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Genome, transcriptome and fermentation analyses based strain improvement to enhance primycin biosynthesis efficiency

PhD Thesis

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INTRODUCTION

Nowadays, the rapid and accelerating spread of resistant bacteria poses serious challenges to health professionals worldwide. Antimicrobial resistance (AMR) is constantly gaining ground due to excessive and unjustified use of antibiotics, which jeopardizes effective action against infections caused by pathogenic microorganisms responsible for a significant proportion of morbidity and mortality [1]. Due to the continuous spread of antimicrobial resistance, the efficacy of antimicrobial drugs is gradually declining, placing a heavy burden on patient care. The World Health Organization has been trying to raise awareness of this problem for years. According to their latest report (2019) AMR related infections already cause at least 700,000 deaths globally every year, including 230,000 deaths from tuberculosis caused by multidrug-resistant *Mycobacterium tuberculosis*. It is estimated that the number of deaths could rise to 10 million by 2050 without taking appropriate actions [2].

During the process of developing multidrug resistance, microorganisms are becoming resistant to several antimicrobial agents with various structures or mechanisms of action to which they were previously sensitive [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) considered as one of the best-known multidrug-resistant (MDR) pathogen responsible for hospital-acquired infections. In Hungarian hospitals it has one of the largest case number of multidrug-resistant infections with nearly 900 registered infections per year. The key to its success is that beside methicillin it can acquire resistance against several commonly used antibiotic groups such as aminoglycosides, macrolides, fluoroquinolones, chloramphenicol and tetracycline.

The severity of the situation is further escalated by the fact that the modern age of antibiotics, beginning with the discovery of penicillin, entered a serious crisis by the end of the century. According to the latest trends in pharmaceutical industry, manufacturers have gradually turned to high-throughput screening of synthetic compound libraries, which has significantly set back the rate of discovery of new antibiotics. The fact that development of AMR is constantly increasing, while the intensity of researches to find new antimicrobial agents is decreasing, urges changes in pharmaceutical industry.

Deepening knowledge about long-existing antibiotics, or reintroducing previously used neglected antibiotics may serves as a new alternative in the fight against MDR pathogens [4]. The first Hungarian antibiotic primycin, discovered almost 70 years ago, is perfectly fit into this concept [5]. Primycin is a non-polyene macrolide lactone exclusively produced by *Saccharomonospora azurea* that possesses high antimicrobial activity against frequent Gram-

positive pathogens, including clinically prevalent multidrug-resistant strains. Due to this property and its bactericidal activity against non-proliferating bacteria, primycin is able to compete effectively with a wide range of bacterial pathogens [6]. Although for topical application it has been used successfully in Hungary for decades, formulation difficulties have not yet allowed its widespread use in clinical practice. Nevertheless, its use, due to its favorable antimicrobial properties, has significant therapeutic potential. Expanding our knowledge regarding the primycin-producing organism and the process of antibiotic synthesis could greatly facilitate further potential product development related to primycin.

Even though a number of initiatives have been launched to reinvigorate the antibiotic research and development pipeline, an application of genome mining approach coupled with transcriptomic analysis and analytical methods is not yet common at industry level. Beside increasing the efficiency of primycin fermentation processes, on the basis of these innovative technologies we also aimed to gain deeper insight into primycin biosynthesis processes via comparative structural and functional analysis of the primycin producing *S. azurea* strains. These researches became possible through a joint research project implemented with the involvement of the University of Pécs and PannonPharma Ltd.

AIMS

According to the principles stated above we have addressed the following goals during our work:

- Revealing primycin producing ability among the members of the *Saccharomonospora* genus.
- Determining antibiotic producing capacity of primycin-producing strains using classical microbiological and analytical methods.
- Increasing the efficiency of the primycin fermentation process by conventional fermentation optimization methods.
    - Investigating the effect of various fatty acid substrates on fermentation yield.
- *In silico* structural characterization of the primycin biosynthetic gene cluster:
    - Identification of type I primycin PKS core biosynthetic genes, precursor genes and additional regulatory genes involving in primycin biosynthesis.
    - Characterization of module and domain organization of the primycin PKS.
    - Structural analysis of catalytic domains responsible for multienzyme substrate specificity.
- Comparative functional analysis of *S. azurea* SZMC 14600 and *S. azurea* DSM 44631 strains:
    - Analysis of RNA-seq data by bioinformatics pipelines.
    - Functional annotation and classification of differentially expressed genes (DEGs) via GO (Gene Ontology) and COG (Clusters of Orthologous Groups) systems.
    - Validation of RNA-seq data by RT-qPCR

MATERIALS AND METHODS

Bacterial strains and culture conditions

Antibiotic production of primycin-producing strains (*Amycolatopsis orientalis* DSM 40040, *Kibdelosporangium aridum* DSM 43828, *Saccharomonospora azurea* SZMC 14600, and *Saccharomonospora azurea* DSM 44631) was achieved by a two-step fermentation process. First bacterial cell suspension (stored at -80°C) was inoculated into seed medium (EI), then the seed culture was transferred into fermentation medium (EF) and incubated under primycin fermentation conditions.

Antimicrobial assay

Antimicrobial activity of n-butanol-ethanol-distilled water 1:1:2 (v/v) extracts of 5 day fermented cells were determined by agar well diffusion assay [7]. In the bioassay 24 h old culture of *Bacillus subtilis* ATCC 6633 test strain was used as indicator. As a reference, standard crystallized primycin-sulphate stock solution was applied. Antibacterial activity was determined by the size of the inhibition zones. Primycin concentrations of extracts were calculated according to the calibration curve fitted trend line equation.

High performance liquid chromatography

Determination of primycin concentrations in fermentation extracts was carried out by high performance liquid chromatography (HPLC) with Diode array detection (DAD) and electrospray-mass spectrometry (ESI–MS). As a reference, standard crystallized primycin-sulphate stock solution was applied. Identification of primycin components was performed based on their mass-to-charge (m/z) ratio. All samples were prepared in three independent biological replicates and measured in technical triplicates. Statistical analysis was completed using two-way analyses of variance (ANOVA) in GraphPad Prism software.

DNA–DNA hybridization

In silico DNA-DNA hybridization (DDH) values among *Saccharomonospora* species were calculated by using the Genome-To-Genome Distance Calculator (GGDC) web server (https ://ggdc.dsmz.de). Distance values were determined by the recommended Formula 2 for incomplete draft genomes [8]. Records of the annotated genomes are displayed by the following accession numbers in GeneBank: *S. azurea* SZMC 14600 - AHBX01000000; *S. azurea* DSM 44631 - AGIU02000000; *S. amisosensis* DSM 45685 - JAAOYM000000000; *S. cyanea* DSM 44103 - AHLY00000000; *S. glauca* DSM 43769 - AGJI00000000; *S. halophila* DSM 44411 -

AICX00000000; *S. marina* DSM 45390 - AHLX01000000; *S. paurometabolica* DSM 44619 - AGIT02000000; *S. piscinae* 06168H-1 - VCEK01000000; *S. saliphila* DSM 45087 - AICY01000000; *S. viridis* DSM43017 - ABUM01000000; *S. xinjiangensis* DSM 44391 - AICV00000000.

Primycin biosynthetic gene cluster

The primycin biosynthetic gene cluster was identified and analyzed by antiSMASH (Antibiotics & Secondary Metabolite Analysis Shell) [9] software. Database searches for homologous biosynthetic genes and proteins were performed using the National Center for Biotechnology Information (NCBI) BLAST server [10].

Primycin PKS gene cluster identification and structural analysis

The primycin type I PKS gene cluster was identified and analyzed by antiSMASH and SBSPKS (Structure Based Sequence Analysis of Polyketide Synthases) [11] softwares, while multiple sequences alignment were performed by CLUSTAL W [12]. Amino acid sequences of primycin PKS domains were compared to amphotericin, azalomycin, erythromycin, rapamycin, stambomycin and thailandin PKS domains. Database searches for homologous proteins in NCBI's non-redundant protein sequence database resulted high level identity with *K. aridum* and *A. orientalis* species, therefore primycin type I PKS gene cluster was identified in those species as well. Records of the analyzed genomes are displayed by the following accession numbers in GeneBank: *K. aridum* DSM 43828 - FWXV00000000; *K. aridum* A82846 - QHKI00000000; *K. aridum subsp. largum* NRRL B-24462 - JNYM01000003; *A. orientalis* B-37 - JXRD01000000; *A. orientalis* DSM 40040 - ASJB00000000.

Transcriptomic analysis

Total RNA was extracted from fermentation medium after five days. RNA quantity was measured by Qubit 2.0 Fluorometer (Thermo Fisher Scientific), and quality was determined by Agilent Bioanalyzer 2100 instrument (Agilent RNA 6000 Nano reagent kit). High quality total RNA samples (RIN > 8.5) from pooled biological replicates were processed using the SOLiD total RNA-Seq Kit (Thermo Fisher Scientific) according to the manufacturer's recommendation.

Bioinformatic analysis

RNA-Seq data was analyzed using Galaxy's open source, web-based platform (https ://usega laxy.org) [13]. Reads of the whole transcriptome of *S. azurea* SZMC 14600 and *S. azurea* DSM

44631 were aligned to S. azurea SZMC 14600 genome using Bowtie2 alignment protocol. Transcriptome assembly and differential expression analysis were performed according to the Cufflinks RNASeq workflow [14]. DEGs represented at least twofold change (> 2 or < − 2 in log2) were functionally annotated using Blast2GO version 5.2.4. software [15]. To determine GO terms, functional annotation of each transcript was performed against the non-redundant (nr) protein database compiled by National Center for Biotechnology Information (NCBI) using BLASTx with 10^{e-3} e-value threshold. The set of DEGs were classified into COG categories based on the Joint Genome Institute (JGI) Integrated Microbial Genomes & Microbiomes (IGM/M) system [16, 17].

Real-time quantitative PCR analysis

Validation of RNA-Seq results using real-time quantitative PCR (RT-qPCR) was performed with the following set of genes: EHK80158.1 - ABC transporter ATP-binding protein, EHK80159.1 - ABC-transporter transmembrane protein, EHK80176.1 - Two-component histidine kinase, EHK80172.1 - Agmatinase, EHK88153.1 - TetR/AcrR family transcriptional regulator, EHK84821.1 - 3-oxoacyl-(acyl-carrier-protein) synthase III and EHK87608.1 - 3-oxoacyl-(acyl-carrier-protein) synthase encoding genes. The expression levels were measured by ABI Prism 7900 Sequence Detection System (Applied Biosystems). Total RNA obtained from three independent fermentation processes were isolated using Quick-RNA MiniPrep Kit (Zymo Research), and quantified by Qubit 2.0 Fluorometer (Thermo Fisher Scientific) using Qubit™ RNA BR Assay Kit (Thermo Fisher Scientific). Reverse transcription was performed by RevertAid Reverse Transcriptase (Thermo Fisher Scientific) applying 1 μg of total RNA according to the manufacturer's instructions. The PCR reactions were performed in the presence of SYBR Green/ROX fluorescence chemistry (Thermo Fisher Scientific) in 25 μL final volume. The relative gene expression was determined by using the ΔΔCt method, relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an endogenous control. Each biological sample was measured in at least three technical replicates. Gene expression correlation between RT-qPCR and RNA-seq data was determined by Pearson correlation coefficient.

RESULTS

Primycin producing ability

In our work, we examined the presence of primycin producing ability via comparative analysis of *Saccharomonospora* species. Although previous works of the Department of General and Environmental Microbiology have shown that *S. cyanea*, *S. glauca*, *S. xinjiangensis* and *S. viridis* species closely related to *S. azurea* were able to grow under primycin fermentation conditions, in their case antibiotic production was not detectable by either agar diffusion bioassay or high-performance liquid chromatography. As part of a comprehensive analysis of *Saccharomonospor*a genus genome sequencing data, the performed *in silico* DNA-DNA hybridization analysis demonstrates the unique role of *S. azurea* within the genus which shows low similarity at genome level with the other species. More detailed analysis of the genome sequencing data revealed that only *S. azurea* possess primycin biosynthesis gene cluster within the genus, however, within the *Pseudonocardiaceae* family we identify further species (*Amycolatopsis orientalis* and *Kibdelosporangium aridum* species) which also have the gene set required for primycin production. For the latter the presence of structural genes responsible for primycin biosynthesis is not associated with active antibiotic synthesis under primycin fermentation conditions.

Although all known *S. azurea* strains is capable of primycin production, significant differences could be observed in yields. In the comparison of *S. azurea* DSM 44631 and *S. azurea* SZMC 14600 strains these differences can reach up to 10x quantity.

The effects of fatty acid substrates on primycin biosynthesis

In order to increase the efficiency of primycin fermentation, we investigated the effect of different fatty acid substrates on primycin production. According to our HPLC/ESI-MS measurements the changing of stearic acid (3 g/L) to palmitic acid (3 g/L) in primycin fermentation medium results significantly higher antibiotic yields. Beside the directly added stearic acid, sunflower oil (1%-7% stearic acid and 4%-9% palmitic acid) is also present in the primycin fermentation media, which also contributes to the elevated yields. We proved that the omission of sunflower oil reduces primycin production.

To answer the question how different quality fatty acid substrates alters primycin yield, a comprehensive analysis of several common fatty acids was performed. According to the obtained results long-chain fatty acids (palmitic acid, stearic acid, lauric acid) proved to be most effective in increasing primycin production, while yields induced by short-chain fatty acids (caproic acid, butyric acid) were significantly lower (<450 mg/L). In contrast, the use of

medium-chain fatty acids (capric acid, enanthic acid) had an antibacterial effect on the producer strain, in their case no primycin production could be detected.

A concentration-dependent comparison of the highest yield inducer palmitic acid, stearic acid and lauric acid revealed that palmitic acid was the most effective component at each of the tested fatty acid concentrations (3 g/L, 4.5 g/L and 6 g/L) regardless of fermentation period. In case of all three fatty acids the highest primycin concentrations could be measured at 4.5 g/L fatty acid concentration (palmitic acid: 2177.19 mg/L, stearic acid: 1388.87 mg/L, lauric acid: 1076 .70 mg/L). Although regardless of the applied fatty acid, 4.5 g/L was the most favorable concentration for primycin production, no significant difference could be observed compared to 3 g/L and 6 g/L concentrations.

Identification of deguanidinoamino-A1 and A1 primycin components by HPLC/ESI-MS

In parallel with the determination of primycin concentrations by HPLC/ESI-MS, the presence of deguanidininoamino-A1 and A1 primycin components, which indicates agmatinase enzyme activity were examined in fermentation extracts. Based on the detected m/z values, we were able to identify deguanidininoamino-A1 and A1 (guanidino-A1) primycin molecules in case of both primycin-producing *S. azurea* strains. According to our findings, deguanidininoamino-A1 primycin accounts for less than 10% of the total amount of primycin A1 (deguanidinoamino-A1 + A1).

Characterization of the primycin biosynthesis gene cluster

During the revision of the *S. azurea* SZMC 14600 draft genome data [19] we could identify the complete primycin biosynthetic gene cluster, which includes type I primycin PKS core biosynthetic genes, as well as precursor and additional regulatory genes essential for the biosynthesis of the biologically active primycin molecule. Database searches for homologous proteins related to primycin biosynthesis in NCBI's non-redundant protein sequence database resulted high level similarity with *K. aridum* and *A. orientalis* species.

Primycin PKS gene cluster identification and structural analysis

Due to the systematic revision of the *S. azurea* SZMC 14600 genome sequencing data, we could determine the detailed structure of the type I primycin PKS multienzyme. As the homology search of the primycin biosynthesis gene cluster resulted high degree of similarity with the biosynthetic genes of *K. aridum* and *A. orientalis* species, we performed comprehensive analysis of type I PKS core biosynthetic genes within these species. Accordingly, we could

identify complete 18-module primycin PKS gene cluster in case of *K. aridum* DSM 43828, *K. aridum* A82846 and *K. aridum* subsp. *largum* NRRL B-24462, and in case of *A. orientalis* B-37 és *A. orientalis* DSM 40040 strains.

Determining substrate specificity of AT domains

In case of primycin PKS, the amino acid sequence alignment of ATs revealed 12 malonyl-CoA specific AT domains having typical GHSx[LVIFAM]G and HAFH motifs. In case of 5 modules methylmalonyl-CoA substrate specific motifs GHSx[QMI]G and YASH were observed. Unlike these unusual butlymalonyl-CoA, pentlymalonyl-CoA or hexylmalonyl-CoA substrate specificity could be observed in module 18 with GHSQG and GAGH conserved sequence motifs.

Characterization of KR domain

According to amino acid sequence alignments, a total of 7 A1-type (2R, 3S) KR domains were identified based on conserved tryptophan (W) residue at position 141, while 10 B1-type (2R, 3R) and 1 type B2 (2S, 3R) KR domain were identified based on leucine and two aspartic acids (LDD) sequence motif (positions 94-96) typical to type B KR.

L-arginine precursor pathway

The 4-guanidinobutanoyl-COA starter unit of the primycin biosynthesis derived from the three-step L-arginine precursor pathway**.** During this synthesis process first 4-guanidinobutyramide, then 4-guanidinobutanoic acid and finally 4-guanidinobutanoyl-CoA is formed from the starting L-arginine molecule. In case of *S. azurea* SZMC 14600 the L-arginine precursor pathway is catalyzed by amine oxidase (EHK88410.1), amidohydrolase (EHK88411.1) and acyl-CoA ligase (EHK88415.1) enzymes. The activated 4-guanidinobutanoyl group is transferred to the loading ACP domain, performed by ACP S-malonyltransferase (EHK80162.1).

Additional genes involved in primycin biosynthesis

Beside the type I primycin PKS core biosynthetic genes and L-arginine precursor pathway related genes 16 additional regulatory genes involving in primycin biosynthesis have been identified. These genes are sharing the highest level of sequence similarity with *K. aridum* biosynthetic genes.

Functional genomics

To gained more insight into difference at gene expression level between high- and low-primycin producer *S. azurea* strains RNA-Seq has been applied. Following the Cufflinks RNA-Seq pipeline, transcriptomic analysis revealed 330 and 356 DEGs in a set of *S. azurea* SZMC 14600 vs. *S. azurea* DSM 44631 respectively at fold change cutoff of 2 in $\log_2$ scale. In case of high-primycin producer *S. azurea* SZMC 14600 strain, out of the 330 identified DEGs 253 were functionally annotated and classified into the three major GO categories, while in case of low-primycin producer strains, out of the 356 identified DEGs, 276 were functionally annotated and classified.

The possible function of DEGs were also predicted and classified by aligning to the COG database. COG assignment resulted 294 and 313 DEGs in comparison of high- and low-primycin producer respectively, corresponding to 22 specific categories.

Among genes potentially related to primycin biosynthesis two ABC multidrug transporter encoding genes EHK80158.1 and EHK80159.1 located adjacent to PKS gene cluster that are linked to defense mechanisms (V) via COG were overexpressed in S. azurea SZMC 14600 with 8.0 and 3.8 log2 fold-change respectively. Similarly, TetR family transcriptional regulators (TFRs), involved in control of a variety of processes such as antibiotic production, efflux pump expression and multidrug and self-resistance were also overexpressed in high-primycin producer strains within a range of 2.08 to 5.23 log2 fold-change.

According to the RNA-Seq analysis, the agmatinase encoding gene belonging to the amino acid transport and metabolism (E) COG category was also up-regulated in the primycin overproducer with 2.62 log2 fold-change. The agmatinase encoding gene (EHK80172.1) is located at the 3′ end of the PKS.

Although comparative analysis of PBGC did not reveal structural differences between *S. azurea* SZMC 14600 and *S. azurea* DSM 44631, receptor histidine kinase (HK) (EHK80176.1) and a cognate response regulator (RR) (EHK80177.1) encoding genes, elements of the two components' signal transduction system (TCS) were significantly up-regulated in high-primycin producer strain. An additional regulatory gene, encoding leucine-responsive regulatory protein (Lrp) was also differentially expressed between the two strains.

It is well known that polyketide and fatty acid (FA) synthesis are evolutionary closely related processes, and the two megasynthase assembly lines use homologous domains and share precursors such as acetyl- and malonyl- CoA. The expression profile of genes encoding 3-oxoacyl-(acyl-carrier-protein) synthases (EHK89245.1, EHK87608.1 and EHK84821.1) that

play key roles regulating the product distribution of FA synthesis were found to express lower levels in high-primycin producer strain.

qRT-PCR analysis

We validated the RNA-Seq data with RT-qPCR using seven gene potentially related to primycin biosynthesis. RT-qPCR expression analyses were in good agreement with the RNA-Seq data, the results can be characterized by a correlation coefficient of 0.71 ($p < 0.05$). Regarding the $\log_2$ transformed gene expression values two-component histidine kinase, ABC transporter ATP-binding protein, ABC-transporter transmembrane protein, agmatinase and the TetR/AcrR family transcriptional regulator encoding genes shown overexpression in the high-primycin producer *S. azurea* SZMC 14600 strain. In contrast, the fatty acid synthesis related 3-oxoacyl-(acyl-carrier-protein) synthase III and EHK87608.1 - 3-oxoacyl-(acyl-carrier-protein) synthase encoding genes were downregulated compared to the *S. azurea* DSM 44631 strain.

SUMMARY

Emerging and re-emerging microbial pathogens, together with their rapid evolution and adaptation against antibiotics, highlight the importance not only of screening for new antimicrobial agents, but also for deepening knowledge about existing antibiotics. Primycin produced by *Saccharomonospora azurea* exhibit advantageous antimicrobial properties against a wide range of Gram-positive bacteria, which serves it a strong therapeutic potential and makes it an ideal target for these purposes.

In this study, we have presented a comprehensive work based on multidisciplinary approaches e.g. traditional microbiology; analytical chemistry; structural, functional and comparative genomics supported by a wide variety of bioinformatic tools in order to improve industrial scale primycin production and gain insight into primycin biosynthesis by comparative analysis of the primycin producer *S. azurea* strains.

Due to our traditional fermentation optimization efforts it turned out that not only quantity but quality of fatty acid substrate play a pivotal role in the rate of primycin fermentation. The most effective precursor molecule for primycin biosynthetic processes was palmitic acid (C16:0). Regarding fatty acid concentration, 4.5 g/L palmitic acid treatment proved to be the most effective in terms of antibiotic yield enhancement. Our results demonstrated that palmitic acid plays an essential role in primycin production and may be used not only as an alternative component of stearic acid in the fermentation media but may also serve as a standard component of a newly designed and highly effective primycin producing fermentation media.

According to the systematic revision of the *S. azurea* SZMC 14600 genome sequencing data, we could characterized the entire primycin biosynthetic gene cluster. Beside the primycin PKS biosynthetic core genes and genes involved in the L-arginine precursor synthesis pathway, we identified 16 additional genes which can be linked to different stages of the primycin biosynthetic processes. Although *in silico* analysis of the newly assembled and characterized primycin PKS gene cluster for *S. azurea* SZMC 14600 did not reveal significant structural differences between the two primycin-producing strains, we found clear evidence of an unusual acyltransferase domain substrate specificity in the module 18 of the primycin PKS.

Our *in silico* analysis to identify the primycin biosynthesis gene clusters supported our previous knowledge that beside *S. azurea* the other members of the *Saccharomonospora* genus do not have the genetic potential to produce primycin. In contrast sequence similarity searching to identify homologous genes and proteins in the NCBI database revealed complete, 18-module containing primycin PKS gene cluster with the associated modifying and regulatory genes in

K. aridum DSM 43828, *K. aridum* A82846, *K. aridum* subsp. *largum* NRRL B-24462, *A. orientalis* B-37 and *A. orientalis* DSM 40040 strains.

Concerning quantitative differences in primycin producing ability, the performed comparative transcriptomic analysis resulted several DEGs, classified into various GO and COG categories. Among them, genes related to fatty acid synthesis, self-resistance, regulation of secondary metabolism and an agmatinase encoding gene responsible for catalyze conversion between primycin A1 and deguanidino-amino-A1 forms of primycin were discussed. In parallel to the performed transcriptomic analysis, the presence of deguanidino-amino-A1 and A1 primycin components referring to agmatinase enzyme activity was also confirmed by high performance liquid chromatography with diode array detection and electrospray-mass spectrometry for both *S. azurea* strains.

As a result our efforts to investigate the primycin biosynthetic gene cluster and the mechanisms lying behind the regulation of primycin biosynthesis provides clues for antibiotic yield- and strain-improvement as well as laying the foundation for rational drug design.

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Bibliography

I. 1. Articles related to the thesis:

1. **Kovács M**, Seffer D, Pénzes-Hűvös Á, Juhász Á, Kerepesi I, Csepregi K, Kovács-Valasek A, Fekete Cs (2020) Structural and functional comparison of *Saccharomonospora azurea* strains in terms of primycin producing ability. World J Microbiol Biotechnol 36:160 doi.org/10.1007/s11274-020-02935-x IF: 3.312 (2020)

2. **Kovács M**, Sefferné Szalai M, Seffer D, Pallos JP, Drávavölgyi G, Kovács-Valasek A, Kerepesi I (2019) Understanding the role of fatty acid substrates on primycin biosynthesis by Saccharomonospora azurea during batch fermentation. Nat Prod Commun 14:1-6 doi.org/10.1177/1934578X19858210 IF: 0.484 (2019)

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II. 1. Poster and oral presentations related to the thesis:

1. **Kovács M**, Seffer D, Pénzes-Hűvös Á, Juhász Á, Kerepesi I, Csepregi K, Kovács-Valasek A, Fekete Cs (2020): Comparative Transcriptome Analysis of *Saccharomonospora azurea* strains in terms of primycin producing ability. Hungarian Molecular Life Science Conference 2021, 5-7 November 2021, Eger, Hungary
2. **Kovács M**, Sefferné Szalai M, Seffer D, Pallos JP, Drávavölgyi G, Kovács-Valasek A, Kerepesi I (2020): Enhancing of primycin production by *Saccharomonospora azurea* with various fatty acid substrates during batch fermentation. Congressus Pharmaceuticus Hungaricus XVI, 10-12. september 2020, Debrecen, Hungary
3. Valasek A, Kiss ÍÉ, Fodor I , **Kovács M**, Urbán P , Jámbor É, Fekete Cs, Kerepesi I (2016) Proteomic insight into the primycin fermentation process of *Saccharomonospora azurea*. 10th Central and Eastern European Proteomic Conference, 11-14. October 2016, Budapest, Hungary
4. Valasek A, Fodor I, Kiss ÍÉ, **Kovács M**, Tóth Zs, Urbán P, Jámbor É, Márk L, Fekete Cs, Kerepesi I (2015) From genomics to proteomics in the field of antibiotic research. Hungarian Molecular Life Sciences 2015, 27-29 March 2015, Eger, Hungary

II. 2. Other oral and poster presentations:

1. Sass V, Imri Á, **Kovács M**, Csicsek G, Ortmann-Ajkai A, Czakó-Vér K (2014) Talajmikrobiológiai vizsgálatok a Bükkhát Erdőrezervátum lékjeiben. Magyar Talajtani Társaság Vándorgyűlése, Keszthely, 2014. szeptember 4-6.

2. Imri Á, **Kovács M**, Kozma P, Csikász-Krizsics A, Árvay Gy, Czakó-Vér K (2012) A vízellátottság meghatározó szerepe a szőlő mikorrhiza kapcsolat alakulásában. A Magyar Mikrobiológiai Társaság 2012. évi Nagygyűlése, Keszthely, 2012. október 24-26.

3. **Kovács M**, Imri Á, Mátyás Á, Kozma P, Árvay Gy, Czakó-Vér K (2012) Mikorrhiza gyökérkolonizáció vizsgálata Jázmin szőlőfajtánál a termőre-fordulás évében. Talajtani Vándorgyűlés, Miskolc, 2012. augusztus 23-24-25.

4. **Kovács M**, Imri Á, Dudás M, Vincze V, Árvay Gy, Czakó-Vér K (2011) Soil dependent efficiency of plant-microbe (wheat-mycorrhiza) interaction at increasing doses of nitrogen fertilizer. 16th International Congress of the Hungarian Society for Microbiology, 20-20 July 2011, Budapest, Hungary

5. Imri Á, **Kovács M**, Tamási K, Czakó-Vér K, Árvay Gy (2011) Evaluation of a potential mycorrhiza inoculum on salt-affected contrasting soils and wheat host. 16th International Congress of the Hungarian Society for Microbiology, 20-20 July 2011, Budapest, Hungary

6. Czakó-Vér K, **Kovács M**, Imri Á, Biró B, Árvay Gy (2011) Mycorrhiza inoculation for improved grapevine production at vineyard conditions. 10th Alps-Adria Scientific Workshop, 14-19 March 2011, Opatija, Croatia

7. **Kovács M**, Árvay Gy, Czakó-Vér K (2010) Szőlő nedvességtartalmának alakulása mikorrhiza oltás mellett szabadföldi körülmények között. XVI. Nemzetközi Környezetvédelmi és Vidékfejlesztési Diákkonferencia, Mezőtúr, 2010. június 30. - július 2.