degrading endocrine-disrupting compound Delor 103.

ZAHRANIČNÍ STÁŽE

02/2016 – 07/2016 **Technical University Graz (Štýrský Hradec, Rakousko)**

Školitel: Prof. Dr. Anton Glieder, Institute of Molecular Biotechnology, DK Molecular Enzymology

Projekt: Development of a library of *Komagataella phaffii* constructs for efficient production of penicillin G acylase.

Financování: ACTION program (OeAD-mbH /ICM Austrian Agency for International Cooperation in Education & Research)

09/2014 – 10/2015 **Zürich University of Applied Sciences (Wädenswil, Švýcarsko)**

Školitel: Prof. Dr. Karin Kovar, Institute of Chemistry and Biotechnology, Section Bioprocess Technology

Projekt: PEGAS: Relationship between *Pichia pastoris* cell physiology and secretion of heterologous penicillin G acylases.

Financování: Scientific exchange programme SCIEX-NMS^{CH} (Conference of the Swiss Universities mandated by Swiss Development & Cooperation agency for Programme implementation and management)

03/2013 – 04/2013 **Fermenta Biotech Ltd. (Thane, Indie)**

Účel: Průmyslová stáž v rámci společného výzkumného projektu soukromé společnosti a domácí laboratoře – transfer metod a technologií.

GRANT (HLAVNÍ ŘEŠITEL), PRŮMYSLOVÉ STIPENDIUM

2014 – 2015 **Metagenome of microbial consortia: a source of molecular evolution in vitro**
Agentura: Grantová agentura Univerzity Karlovy (GAUK), ČR
Náplň: Studium bakteriálních konsorcií v půdě zatížené odpadem z produkce beta-laktamových antibiotik a studium jejich biotechnologického potenciálu. Konstrukce chimerních enzymů za využité environmentální DNA.

2012 – 2014 **Průmyslové stipendium**
Společnost: Fermenta Biotech Ltd. (Thane, Indie)
Cíl: Podpora vědeckých aktivit spojených s výzkumným projektem domácí laboratoře a soukromé společnosti. Cestovní a konferenční náklady.

PRACOVNÍ ZKUŠENOSTI

Současné angažmá (04/ 2020 – dosud)

Vědecký koordinátor – Onkogynekologické centrum, Gynekologicko-porodnická klinika 1. lékařské fakulty Univerzity Karlovy a Všeobecné fakultní nemocnice v Praze

Projektový management

03/2019–08/2019 Projektová ředitelka – GHC Research institute s.r.o., Praha, ČR.
Dohled nad projekty: Humánní genetika, veterinární genetika, mikrobiom.

05/2018–03/2019 Vedoucí oddělení veterinární genetiky/ Vědecký pracovník – GHC Research institute s.r.o.

01/2018–04/2018 Vedoucí laboratoře: Laboratoř molekulární genetiky, Česká zemědělská univerzita, ČR.

01/2013–06/2015 Projektový manažer veřejných vzdělávacích kurzů pro Circle Education s.r.o., Praha, ČR.

10/2008–09/2014 Koordinátor logistiky pro www.scio.cz, s.r.o., Praha, ČR.

Akademický výzkum:

2013-2017 Vědecký pracovník, PhD. student – Laboratoř Enzymových Technologií, Mikrobiologický ústav v.v.i., Česká Akademie věd, Praha, ČR; Bioprocess Development section, Zürich University of Applied Sciences, Wädenswil, Švýcarsko. Zaměření: molekulární genetika, mikrobiologie, biotechnologie.

2008-2013 Laborant/ Vědecký pracovník – Laboratoř environmentální biotechnologie, Mikrobiologický ústav v.v.i., Česká Akademie věd, Praha, ČR. Zaměření: molekulární biologie, ekologie.

Výzkum v soukromé sféře: 2016 Vědecký asistent v Austrian Centre of Industrial Biotechnology GmbH, Štýrský Hradec, Rakousko. Zaměření: molekulární biologie, biotechnologie. Krátkodobé zaměstnání na projektu.

Vedení bakalářských a magisterských studentů: Molekulární biologie/ Genetika a Bioprocess technology (studenti z Utrecht University, Nizozemsko; Univerzita Karlova v Praze, ČR; Zürich University of Applied Sciences, Švýcarsko; Technical University Graz, Rakousko).

Členka organizačního týmu: od 2019, projekt „Enviromeetup" – pravidelné přednášky pro veřejnost na ekologická, environmentální a biotechnologická témata. Paralelní Polis, Praha, ČR (z důvodu pandemie SARS-CoV-2 dočasně pozastaveno).

PUBLIKACE

- Borčinová, M., Pitkina, A., Marešová, H., Štěpánek, V., Palyzová, A., Kyslík, P. (2020): Characteristics of microbial community of soil subjected to industrial production of antibiotics. Folia Microbiologica 65:1061–1072.
- Borcinova, M., Raschmanova, H., Zamora, I., Looser, V., Maresova, H., Hirsch, S., Kyslik, P., Kovar, K. (2020) Production and secretion dynamics of prokaryotic Penicillin G acylase in *Pichia pastoris*. Applied Microbiology and Biotechnology 104: 5787-5800.
- Raschmanová, H., Zamora, I., Borčinová, M., Meier,P., Weninger, A., Mächler, D., Glieder, A., Melzoch, K., Knejzlík, Z., Kovar, K. (2019): Single-Cell Approach to Monitor the Unfolded Protein Response During Biotechnological Processes With *Pichia pastoris.* Frontiers in Microbiology 10:335.
- Zahradník, J., Plačková, M., Palyzová, A., Marešová, H., Kyslíková, E., Kyslík, P. (2017): Draft Genome Sequence of *Pantoea agglomerans* JM1, a Strain Isolated from Soil Polluted by Industrial Production of Beta-Lactam Antibiotics That Exhibits Valacyclovir-Like Hydrolase Activity. Genome announcements 5(38): e00921-17.
- Marešová, H., Palyzová, A., Plačková, M., Grulich, M., Vyasarayani R.W., Štěpánek, V., Kyslíková, E., Kyslík, P. (2017): Potential of Pichia pastoris for the production of industrial penicillin G acylase. Folia Microbiologica 62 (5): 417–424.
- Marešová, H., Plačková, M., Grulich, M., Kyslík, P. (2014): Current state and perspectives of penicillin G acylase-based biocatalyses. Applied Microbiology and Biotechnology 98(7):2867-79.
- Bečka, S., Štěpánek, V., Vyasarayani, R.W., Grulich, M., Maršálek, J., Plhačková, K., Dobišová, M., Marešová, H., Plačková, M., Valešová, R., Palyzová, A., Datla, A., Ashar, T.K., Kyslík P. (2013): Penicillin G acylase from *Achromobacter* sp. CCM 4824. Applied Microbiology and Biotechnology 98(3): 1195–1203.
- Plačková, M., Svobodová, K., Cajthaml, T. (2012): Laccase activity profiling and gene expression in PCBs degrading cultures of *Trametes versicolor*. International Biodeterioration & Biodegradation 71: 22 – 28.
- Svobodová, K., Plačková, M., Novotná, V., Cajthaml, T. (2009): Estrogenic and androgenic activity of PCBs, their chlorinated metabolites and other endocrine disruptors estimated with two *in vitro* yeast assays. Science of the Total Environment 407 (22): 5921 – 5925.

Další publikace (bez IF):

- Plačková, M., Krainer, F., Kyslik, P., Glieder, A.: Effect of multivariate engineering and co-expression of helper factors on an efficient production of penicillin G acylase in Pichia pastoris. Koncepční studie pro podporu grantové žádosti H2020.
- Plačková, M. and Fitze, M. (2015): PACE: *Pichia pastoris* as an innovative platform for Penicillin G acylase production. Report zpracovaný jako „new business opportunity", NBO course, Zürich University of Applied Sciences, Švýcarsko.

KONFERENCE

- Raschmanová, H., Meier, P., Borčinová, M., Looser, V., Neutsch, L., Melzoch, K., Knejzlík, Z., Kovar, K. (2017): Method for monitoring the unfolded protein response (UPR) in Pichia pastoris overproducing different recombinant proteins. BioTech 2017, Wädenswil, Switzerland
- Plačková, M. (2016): Position of junior scientists in the Czech Republic and abroad: No pain no Gain? Conference KRECon 2016, Praha (ČR). Pozvaný řečník.
- Plačková, M., Pitkina, A., Marešová, H., Kyslík, P. (2015): Influence of the decades-lasting industrial production of β-lactams on the soil microbiome. Fulltext on the conference 6th Congress of European Microbiologists (FEMS 2015) June 7-11, 2015. Maastricht, Netherlands.
- Plačková, M., Marešová, H., Brühlman, B., Raschmanová, H., Knejzlík, Z., Kyslík, P., Kovar, K.: Production of the penicillin G acylase in *Pichia pastoris*. Fulltext on the conference 8th Conference on Recombinant Protein Production April 22-24, 2015. Palma, Mallorca.

KURZY S MEZINÁRODNÍ ÚČASTÍ, ČLENSTVÍ V ORGANIZACÍCH, JAZYKOVÉ SCHOPNOSTI

Jazyky:	Anglický jazyk (plynně slovem i písmem, FCE certifikát), Francouzský jazyk (mírně pokročilý), Německý jazyk (začátečník)
2020	Kurz programovacího jazyka Java
2012	Kurz vědeckého psaní, Univerzita Karlova v Praze, ČR
2011 – dosud	Členství v Československé společnosti mikrobiologické.
Další:	Řidičský průkaz A, B

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Detailní rozpis publikací týkajících se disertační práce

Paper I

Characteristics of microbial community of soil subjected to industrial production of antibiotics.

Borčinová, M.; Pitkina, A.; Marešová, H.; Štěpánek, V.; Palyzová, A.; Kyslík, P. Folia Microbiologica 2020, 65, 1061–1072, doi: 10.1007/s12223-020-00819-z.

IF_{2019} 1.730

In this study, we focused on characterization of microbial consortia exposed to long-term selection pressure caused by penicillin G production with the aim of examining the diversity and composition of these communities and exploring their biotechnological potential.

For the analyses, the soil from the area of pharmaceutical plant Biotika, a.s. (Slovenská Ľupča, Slovakia), which has been producing penicillin G since 1956, was sampled. For control purposes we also sampled the soil from the same geographical area outside the mentioned plant. Metagenomic DNA from both on-site and control samples was used to analyse and compare the composition of the respective microbial communities by analysing V4 hypervariable region of 16S rRNA gene by using Illumina MiSeq platform.

Consequently, metagenomic DNA from the on-site samples was also used for creation of *E. coli* T1R-based fosmid library. The aim was to unravel the biotechnological potential of the communities in terms of enzymes involved in biotransformation of beta-lactam antibiotics, i.e. penicillin G acylase and alpha amino acid ester hydrolase.

This study offers new insights into the changes in microbial communities of soils exposed to anthropogenic activity and indicates that those soils may represent a hotspot for biotechnologically interesting targets.

Author statement: Contribution of the author: 70%. For this publication, I was involved in all of the steps of the publication preparation. I conceptualized the study and collected the respective samples. Subsequently, I planned the experimental part of the study and worked on the sequencing analyses of the samples. I was also responsible for the statistical analyses of the data and for the interpretation of the results. I also drafted the manuscript.

Paper II

Draft genome sequence of *Pantoea agglomerans* JM1, a strain isolated from soil polluted by industrial production of beta-lactam antibiotics that exhibits valacyclovir-like hydrolase activity.

Zahradník, J.; Plačková, M.; Palyzová, A.; Marešová, H.; Kyslíková, E.; Kyslík, P. Genome Announcements 2017, 5, e00921-00917, doi:10.1128/genomeA.00921-17.

IF_{2018} 0.89

In this study, we were screening cultivable organisms from the on-site soil samples within the area of pharmaceutical plant Biotika, a.s. (Slovenská Ľupča, Slovakia) for the presence of microorganisms exhibiting PGA- or AEH- like activity.

Out of the screened isolates, one was weakly positive for AEH activity. Genome of this strain was sequenced, assembled and described. Genes with a predicted PGA- or AEH-like activity were identified in the genome of this strain and were cloned and expressed in *E. coli* BL21. Using this approach, we discovered a new protein with alpha/beta hydrolase fold that was remotely homologous to human valacyclovirase gene (member of AEH-enzyme family).

The study brought new information on genes encoding novel enzymes with industrial potential and further supported the theory that microbial consortia from soils polluted by antibiotics are a potent source of microorganisms with industrially usable characteristics.

Author statement: Contribution of the author: 30%. For this publication I was responsible for the selection of the strain. I performed the cultivation experiments and screened for the strains in possession of the desired characteristics (production of enzyme biotransforming beta-lactam antibiotics; i.e. PGA or AEH). After I selected the strain in question, I also performed pilot PCR gene mining experiments using the method of sequence homology. I was also involved in the manuscript corrections.

Paper III

Current state and perspectives of penicillin G acylase-based biocatalyses.

Maresova, H.; Plackova, M.; Grulich, M.; Kyslik, P. Applied Microbiology and Biotechnology 2014, 98, 2867-2879, doi: 10.1007/s00253-013-5492-7.

IF_{2019} 3.530

This publication is a review article focused on the enzyme Penicillin G acylase (PGA) and its potential for the industrial biocatalyses. In the course of more than 60-year history, PGA has gained a unique position among the enzymes used in bioprocesses and for biotransformation of beta-lactam antibiotics, especially in the production of beta-lactam nuclei from penicillin G and glutaryl-7-aminocephalosporanic acid. A portfolio of other PGA traits required for enzymes with high industrial potential was summarized and discussed in terms of the current industrial utilization of these traits and their potential for other biotechnological applications. PGA was also compared with enzymes competing with PGA in the syntheses of semisynthetic beta-lactam antibiotics (alpha amino acid ester hydrolases, penicillin V acylases, and cephalosporin acylases).

The performed review of literature showed that PGA has a great potential to go beyond the beta-lactam biocatalyses and has the potential to be used in synthetic reactions, in the production of achiral and chiral compounds, or in the pro-drug activation. On the other hand, our review also revealed that even though a great number of PGAs of different origins has been described, only a limited number of production strains was at the time available for industrial-scale production of PGA; all of them based on prokaryotic host, namely *E. coli*.

Author statement: Contribution of the author: 30%. For this publication, I participated in the literature review and in writing the manuscript, namely I drafted the section concerning the description of the enzymes competing with PGA for syntheses of semisynthetic beta-lactam antibiotics. I was also involved in the manuscript corrections.

Paper IV

Potential of *Pichia pastoris* for the production of industrial penicillin G acylase.

Maresova, H.; Palyzova, A.; Plackova, M.; Grulich, M.; Rajasekar, V.W.; Stepanek, V.; Kyslikova, E.; Kyslik, P. Folia Microbiologica 2017, 62, 417-424, doi:10.1007/s12223-017-0512-0.

IF_{2019} 1.730

In this study we focused on construction and characterization of two *P. pastoris* based production systems for intracellular and extracellular production of PGA from *Achromobacter* sp. CCM 4824. Prokaryotic *pga* gene was codon optimized for the use in the yeast host and was cloned into the commercial vectors p*PICZ* and p*PICZα* for intracellular and extracellular production, respectively.

P. pastoris X33 was consequently transformed with the prepared plasmids and the created transformants were screened for those with the best PGA-production performance and characterized. A set of fed-batch 6 L stirred bioreactor cultivations with the prepared strains was consequently performed using the in-study optimized media.

The performed bioreactor fed-batch cultivations revealed that the strain producing PGA intracellularly yielded a comparable amount of enzyme as industrially established *E. coli* production systems. On the contrary, equivalent bioreactor cultivation with the strain constructed for extracellular production of PGA revealed secretory bottleneck of the production strain, whereby only approx. 40% of the produced enzyme was secreted into the culture supernatant while the majority was retained intracellularly.

This study laid, for the first time, the basis for extracellular PGA production in *P. pastoris*. Even though the potential of *P. pastoris* as a production host for PGA was established in principle, the secretory bottleneck needed to be addressed in further studies.

Author statement: Contribution of the author: 20%. During the experimental part of this study, I was working on the bioreactor cultivations and I was performing the enzymatic assays. I was also involved in the data analyses and in the manuscript preparation and corrections.

Paper V

Production and secretion dynamics of prokaryotic Penicillin G acylase in *Pichia pastoris*.

Borcinova, M.; Raschmanova, H.; Zamora, I.; Looser, V.; Maresova, H.; Hirsch, S.; Kyslik, P.; Kovar, K. Applied Microbiology and Biotechnology 2020, 104, 5787-5800, doi:10.1007/s00253-020-10669-x.

IF_{2019} 3.530

In this study we continued with the development of the *P. pastoris* production system for extracellular production of PGA. We performed a detailed study dealing with the optimization of the production process as well as with the quantification of the time-dependent specific rate of PGA secretion and its interdependence with intracellularly retained PGA and biomass growth.

The strain producing PGA extracellularly (developed in the Paper IV, Maresova *et al.*, 2017) was cultivated in a series of 6 L stirred bioreactor fed-batch cultivations. Those cultivations were performed at different specific growth rates, which were maintained by exponentially increasing the feeding of methanol. Detailed analyses of the production process as well as of cells and their analysts were performed, including substrate analyses, protein analyses, and cell viability and lysis analyses. In order to study the evolution of specific productivity of the system over the course of cultivation in great detail, a descriptive mathematical model was developed. This advanced data interpolation and fitting tool allowed us to describe dynamic changes in specific productivity (q_p) and specific rate of product secretion ($q_{p,extra}$) in considerable depth.

The key achievement of the study is a description of the temporal change in the rate of specific product formation during the production phase of *P. pastoris* fed-batch cultivation, when producing PGA under the control of the p*AOX1* promoter. We also showed that the stress caused by heterologous PGA production induced cellular imbalance leading to the selective translational arrest as a response to the oversaturation of the secretory pathway.
The study represents a significant contribution to understanding the dynamic changes in q_p over time and may generate opportunities for expanding the biotechnological application potential of the *Pichia-pAOX1* system for difficult-to-produce products.

Author statement: Contribution of the author: 80%. For this publication, I was involved in all of the steps of the publication preparation. I conceptualized the study, and I planned the experimental part of the study. I performed all of the bioreactor cultivations, including sample analyses and interpretation. I was also responsible for the analyses of the resulting data and for writing the manuscript.

Paper VI

Single-cell approach to monitor the unfolded protein response during biotechnological processes with *Pichia pastoris*

Raschmanova, H.; Zamora, I.; Borcinova, M.; Meier, P.; Weninger, A.; Machler, D.; Glieder, A.; Melzoch, K.; Knejzlik, Z.; Kovar, K. Frontiers in Microbiology 2019, 10, 335, doi:10.3389/fmicb.2019.00335.

IF_{2019} 4.235

In this study, production and secretion of three different recombinant proteins (PGA from *E. coli*, lipase B from *Candida Antarctica*, and xylanase A from *Thermomyces lanuginosus*) by *P. pastoris* was investigated along with up-regulation of the unfolded protein response pathway (UPR) and cell viability, which were assessed at a single-cell level in living cells and at-line with flow cytometry.

For the purpose of the study, a new strain carrying PGA production cassette as well as UPR-reporter cassette was constructed (analogously for lipase B and xylanase A). The constructed strain was cultivated in 6 L stirred bioreactor fed-batch cultivation and intracellular and extracellular product concentrations were measured along with measurements of the cell viability and the upregulation of UPR using flow-cytometry. Subsequently, the cells´ viability and the levels of UPR up-regulation were put in the context of production patterns.

The resulting data brought novel insight into the development of heterogeneity in a recombinant *P. pastoris* population during a biotechnological process. They also provided us with important information about UPR upregulation in context of the dynamic changes of specific productivity of PGA-*P. pastoris* system described in the previous study (Paper V, Borcinova *et al.*, 2020).

This study represents a first trial in which UPR up-regulation was studied at a single-cell level and in a non-invasive manner. By understanding the relationship between the protein production/secretion and the tuning of the UPR, this monitoring system based on fluorescence measurement can be utilized in future bioprocess control and optimizations.

Author statement: Contribution of the author: 30%. For this publication, I was involved in the PGA production strain design and preparation. I performed the respective bioreactor cultivations with the created strain and I was involved in sample analyses. I was also involved in the manuscript preparation and corrections.

Pilot study VII

Effect of multivariate engineering and co-expression of helper factors on an efficient production of penicillin G acylase in *Pichia pastoris*

Borčinová, M.; Krainer, F.; Kyslík, P.; Glieder, A. Concept study performed for the European Research Council H2020 grant application.

This concept study dealt with the development and studying of various *P. pastoris* strains for PGA production. By employing an integrated process of identifying essential properties of the production strains, the aim was to acquire an in-depth view of the bottlenecks and limitations of PGA production by *P. pastoris*.

Gene codon usage, promoter choice and regulation, the type of transport to endoplasmic reticulum, the effect of increasing gene dosage, and finally the influence of overproduction of the proteins involved in the protein maturation and secretion were analysed. Overall, 40 different constructs were prepared during the course of the study. In a series of cultivation experiments performed by the high throughput method in 96-deep well plates, the uncoupled effect of the mentioned studied parameters was quantified, and putative activity landscapes were generated.

This study represents a pilot analysis of the rate limiting steps in the maturation and secretion of PGA in *P. pastoris* that evidences the potential of this host for PGA production. The obtained knowledge should give base for the development of a tailored strain capable of efficient production and secretion of PGA.

Author statement: Contribution of the author: 80%. For this study, I was involved in all of the steps of the study preparation. I conceptualized the study and planned the experimental part of the study. I constructed all of the studied strains and performed all of the cultivations, including sample analyses and interpretation. I was also responsible for the analyses of the resulting data.

V Praze dne 14.12.2020
Martina Borčinová