

4TH NATIONAL CONFERENCE OF YOUNG BIOTECHNOLOGISTS

ABSTRACT BOOK

„FIBOK 2020”



Debrecen, 2020

4TH NATIONAL CONFERENCE OF YOUNG BIOTECHNOLOGISTS

**„FIBOK 2020” ONLINE CONFERENCE
ABSTRACT BOOK**

**organized by the
HUNGARIAN ACADEMY OF SCIENCES
and the
UNIVERSITY OF DEBRECEN**

**in
November of 2020**



**Edited by
Tünde Pusztahelyi
Levente Czeglédi
Éva Domokos-Szabolcsy
Tamás Emri**

The Committee on Agricultural Biotechnology of the Section of Agricultural Sciences of the Hungarian Academy of Sciences

in co-organization with

-the Intersectional Committee on Microbiology of the Section of Biological Sciences, HAS

-the Committee on Molecular Biology, Genetics, and Cell Biology of the Section of Biological Sciences, HAS

-the Committee on Food Sciences of the Section of Chemical Sciences, HAS; and

-the Society for Innovational Agricultural Biotechnology, Hungary

proudly presents the 4th National Conference of Young Biotechnologists.

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KOVÁCS Szilvia (DE MÉK Agrárműszerközpont)

TENTATIVE PROGRAMME

5th NOVEMBER 2020: PLANT BIOTECHNOLOGY

Chair: FEHÉR Attila, SZBK Növénybiológiai Intézet, Szeged

Co-Chair: DOMOKOS-SZABOLCSY Éva, DE MÉK, Debrecen

9:00 Debreceni Egyetem köszöntője: DOBRÁNSZKY JUDIT, DE AKIT

9:05 MTA Agrártudományi Osztály Mezőgazdasági Biotechnológiai Tudományos Bizottsági köszöntő, bevezető: FEHÉR ATTILA, SZBK Növénybiológiai Intézet, Szeged

9:20 Plenary lecture: GYÖRGYÉY János, IMBE, SZBK Növénybiológiai Intézet, Szeged. A NÖVÉNYI GÉNSZERKESZTÉS GYAKORLATI ALKALMAZÁSÁNAK TRENDJEI A „GMO-MIZÉRIA” TÁRSADALMI KÉRDÉSEI KÖZEPETTE

9:45 Kinga BALASSA¹- György BALASSA¹– Asztéria ALMÁSI² – Orsolya Kinga GONDOR³ - Tibor JANDA³ - Szabolcs RUDNÓY¹ ¹Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, H-1117, Budapest, Pázmány Péter sétány 1/C, Hungary; ²Department of Plant Pathology, Centre for Agricultural Research, H-1022 Budapest, Herman Ottó út 15, Hungary; ³ Institute of Agriculture, Centre for Agricultural Research, H-2462 Martonvásár, Brunszvik utca 2, Hungary UNCOVERING CHANGES IN THE ETHYLENE BIOSYNTHETIC PATHWAY OF SWEET CORN IN THE CASE OF SMALL-RNA-PRETREATMENT PRIOR TO AN MDMV-INFECTION

10:05 György BALASSA¹ - Kinga BALASSA¹ - Tibor JANDA² - Szabolcs RUDNÓY¹ ¹Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, H-1117, Budapest, Pázmány Péter sétány 1/C, Hungary; ² Institute of Agriculture, Centre of Agricultural Research, Hungarian Academy of Sciences, H-2462 Martonvásár, Brunszvik utca 2, Hungary. THE EFFECT OF DROUGHT AND SALICYLIC ACID ON THE RNA INTERFERENCE GENE EXPRESSION PATTERN IN MAIZE

10:25 Döme BARNA¹ – Miklós Gábor FÁRI¹ – Ibolya O. TÓTH¹ – Nóra BÁKONYI¹ ¹ University of Debrecen; Faculty of Agricultural and Food Sciences and Environmental Management, Department of Crop Sciences, Institute of Agricultural Botany, Physiology and Biotechnology H-4032 Debrecen Böszörményi str 138., Hungary PRELIMINARY RESULTS ON THE TOTAL PHENOLIC CONTENT OF ALFALFA BROWN JUICE

10:45 Zoltán KOVÁCS¹ – Tarek ALSHAAL^{1,2} – Nevien ELHAWAT^{1,3} – László KASZÁS¹ – Judit Ágnes KOROKNAI¹ – József PROKISCH⁴ – Miklós Gábor FÁRI¹ – Éva DOMOKOS-SZABOLCSY¹ ¹Agricultural Botany, Crop Physiology and Biotechnology Department., University of Debrecen, Debrecen, Böszörményi str. 138., H-4032, Hungary, ² Soil and Water Department, Faculty of Agriculture, University of Kafr El Sheikh, Qism

Kafr El-Shaikh, Kafr Al Sheikh, Kafr El Sheikh Governorate, Egypt, ³Department of Biological and Environmental Sciences, Faculty of Home Economics, Al-Azhar University, Al Mokhaym Al Daem, Nasr City, Cairo, Egypt; ⁴Faculty of Agricultural and Food Sciences and Environmental Sciences, Institute of Animal Sciences, Biotechnology and Nature Conservation, Non-independent Department of Animal Husbandry, University of Debrecen, Debrecen, Böszörményi str. 138., H-4032, Hungary. AGRONOMIC FORTIFICATION TO ENHANCE THE ORGANIC SELENIUM CONTENT OF ALFALFA GREEN BIOMASS

11:05 Iman MIRMAZLOUM^{1,2*} – Zsolt PÓNYA³ – Réka OSZLÁNYI¹ – Anita SZEGŐ¹ – Shahid JAMAL¹ – Oyuntogtokh BAT-ERDENE¹ – István PAPP¹ ¹ Department of Plant Physiology and Plant Biochemistry, Faculty of Horticultural Science, Szent István University. Budapest, Hungary; ² Food Science Innovation Centre, Kaposvár University. Kaposvár, Hungary; ³ Department of Plant Production and Plant Protection. Kaposvár University. Kaposvár, Hungary. ELEVATED FERTIGATION LEVEL INDUCES OXIDATIVE STRESS AND THE EXPRESSION OF A DEHYDRIN GENE IN CUCUMBER F1 HYBRIDS

LUNCH BREAK

12:20 Zsuzsanna GALBÁCS NAGYNÉ¹ – Éva VÁRALLYAY ¹ ¹ Agricultural Biotechnology Institute, Molecular Plant Pathology Group H- 2100 Gödöllő, Szent-Györgyi Albert u. 4., Hungary. INVESTIGATION CAUSATIVE AGENTS OF TREE DECLINE IN APRICOT AND APPLE ORCHARDS IN HUNGARY

12:40 Norbert HÍDVÉGI¹ – Andrea GULYÁS¹ – Erzsébet KISS² ¹University of Debrecen IAREF Research Institute of Nyíregyháza, H-4400 Nyíregyháza Westsik Vilmos street 4-6., Hungary; ²Szent István University Institute of Genetics, Microbiology and Biotechnology H-2100 Gödöllő Páter Károly street 1. Hungary PROMOTERS OF SPATULA AND SPIRAL GENES IN FRAGARIA VESCA

13:00 Máté SÁGI-KAZÁR¹ – Barnabás CSEH¹ – Brigitta MÜLLER¹ – Kálmán SZENTHE² – Brigitta TÓTH³ – Ádám SOLTÍ¹; ¹Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter sétány. 1/C., 1117 Budapest, Hungary; ²RT-Europe Nonprofit Research Ltd., Vár tér 2, E Building, 9200 Mosonmagyaróvár, Hungary; ³Department of Agricultural Botany, Crop Physiology and Biotechnology, University of Debrecen, Böszörményi út 138, Debrecen, 4032, Hungary THE REGULATIVE EFFECTS OF IRON NUTRITION ON CHLOROPLAST FERRIC-CHELATE OXIDOREDUCTASE 7

POSTER SECTION

13:20 Eszter BALOGH¹ – Ákos BOLDIZSÁR¹ – Balázs KALAI¹ – Krisztián GIÉRCZIK¹ – Zsolt GULYÁS² – Mohamed AHRES¹ – Gabriella SZALAI³ – Gábor GALIBA¹ – Gábor KOCSY¹ Agricultural Institute, Centre for Agricultural Research; Department of Plant Molecular Biology ² Agricultural Institute, Centre for Agricultural Research; Department of Applied Genomics ³ Agricultural Institute, Centre for Agricultural Research; Department of Plant Physiology 2462 Martonvásár, Brunszvik str 2., Hungary MODULATION OF WHITE LIGHT

SPECTRUM AFFECTS DIURNAL CHANGES IN GLUTATHIONE-DEPENDENT REDOX ENVIRONMENT AND EXPRESSION OF ANTIOXIDANT GENES IN BARLEY

13:25 Oyuntogtokh BAT-ERDENE - Iman MIRMAZLOUM - Anita SZEGŐ - István PAPP. Department of Plant Physiology and Plant Biochemistry, Szent István University, 1118 Budapest, Menesi street 44, Hungary. GROWTH PROMOTION IN PERLITE BASED CULTIVATION UPON SILICON TREATMENT IS PARALLELED BY EXPRESSION OF SILICATE TRANSPORTER GENES

13:30 Péter BENKŐ^{1,2} – Attila FEHÉR¹ – Katalin GÉMES^{1,1} ¹Department of Plant Biology and ²Doctoral School of Biology, University of Szeged, 52. Közép fasor, H-6726, Szeged, Hungary. POLYAMINES REGULATORY EFFECTS DURING TOBACCO POLLEN GERMINATION AND POLLEN TUBE ELONGATION

13:35 Zalán CZÉKUS^{1,2} – András KUKRI¹ – Iqbal NADEEM^{1,3} – Boglárka POLLÁK¹ – Atina MARTICS¹ – Dóra CSÓRÉ¹ – Attila ÖRDÖG¹ – Péter POÓR¹ ¹University of Szeged, Department of Plant Biology, 6726 Szeged, Közép fasor 52, Hungary ²Doctoral School of Biology, University of Szeged ³Doctoral School of Environmental Sciences, University of Szeged DAYTIME-DEPENDENT REGULATION OF FLAGELLIN-INDUCED DEFENCE RESPONSES IN TOMATO PLANTS

13:40 Bettina HUPP^{1,2} – Gergely MARÓTI^{1,1} Plant Biology Institute, Biological Research Center, H–6726 Szeged, Temesvári krt. 62., Hungary; ² Doctoral School in Biology; Faculty of Science and Informatics, University of Szeged, H–6726 Szeged, Hungary INVESTIGATION OF THE GROWTH AND HYDROGEN PRODUCTION OF MICROALGAE STRAINS IN STARCH-CONTAINING MEDIA

13:45 Jeny JOSE^{1,2} – Zsófia BÁNFALVI¹ ¹NARIC Agricultural Biotechnology Institute, Szent-Györgyi A. u. 4., 2100 Gödöllő, Hungary; ²Szent István University, Páter Károly u. 1., 2100 Gödöllő, Hungary EXPRESSIONAL AND FUNCTIONAL ANALYSIS OF GIGANTEA IN POTATO (SOLANUM TUBEROSUM L.)

13:50 Nikolett KASZLER² - Katalin GÉMES^{1,1} ¹Department of Plant Biology and ²Doctoral School of Biology, University of Szeged, 52. Közép fasor, H-6726, Szeged, Hungary SILVER NITRATE DECREASED THE EFFICIENCY OF DIRECT ORGANOGENESIS THROUGH THE DOWNREGULATION OF POLYAMINE BIOSYNTHESIS GENES IN ARABIDOPSIS THALIANA

13:55 Zsófia KOVÁCS¹ – Antal SZŐKE¹ – Gábor CSILLÉRY² – Erzsébet KISS¹ – Anikó VERES^{1,1} ¹Szent Istvan University, Genetics Microbiology and Biotechnology, 2100 Páter Károly u. 1, Hungary ² PepGen Kft., 1114 Bartók Béla street 41, Hungary STUDY OF GENES GOVERNING THE ANTHOCYANIN BIOSYNTHETIC PATHWAY IN PURPLE CAPSICUM MUTANTS

14:00 Árpád MOLNÁR¹ – Péter BORBÉLY¹ – Zsuzsanna KOLBERT¹ ¹ Department of Plant Biology, University of Szeged; 6726 Szeged, Közép Fásor 52., Hungary IN VITRO MICROPROPAGATION OF STEVIA REBAUDIANA BERTONI ON DIFFERENT MEDIA

14:05 Roumaissa OUNOKI¹ – Ferenc ÁGH^{1,2} – Richard HEMBROM¹ – Bernadett SZÖGI-TATÁR² – Andrea BÖSZÖRMÉNYI² – Katalin SOLYMOSI^{1,1} ¹ Department of Plant Anatomy, ELTE Eötvös Loránd University, H-1117 Budapest Pázmány P. s. 1/C., Hungary ² Department of Pharmacognosy, Semmelweis University, H-1085 Budapest Üllői út 26., Hungary THE EFFECT OF SALT STRESS ON THE VEGETATIVE PROPAGATION, PHOTOSYNTHESIS AND ESSENTIAL OIL COMPOSITION OF SPEARMINT

14:10 Viktor SZENTPÉTERI¹ – Kamirán Áron HAMOW² – Zsuzsanna AMBRÓZY² – Szilvi RÁTH³ – Katalin POSTA² ¹ Institute of Genetics, Microbiology and Biotechnology (GMBI) Szent István University H-2100 Gödöllő Páter Károly st 1., Hungary ² Plant Protection Institute, Centre for Agricultural Research, H-2462 Martonvásár, Hungary ³ Institute of Horticultural Technology, Szent István University H-2100 Gödöllő, Páter Károly st. 1. Hungary SALICYLIC ACID EXPRESSION CHANGES TO THIAMETHOXAM TREATMENT IN MYCORRHIZAL TOMATO PLANTS

14:15 Andrea Kitti TÓTH-LENCSE¹ – Antal KNEIP² – Glodia KGOBE¹ – Almash JAHAN¹ – Carolyn NYITRAI¹ – Anikó VERES¹ – Antal SZŐKE¹ – Pál KOZMA³ – György BISZTRAY⁴ – Erzsébet KISS¹ ¹ Szent István University, Institute of Genetics, Microbiology and Biotechnology, Gödöllő, Hungary ² Tokaj Wine Region Research Institute for Viticulture and Oenology, Tarcas, Hungary ³ University of Pécs, Institute of Viticulture and Enology, Pécs, Hungary ⁴ Szent István University, Department of Viticulture, Budapest, Hungary MARKER BASED GENOTYPING OF FURMINT AND OLASZRIZLING VARIETIES AND CLONES

14:20 Máté TÓTH¹ – Péter KALÓ¹ – Beatrix HORVÁTH¹ – Zoltán SZABÓ¹ – Zoltán TÓTH¹ ¹ Agricultural Biotechnology Centre, NARIC, Szent-Györgyi Albert str. 4., 2100 Gödöllő, Hungary ALTERNATIVE METHOD FOR THE TRANSFORMATION OF CAPSICUM SPECIES

14:40 CLOSING OF THE SECTION

12TH NOVEMBER 2020: MEDICAL BIOTECHNOLOGY

Chair: DELI Mária Szegedi Biológiai Kutatóközpont, Biofizikai Intézet, Biológiai Barrierék Kutatócsoport, Szeged

Co-Chair: PUSZTAHELYI Tünde, DE MÉK, Debrecen

9:00 MTA Agrártudományi Osztály Mezőgazdasági Biotechnológiai Tudományos Bizottsági köszöntő, bevezető: FEHÉR Attila, SZBK Növénybiológiai Intézet, Szeged

9:20 Plenary lecture: DELI Mária, Szegedi Biológiai Kutatóközpont, Biofizikai Intézet, Biológiai Barrierék Kutatócsoport, Szeged. TENYÉSZETES MODELLEK ALKALMAZÁSA AZ ORVOSI BIOTECHNOLÓGIÁBAN: FOKOZOTT ÉS CÉLZOTT GYÓGYSZERBEVITEL

9:45 Klára FODOR¹ – Nikoletta DOBOS¹ – Andrew V. SCHALLY² – Zita STEIBER³ – Gábor HALMOS^{1,2} ¹ 4032, Debrecen, Nagyerdei krt. 98, Hungary, University of Debrecen, Faculty of Pharmacy, Department of Biopharmacy ² Miami, FL, USA, Veterans Affairs Medical Center; Endocrine, Polypeptide and Cancer Institute; University of Miami, Miller School of Medicine, Department of Pathology and Department of Medicine, Divisions of Oncology and Endocrinology, Sylvester Comprehensive Cancer Center, ³4032, Debrecen, Nagyerdei krt. 98, Hungary, University of Debrecen, Clinical Centre, Department of Ophthalmology THE TARGETED LHRH ANALOG AEZS-108 ALTERS EXPRESSION OF GENES RELATED TO ANGIOGENESIS AND DEVELOPMENT OF METASTASIS IN UVEAL MELANOMA

10:05 Zsófia MOLNÁR^{1,2} – Zsófia BATA^{1,2} – Beáta G. VÉRTESSY^{2,3} – László POPPE^{1,4} ¹ Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rkp. 3. H-1111, Budapest, Hungary. ² Institute of Enzymology, HAS-Research Center of Natural Sciences, Budapest, H-1117 Magyar tudósok krt. 2. Budapest, Hungary, ³ Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Műegyetem rkp. 3. H-1111, Budapest, Hungary, ⁴ Biocatalysis and Biotransformation Research Centre, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University of Cluj-Napoca, Arany János Str. 11, RO-400028 Cluj-Napoca, Romania. X-RAY CRYSTALLOGRAPHY, FUNCTIONAL DYNAMICS, AND STRUCTURE-BASED ENGINEERING OF CONSERVED SUBSTRATE CHANNELS TO ALTER MIO-ENZYMES

10:25 Attila VÁMOS¹ – Boglárka Ágnes VINNAI¹ – Ágnes KLUSÓCZKI¹ – László FÉSÜS¹ – Endre Károly KRISTÓF¹ ¹ Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, H-4032 Debrecen Egyetem square 1., Hungary GENE EXPRESSION PATTERN OF HUMAN PRIMARY ADIPOCYTES DURING BEIGE TO WHITE TRANSITION

10:45 Krisztina POHÓCZKY^{1,2} – Nikolett SZENTES² – Valéria TÉKUS² – Bálint BOTZ² – Ágnes KEMÉNY^{2,3} – Serena SENSI⁴ – Ádám DÉNES⁵ – Andreas GOEBEL^{4,6} – Zsuzsanna HELYES^{2,7} ¹ University of Pécs, Faculty of Pharmacy, Department of Pharmacology H-7624 Pécs, Szigeti út 12. ² University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy & János Szentágotthai Research Centre, Centre for Neuroscience H-7624 Pécs, Szigeti út 12.- Ifjúság út 20 ³ University of Pécs, Medical School, Department of Medical Biology H-7624 Pécs, Szigeti út 12. ⁴ Department of Translational Medicine, University of Liverpool Brownlow Hill, Liverpool, United Kingdom ⁵ Momentum Laboratory of Neuroimmunology, Institute of Experimental Medicine, H-1083 Budapest, Szigony u. 43 ⁶ The Walton Centre NHS Foundation Trust, Brownlow Hill, Liverpool, United Kingdom ⁷ PharmInVivo Ltd., H-7629 Pécs, Szondy György u. 10 CENTRAL SENSITIZATION AND NEUROINFLAMMATION ARE MEDIATED BY TNF- AND IL1-DRIVEN

PATHWAYS IN A TRANSLATIONAL MOUSE MODEL OF COMPLEX REGIONAL PAIN SYNDROME (CRPS)

11:05 Rita HIRMONDÓ¹ – Éva Viola SURÁNYI^{1,2} – Dániel MOLNÁR¹ – Beáta G. VÉRTESSY^{1,2} – Judit TÓTH¹

¹Institute of Enzymology, Research Centre for Natural Sciences, 1117 Budapest, Magyar Tudósok krt. 2. Hungary

²Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, 1113 Budapest, Műegyetem tér 1-3. Hungary INVESTIGATION OF THE MUTATOR EFFECTS OF VARIOUS GENOTOXIC STRESSES PROVIDES INSIGHT INTO THE MECHANISM OF DRUG RESISTANCE DEVELOPMENT IN MYCOBACTERIA

11:25 Katalin PETRÉNYI - Ildikó BACSKAI, Biotech Hungary Kft. H-2310 Szigetszentmiklós Gyári Str. 33., Hungary NEW ENGLAND BIOLABS – „BY SCIENTISTS FOR SCIENTISTS”

LUNCH BREAK

13:00 Ádám HORVÁTH^{1,2} – Tünde BIRÓ-SÜTŐ^{1,2} – Boglárka KÁNTÁS^{1,2} – Maja PAYRITS^{1,2} – János EROSTYÁK^{2,3} – Géza MAKKAI^{2,3} – Zsuzsanna HELYES^{1,2} – Éva SZŐKE^{1,2}, ¹University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, H-7624 Pécs, Szigeti str 12., Hungary; ²University of Pécs, Szentágotthai Research Centre, H-7624, Ifjúság str 20., Hungary; ³University of Pécs, Faculty of Sciences, Institute of Physics, Department of Experimental Physics, H-7624 Ifjúság str 6., Hungary. IS THE CELL MEMBRANE MODIFICATION A NOVEL POSSIBILITY IN THE PAIN MANAGEMENT?

POSTER SECTION

13:20 Klára FODOR¹ – Nikoletta DOBOS¹ – János NAGY² – Gábor MÉHES³ – Gábor HALMOS¹, ¹University of Debrecen, Faculty of Pharmacy, Department of Biopharmacy, H-4032, Debrecen, Nagyerdei krt. 98, Hungary ²University of Debrecen, Clinical Center, Department of Radiotherapy, H-4032, Debrecen, Nagyerdei krt. 98, Hungary, ³University of Debrecen, Faculty of Medicine, Department of Pathology H-4032, Debrecen, Nagyerdei krt. 98, Hungary. CORRELATIONS BETWEEN THE EXPRESSION OF ANGIOGENIC FACTORS AND STEM CELL MARKERS IN HUMAN UVEAL MELANOMA

13:25 Viktória BAKSA¹ – Alexandra KISS¹ – Lenke POLYÁK¹ – Melinda SZIGETI-TURÁNI¹ – Gábor SZEMÁN-NAGY¹ ¹Department of Biotechnology and Microbiology, University of Debrecen, 4032 Debrecen, Egyetem Tér 1, Hungary EXAMINATION OF LONGITUDINAL CORRELATION OF MULTI-WALLED CARBON NANOTUBES (MWCNTS), OBSERVATION OF THEIR GENO- AND CYTOTOXIC EFFECTS DURING CORNEAL REGENERATION

13:30 József KIRÁLY¹ – Erzsébet SZABÓ¹ – Zsolt FEJES² – Béla NAGY² – Petra FODOR¹ – Zsuzsanna SZABÓ¹ – Gábor HALMOS¹ ¹4032, Debrecen, Nagyerdei krt. 98, Hungary, University of Debrecen, Faculty of

Pharmacy, Department of Biopharmacy ²4032, Debrecen, Nagyerdei krt. 98, Hungary, University of Debrecen, Faculty of Medicine, Institute of Laboratory Medicine ³4032, Debrecen, Nagyerdei krt. 98, Hungary, University of Debrecen, Clinical Center, Department of Urology EFFECT OF SHIKONIN ON THE EXPRESSION OF ONCOGENIC miRNAs IN HUMAN RENAL CANCER CELL LINES

13:35 Pál SALAMON^{1,2} – Csongor ORBÁN² – Ildikó MIKLÓSSY² – Szabolcs LÁNYI² – Beáta ALBERT^{1,2}
¹University of Pécs, Faculty of Sciences, Institute of Chemistry H-7624 Pécs, Ifjúság útja 6, Hungary; ²Sapientia Hungarian University of Transylvania, Faculty of Economics, Socio-Human Sciences and Engineering, Miercurea Ciuc, Department of Bioengineering, 530104 Miercurea Ciuc Piața Libertății nr. 1, Harghita County, Romania. ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN CELL DEATH CONTROL

14:00 CLOSING OF THE SECTION

19TH NOVEMBER 2020: MICROBIAL AND BIOMASS BIOTECHNOLOGY

Chair: OLASZ Ferenc, NAIK, Gödöllő

Co-chair: EMRI Tamás, DE TTK, Debrecen

9:00 MTA Agrártudományi Osztály Mezőgazdasági Biotechnológiai Tudományos Bizottsági köszöntő, bevezető

9:20 Plenary lecture: PÓCSI István, Debreceni Egyetem, Természettudományi és Technológiai Kar, Biotechnológiai Intézet, Molekuláris Biotechnológiai és Mikrobiológiai Tanszék, Debrecen. OMIKAI ADATGYŰJTÉS ÉS FELHASZNÁLÁS A GOMBA-BIOTECHNOLÓGIÁBAN

9:45 Dóra BALÁZS¹ – Tamás MARIK¹ – András SZEKERES¹ – Chetna TYAGI¹ – Ágnes SZEPESI² – László BAKACSY² – Csaba VÁGVÖLGYI¹ – László KREDICS¹ ¹Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Közép fasor 52., Hungary ² Department of Plant Biology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Közép fasor 52., Hungary PEPTAIBOL PROFILES OF THE CLINICALLY RELEVANT SECTION LONGIBRACHIATUM AND THE BIOACTIVITY OF TRICHODERMA REESEI PEPTAIBOLS

10:05 Dániel MOLNÁR^{1,2} – Hanna LÓCZI^{1,3} – Éva Viola SURÁNYI^{2,3} – Rita HIRMONDÓ¹ – Beáta G. VÉRTESSY^{1,3} – Judit TÓTH¹ ¹Research Centre for Natural Sciences, Institute of Enzymology, H-1117 Budapest Magyar Tudósok körútja 2., Hungary ² Eötvös Loránd University, Department of Biochemistry, H 1117 Budapest Pázmány Péter sétány 1/C., Hungary ³ Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Sciences, H 1111 Budapest Szent Gellért tér 4., Hungary. THE EFFECTS OF GENOTOXIC STRESS FACTORS ON DNA REPAIR SYSTEM IN MYCOBACTERIA

10:25 Noémi Nikolett GÖNCZI¹ – Orsolya STRANG¹ – Gábor RÁKHELY^{1,2} – Kornél L. KOVÁCS^{1,3}
¹Department of Biotechnology, University of Szeged, Közép fasor 52, 6726 Szeged, Hungary;² Institute of Biophysics, Biological Research Center, Temesvári krt. 62, 6726 Szeged, Hungary;³ Department of Oral Biology and Experimental Dental Research, University of Szeged, Tisza L. krt. 64, 6720 Szeged, Hungary.
INTERACTIONS BETWEEN PROBIOTIC AND ORAL PATHOGENIC STRAINS

11:45 Tibor KERESZTÉNYI^{1*} – Balázs LIBISCH^{1*} – Beáta VITÁNYI¹ – Zoltán KERÉNYI² – Róbert KOCSIS² – Ferenc OLASZ¹ – Péter PAPP^{1,1} Department of Genetics, Microbiology Laboratory, National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute, Gödöllő, Szent-Györgyi A. str. 4., Hungary.
²Hungarian Dairy Research Institute Ltd., Mosonmagyaróvár, Lucsony str. 24., Hungary. INTESTINAL MICROBIOTA OF WILD BOARS AS POTENTIAL SOURCE OF BENEFICIAL MICROORGANISMS

11:05 Csilla SZŰCS¹ – Etelka KOVÁCS¹ – Zoltán BAGI¹ – Gábor RÁKHELY^{1,2} – Kornél L. KOVÁCS^{1,3}
¹Department of Biotechnology, University of Szeged, H-6726 Szeged, Közép fasor 52., Hungary.²Institute of Biophysics, Biological Research Centre, H-6726 Szeged, Temesvári krt. 62., Hungary.³Department of Oral Biology and Experimental Dental Research, University of Szeged, H-6720 Szeged, Tisza Lajos körút 64-66., Hungary
ENHANCING BIOGAS PRODUCTION FROM AGROINDUSTRIAL WASTE PRE-TREATED WITH FILAMENTOUS FUNGI

11:25 József NYÁRI¹ – Balázs KAKUK¹ – Zoltán BAGI¹ – Gábor RÁKHELY^{1,2} – Kornél L. KOVÁCS^{1,3}
¹University of Szeged, Department of Biotechnology, Szeged, Hungary; ²Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary; ³University of Szeged, Department of Oral Biology and Experimental Dental Research, Szeged, Hungary
USE OF ENSILED GREEN WILLOW BIOMASS IN BIOGAS FERMENTERS

LUNCH BREAK

13:00 Petra MÉSZÁROS^{1,2,3} – Rita HÍRMONDÓ¹ – Beáta G. VÉRTESSY^{1,2} – Judit TÓTH^{1,3} ¹ Institute of Enzymology, Research Centre for Natural Sciences, 1117 Budapest, Magyar Tudósok Krt. 2. Hungary; ²Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, 1113 Budapest, Műegyetem Tér 1-3. Hungary; ³ Department of Biochemistry, Eötvös Loránd University, Faculty of Science, 1117 Budapest, Pázmány Péter Sétány 1/A. EXAMINATION OF THE ROLE OF MYCOBACTERIAL DUTPASE IN HOMOLOGUES RECOMBINATION

13:20 Zsófia BERKL¹ – Mónika MOLNÁR¹ – Éva FENYVESI² – Imre NÉMETH¹ – Kata BUDA¹ – Ildikó FEKETE-KERTÉSZ¹ – Rita MÁRTON¹ – Lajos SZENTE² ¹ Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest, Hungary; ² CycloLab Cyclodextrin R & D Laboratory Ltd., Budapest, Hungary. CYCLODEXTRIN-MEDIATED QUORUM QUENCHING IN ALIIVIBRIO FISCHERI MODEL SYSTEM

13:40 Anita KURILLA^{1,2} – Anita SZŐKE³ – Levente KONTRA⁴ – Krisztina KÁLDI³ – Tibor CSORBA¹ – Dániel SILHAVY⁵ ¹ National Agricultural Research and Innovation Centre – Agricultural Biotechnology Institute; Department of Virology, 2100 Gödöllő Szent-Györgyi Albert str 4., Hungary. ² Doctoral School of Biology, ELTE; 1117 Budapest Pázmány Péter str., Hungary. ³ Semmelweis University; Institute of Physiology; Department of Chronophysiology 1094 Budapest Tűzoltó str 37-47., Hungary. ⁴ National Agricultural Research and Innovation Centre – Agricultural Biotechnology Institute; Department of Genomics, 2100 Gödöllő Szent-Györgyi Albert str 4., Hungary. ⁵ Biological Research Centre; 6726 Szeged Temesvári krt 62., Hungary. IDENTIFICATION OF PRD-2 GENE THAT LINKS CIRCADIAN RHYTHM AND NONSENSE-MEDIATED DECAY IN NEUROSPORA CRASSA

14:00 Cs. Barnabás GILA^{1,2} – Károly ANTAL³ – István PÓCSI¹ – Tamás EMRI¹. ¹ Department of Molecular Biotechnology and Microbiology, Institute of Biotechnology, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary. ² Doctoral School of Nutrition and Food Sciences, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary. ³ Department of Zoology, Eszterházy Károly University, Eszterházy tér 1., H-3300 Eger, Hungary TRANSCRIPTOMIC ANALYSIS OF CARBON LIMITATION STRESS RESPONSES IN ASPERGILLUS NIDULANS

POSTER SECTION

14:20 Cintia ADÁCSI¹ – Tünde PUSZTAHELYI² ¹ Doctoral School of Nutrition and Food Sciences, University of Debrecen H-4032, Böszörményi út 138. Debrecen, Hungary ² Central Laboratory of Agricultural and Food Products, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, H-4032, Böszörményi út 138. Debrecen, Hungary INVESTIGATION OF AFLATOXIN M1 BINDING CAPACITY OF PROBIOTIC BACTERIAL CULTURES AND THEIR PREPARATIONS

14:25 Viola Zsuzsanna ANGYAL^{1,2} – Viola Éva SURÁNYI^{1,2} – Beáta VÉRTESSY^{1,2} – Judit Eszter SZABÓ^{1,2} ¹ Department of Applied Biotechnology and Food Sciences, Budapest University of Technology and Economics, Budapest, 1111, Hungary, Műegyetem rkp. 3. ² Institute of Enzymology, Research Centre for Natural Sciences, Budapest 1117, Hungary, Magyar tudósok krt. 2. ESTABLISHMENT OF A MODEL SYSTEM FOR STUDYING THE EFFECT OF BACTERIOPHAGE DUTPASE ACTIVITY ON HORIZONTAL GENE TRANSFER IN STAPHYLOCOCCUS AUREUS

14:30 Livia DÁLYAI¹ – Madina KHAMITOVA¹ – Lajos ÁCS-SZABÓ¹ – Ida MIKLÓS¹ – Hajnalka CSOMA¹ ¹ University of Debrecen, Institute of Biotechnology, Department of Genetics and Applied Microbiology, H-4032 Debrecen, Egyetem tér 1., Hungary DETECTION OF PULCHERRIMIN GENE CLUSTER IN THE METSCHNIKOWIA FRUCTICOLA TYPE STRAIN (CBS 8853^T)

14:35 Evelin SÁNTA-BELL¹ – Zsófia MOLNÁR^{1,2} – Gábor HORNYÁNSZKY^{1,3} – Diána BALOGH-WEISER^{1,3,4} – László POPPE^{1,3,5} ¹ Budapest University of Technology and Economics, Department of Organic Chemistry and Technology, Műegyetem rkp. 3, H-1111 Budapest, Hungary ² Institute of Enzymology, HAS-Research Center of Natural Sciences, Magyar tudósok körútja 2, H-1117 Budapest, Hungary ³ Synbiocat Ltd., Szilasliget u. 3, H-1172 Budapest, Hungary ⁴ Budapest University of Technology and Economics, Department of Physical Chemistry and Materials Science, Műegyetem rkp. 3, H-1111 Budapest, Hungary ⁵ Biocatalysis and Biotransformation Research Centre, Faculty of Chemistry and Chemical Engineering, Babeş-Bolyai University of Cluj-Napoca, Arany János Str. 11, RO-400028 Cluj-Napoca, Romania SELECTIVE IMMOBILIZATION OF A RECOMBINANT PHENYLALANINE AMMONIA-LYASE FROM FERMENTATION MEDIA

14:40 Galiya AKHMETOVA^{1,2} – Dániel G. KNAPP¹ – Aldabergen KIYAS² – Vladimir ZABOLOTSKICH² – Gábor M. KOVÁCS¹ ¹Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Budapest, Hungary, Pázmány Péter Sétány 1/c, H-1117, Budapest, Hungary ²A.I. Barayev “Scientific Production Centre for Grain Farming,” 021601, St. Barayev 15, Shortdandy, Kazakhstan THE SCREENING OF ENDOPHYTES FROM AGRICULTURAL AND NON-AGRICULTURAL CROPS IN NORTHERN KAZAKHSTAN

14:45 Annamária GERŐCS¹ – János MÁJER² – Barna SZÓKE² – Frederico MAGALHÃES³ – Brian GIBSON³ – Ferenc OLASZ¹ ¹Agricultural Biotechnology Institute, H-2100 Gödöllő Szent-Györgyi Albert str 4., Hungary ²Research Institute for Viticulture and Enology, H-8261 Badacsonytomaj Római str 181., Hungary ³VTT Technical Research Centre of Finland Ltd, FI-02044 Espoo Tietotie 2., Finland OENOLOGICAL AND MOLECULAR ANALYSIS OF TWO INDIGENOUS SACCHAROMYCES POTENTIAL HYBRID STRAINS FROM BADACSONY WINE AREA

14:50 Ildikó IMREFI¹ – Petra LENGYEL¹ – Gábor M. KOVÁCS¹ – Dániel G. KNAPP¹ ¹Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, H-1117 Budapest, Pázmány Péter sétány 1/C., Hungary HABITAT ADAPTATION OF FUNGAL ROOT ENDOPHYTES —LINEAGES IN GRAMINEOUS CROPS ON AGRICULTURAL AREAS

14:55 Péter János BEREK-NAGY¹ – Gergő TÓTH² – András DARCSI³ – Dániel G. KNAPP¹ – Szilvia BŐSZE⁴ – Imre BOLDIZSÁR¹ – Gábor M. KOVÁCS¹ ¹Department of Plant Anatomy, Institute of Biology, Eötvös Loránd University, Pázmány Péter sétány 1/C., H-1117 Budapest, Hungary ²Department of Pharmaceutical Chemistry, Semmelweis University, Hőgyes Endre utca 9., H-1092 Budapest, Hungary ³National Institute of Pharmacy and Nutrition, Zrínyi utca 3., H-1051 Budapest, Hungary ⁴MTA-ELTE Research Group of Peptide Chemistry, Eötvös Loránd University, Pázmány Péter sétány 1/A., H-1117 Budapest, Hungary SECONDARY METABOLITES OF FLAVOMYCES FULOPHAZII, A ROOT ENDOPHYTE OF SEMIARID SANDY GRASSLANDS

15:00 Judit E. SZABÓ^{1,2} – Viola Zs. ANGYAL^{1,2} – Gábor T. KOVÁCS^{1,2} – Bernadett MIHÁLY^{1,2} – Orsolya DOBAY³ – Beáta VÉRTESSY^{1,2} ¹Department of Applied Biotechnology and Food Sciences, Budapest University of Technology and Economics, Budapest, 1111, Hungary ² Institute of Enzymology, Research Centre for Natural

Sciences, Budapest 1117, Hungary, ³ Institute of Medical Microbiology, Semmelweis University, Budapest 1089, Hungary, URACIL-DNA REPAIR INFLUENCING GENES OF MOBILE GENETIC ELEMENTS DO NOT COUNTERBALANCE THE LACK OF GENOMIC dUTPASE IN STAPHYLOCOCCUS AUREUS

15:05 Kinga Edina VARGA¹ – Zsigmond BENKŐ¹ – Yingying HUANG² – István PÓCSI¹ – István MOLNÁR²
¹Department of Molecular Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, H-4032 Egyetem tér 1, Debrecen, Hungary; ²Southwest Center for Natural Product Research, University of Arizona, Tucson, AZ 85706 ADAPTATION OF THE CRISPR GENOME EDITING SYSTEM FOR SYNTHETIC MICROBIOLOGY

15:10 Ágnes JAKAB¹ – Anita SZABÓ¹ – Tamás EMRI¹ – Viktor DOMBRÁDI² – István PÓCSI¹
¹Department of Molecular Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1., 4032 Debrecen, Hungary; ²Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Egyetem tér 1., 4032 Debrecen, Hungary. THE COMBINED EFFECTS OF THE PROTEIN PHOSPHATASE Z1 GENE DELETION AND OXIDATIVE STRESS ON THE GENE EXPRESSION OF THE GLUCOCORTICOID TREATED CANDIDA ALBICANS

15:15 Imre NÉMETH – Fanni BODÓ – Sára SZIKSZAI- Mónika MOLNÁR Budapest University of Technology and Economics; Department of Applied Biotechnology and Food Science, H-1111 Budapest Műegyetem rkp. 3., Hungary EFFECTS OF ZINC OXIDE AND TITANIUM DIOXIDE NANOPARTICLES ON *PSEUDOMONAS AERUGINOSA* AND *SERRATIA MARCESCENS* BIOFILM FORMATION

15:30 CLOSING OF THE SECTION

27TH NOVEMBER 2020: ANIMAL BIOTECHNOLOGY

Chair: GÓCZA Elen, NAIK, MBK, Gödöllő

Co-chair: CZEGLÉDI Levente, DE MÉK, Debrecen

9:00 MTA Agrártudományi Osztály Mezőgazdasági Biotechnológiai Tudományos Bizottsági köszöntő, bevezető

9:20 Plenary lecture: URBÁNYI Béla, Szent István Egyetem, MKK-TEMI, Halgazdálkodási Tanszék, Gödöllő.
A HALAKBAN REJLŐ LEHETŐSÉGEK A GENOMIKA ÉVSZÁZADÁBAN

9:45 Zsófia FEKETE^{1,2} – Levente KONTRA^{1,3} – Viktor STÉGER¹ – László HIRIPI¹ – Tibor NAGY^{1,3} – Zoltán László NÉMETH⁴ – Áron SZENES⁴ – Nóra NINAUSZ¹ – Adrienn PÁSZTOR¹ – Bálint ÉGERHÁZI¹ – Tímea PINTÉR¹ – Mátyás SCHILLER¹ – Endre BARTA^{1,3}
¹National Agricultural Research and Innovation Centre – Agricultural Biotechnology Institute; H-2100 Gödöllő, Szent-Györgyi Albert street 4, Hungary.²Szent István

University, H-2100 Gödöllő, Páter Károly street 1., Hungary.³University of Debrecen; H-4032 Debrecen, Egyetem square 1., Hungary; ⁴University of Veterinary Medicine Budapest; H-1078 Budapest, István street 2., Hungary.
GENOME WIDE STUDY OF WILD EUROPEAN RABBIT (ORYCTOLAGUS CUNICULUS) POPULATIONS IN HUNGARY

10:05 Lilla DÉNES¹ – Ines RUEDAS-TORRÉS² – Gyula BALKÁ¹ ¹ Department of Pathology, University of Veterinary Medicine, 1078 Budapest, István Str. 2., Hungary. ² Department and Clinic of Food Animal Medicine, University of Veterinary Medicine, 2225 Üllő, Dóra major, Hungary. IDENTIFICATION OF ATYPICAL PORCINE PESTIVIRUS IN HUNGARIAN HERDS

10:25 Lilla BODROGI¹ – Tímea PINTÉR^{1,2} – Nándor LIPTÁK¹ – Réka BALÁZS² – Elen GÓCZA¹ ¹Department of Animal Biotechnology, Agricultural Biotechnology Institute; NARIC, Szent-Györgyi Albert str. 4., 2100 Gödöllő, Hungary ²Faculty of Agricultural and Environmental Science, Szent István University, Páter Károly str. 1, 2100 Gödöllő, Hungary EFFECTS OF T2 MYCOTOXIN TREATMENT ON RABBIT EMBRYO DEVELOPMENT IN VITRO

10:45 Nóra NINAUSZ¹ – Péter FEHÉR¹ – Péter KEMENSZKY² – Viktor STÉGER¹ ¹ Agricultural Biotechnology Center, NAIK, 2100 Gödöllő, Szent-Györgyi Albert Str. 4, Hungary ² Faculty of Forestry, University of Sopron, 9400 Sopron, Bajcsy-Zsilinszky Str. 4., Hungary GOLDEN JACKAL'S WHITE FUR IS CAUSED BY MC1R MUTATION FOUND IN DOGS

11:05 Nikolett TOKODYNÉ SZABADI^{1,2} – Roland TÓTH² – Bence LÁZÁR^{2,3} – Eszter PATAKINÉ VÁRKONYI³ – Krisztina LIPTÓI³ – Elen GÓCZA² ¹Doctoral School of Animal Science, KU, Guba S. str. 40., 7400 Kaposvár, Hungary ²ABC, NARIC, Szent-Györgyi A. str. 4., 2100 Gödöllő, Hungary ³National Centre for Biodiversity and Gene Conservation, Isaszegi road 200., 2100 Gödöllő, Hungary. DEVELOPMENT AN EFFECTIVE METHOD TO PREVENT THE NEGATIVE EFFECTS OF HEAT STRESS BY STUDYING THE MRNA AND MIRNA EXPRESSION PROFILE SUBSEQUENT A POST-HATCH HEAT-TREATMENT

11:25 Anna Georgina KOPASZ – Dávid PUSZTAI – Liza HU DOBA – Réka KARKAS – András BLASTYÁK – Gergely IMRE – Lajos MÁTÉS. Institute of Genetics, Biological Research Centre, Szeged Temesvári krt. 62., 6726 Hungary. ESTABLISHMENT OF AN RNA INTERFERENCE BASED GENE SILENCING SYSTEM IN A SOMATICALLY TRANSGENIC MOUSE MODEL

LUNCH BREAK

POSTER SECTION

12:30 Eszter ANGYAL¹ – Gabriella NOVOTNI-DANKÓ¹ – Boglárka VINCZE² ¹Department of Animal Husbandry, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen,

Böszörményi street 138., 4032, Debrecen, Hungary ²Department of Obstetrics and Food Animal Medicine Clinic, University of Veterinary Medicine Budapest, István street 2., Budapest, Hungary OPU (OVUM PICK-UP) AND ICSI (INTRACYTOPLASMIC SPERM INJECTION) TECHNIQUES IN MARES

12:35 Katalin BALOG¹ – Zoltán BAGI² – Bianka TÓTH² – Szilvia KUSZA³ ¹ University of Debrecen Faculty of Science and Technology; H-4032 Debrecen, Egyetem tér 1., Hungary ² University of Debrecen, Institute for Agricultural Research and Educational Farm, H-4032 Debrecen, Böszörményi út 138., Hungary ³ University of Debrecen Faculty of Agricultural and Food Sciences and Environmental Management Department of Animal Science, Biotechnology and Nature Conservation, Laboratory of Animal Genetics; H-4032 Debrecen, Böszörményi út 138., Hungary GENETIC DIVERSITY OF FIVE HUNGARIAN COMMON CARP (CYPRINUS CARPIO L.) LANDRACES BASED ON MITOCHONDRIAL DNA – INITIAL RESULTS

12:45 Tímea PINTÉR^{1,2} – Lilla BODROGI – ^{1.} Zoltán BAGI³ – Szilvia KUSZA⁴ ¹Department of Animal Biotechnology, Agricultural Biotechnology Institute; NARIC, Szent-Györgyi Albert str. 4., 2100 Gödöllő, Hungary ²Faculty of Agricultural and Environmental Science, Szent István University, Páter Károly str. 1, 2100 Gödöllő, Hungary ³Department of Biotechnology, University of Szeged, Közép fasor 52, 6726 Szeged, Hungary ⁴Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Böszörményi str. 138. 4032 Debrecen, Hungary ADAPTATION OF SHEEP BREEDS TO SEASONAL HEAT STRESS IN HUNGARY

12:50 Loretta SÁRVÁRI¹ – Károly TEMPFLI¹ – Klaudia SZALAI¹ – Eszter ZSÉDELY¹ – Erika LENCSÉSVARGA¹ – Anita ALMÁSI² – Ágnes BALI PAPP¹ ¹ Department of Animal Sciences, Széchenyi István University, H-9200 Mosonmagyaróvár, 2 Vár square, Hungary ² Bábolna TETRA Kft., H-2943 Bábolna, 16 Radnóti str, Hungary SORCS2 GENOTYPE IS ASSOCIATED WITH PLUMAGE TRAITS IN LAYING CHICKEN HYBRIDS

12:55 Gabriella SKODA¹ – Andrea KERÉKES¹ – Orsolya Ivett HOFFMANN¹ – Nándor LIPTÁK¹ – Levente SZEREDI² – Tamás DONKÓ³ – Zsuzsanna BŐSZE¹ – László HIRIPI¹ ¹Department of Animal Biotechnology, ABI, NARIC H-2100 Gödöllő Szent-Györgyi Albert str 4., Hungary; ²Laboratory for the Pathology of Mammals, Wildlife and Poultry, National Food Chain Safety Office H-1143 Budapest Tábornok str. 2., Hungary ³Institute of Diagnostic Imaging and Radiation Oncology, Faculty of Agricultural and Environmental Sciences, Kaposvár University H-7400 Kaposvár Guba Sándor str 40., Hungary NEW PHENOTYPE OF MYOSTATIN MUTANT RABBIT CREATED BY CRISPR/CAS9 SYSTEM

13:00– Lilla DÉNES¹ - Gyula BALKAI¹ – Amadeu GRIOL² – Sergio PERANSI³ – Manuel RODRIGO³ – Alessandro GIUSTI⁴ – Ioannis BOSSIS⁵ – Grzegorz WOZNIAKOWSKI⁶ – Sabato D'AURIA⁷ – Antonio VARRIALE⁷ ¹ University of Veterinary Medicine, Budapest, Hungary; ²Universitat Politècnica de València Nanophotonics Technology Center, Valencia Spain; ³Lumensia Sensors S.L., Valencia, Spain; ⁴CyRIC, Cyprus Research and Innovation Centre, Nicosia, Cyprus; ⁵Agricultural University of Athens, Greece; ⁶National

Veterinary Research Institute, Pulawy, Poland; ⁷Institute of Food Science, CNR, Naples, Italy A FIELD-BASED
DIAGNOSTIC DEVICE FOR SWINE VIRAL DISEASES: THE SWINOSTICS H2020 PROJECT

13:20 CLOSING OF THE SECTION

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PLENARY LECTURES

TENYÉSZETES MODELLEK ALKALMAZÁSA AZ ORVOSI BIOTECHNOLÓGIÁBAN: FOKOZOTT ÉS CÉLZOTT GYÓGYSZERBEVITEL

DELI Mária

Szegedi Biológiai Kutatóközpont, Biofizikai Intézet, Biológiai Barrierek Kutatócsoport,
Szeged

A biológiai gátrendszerek tenyészetes modelljei fontos eszközei a gyógyszeripari kutatásoknak. A bél- és a légúti hámok, valamint a vér-agy gát példáján bemutatom ezeknek a modell rendszereknek az alkalmazását (1) hatóanyagok sejtes toxicitásának és barrieren való átjutásának tesztelésére, (2) fokozott gyógyszerátjutást lehetővé tevő eljárások kidolgozására és (3) hatóanyagok célzott agyi bejuttatásának kifejlesztésére. Az előadásban szó lesz a tenyészetes modellrendszerek chip eszközökön létrehozott új nemzedékéről, és a gátrendszereken keresztüli fokozott, illetve célzott gyógyszerátjuttatást biztosító nanorendszerekről és nanorészecskékről.

A NÖVÉNYI GÉNSZERKESZTÉS GYAKORLATI ALKALMAZÁSÁNAK TRENDJEI A „GMO-MIZÉRIA” TÁRSADALMI KÉRDÉSEI KÖZEPETTE

GYÖRGYÉY János

IMBE, SZBK Növénybiológiai Intézet, Szeged

A génszerkesztési módszerek között a CRISPR 2012-es berobbanása hasonló minőségi ugrást eredményezett, mint a PCR elterjedése úgy két évtizeddel korábban. Mára nap, mint nap jelennek meg az új génszerkesztési eredmények a növényi géntechnológia területén is, köztük nagy számban olyanok, melyek a gyakorlati hasznosítás ígérését hordozzák. Vannak ezek között betegség-rezisztenciát adó fejlesztések, újabb gyomirtószer-tűrő vonalak éppúgy, mint radikálisan csökkentett gluténtartalmú búzafajták. Ezekből mutat be példákat az előadás első része.

A növényi biotechnológiával foglalkozó kutató-fejlesztő közösség évekig remélte, hogy a génszerkesztés elterjedésével a klasszikus értelemben vett GMO-k körüli áldatlan patthelyzet végre feloldódik Európában és a tudományos eredményekre, a rációra alapozott szabályozás születhet. Az anti-GMO szervezetek aktív tevékenységének köszönhetően azonban még a természetes mutánsokkal mindenben megegyező génszerkesztett mutánsokra is rákerült a GMO stigma, és ezt az Európai Bíróság 2018-as elvi állásfoglalást jelentő döntése is megerősítette, még nyilvánvalóbbá téve, hogy az EU közel húsz éves géntechnológiai irányelvei súlyosan idejétmúltak és alapvető reformra szorulnak. A fenti ellenmondásokat és problémákat boncolja a az előadás második fele.

OMIKAI ADATGYŰJTÉS ÉS –FELHASZNÁLÁS A GOMBA-BIOTECHNOLÓGIÁBAN

PÓCSI István

Debreceni Egyetem, Természettudományi és Technológiai Kar, Biotechnológiai Intézet,
Molekuláris Biotechnológiai és Mikrobiológiai Tanszék, Debrecen

Az omikai eszközökkel történő adatgyűjtés, az adatok adatbázisokba való foglalása, illetve ezek bányászata mára a korszerű, molekuláris szemléletű gomba-biotechnológiai kutatások alapját képezik. Az összehasonlító genomikai, transzkriptomikai, proteomikai és metabolomikai kutatások által biztosított nagy mennyiségű adat új típusú megközelítéseket tesz lehetővé az ipar számára fontos fonalas gombák és élesztők genetikai módosításában és törzsfejlesztésében. Az előadás kiemelten az általunk végzett gomba stresszbiológiai kutatások keretében nyert omikai adatokat elemzi a gyakorlati felhasználhatóság szempontjából különös tekintettel a környezeti stresszhatásoknak (nehézfém, oxidatív és ozmotikus stressz) fokozottan ellenálló ipari és bioremediációt végző gombatörzsek fejlesztési lehetőségeire. Ezen túlmenően bemutatásra kerülnek a patogén gombák életmódjának és tulajdonságainak a mélyebb megértésére irányuló omikai kutatásaink is, amelyek reményeink szerint a jövőben új gombaellenes szerek létrehozását, illetve új biológiai kontroll stratégiák kidolgozását teszik majd lehetővé.

A HALAKBAN REJLŐ LEHETŐSÉGEK A GENOMIKA ÉVSZÁZADÁBAN

URBÁNYI Béla

Szent István Egyetem, MKK-TEMI, Halgazdálkodási Tanszék, Gödöllő

A halak (Pisces) a gerincesek legfejletlenebb, de legnagyobb fajsámú osztálya. Ezen szélsőséges tulajdonságok számos olyan genetikai-biotechnológiai-biotechnikai módszer, eljárás és technológia megvalósítását tették lehetővé a halakban, amelyeket nem, vagy csak korlátozottan lehet végrehajtani a fejlettebb gerinceseken. Ez részben annak is köszönhető, hogy a ploiditásban is elképesztő változatosságot mutatnak (triploid-tetraploid fajok), speciális technikák vethetők be a tenyésztésben (androgenezis, ginogenezis), és a különleges szaporodási sajátosságok megértése is tudományos és gazdasági jelentőségűvé váltak. Mindezeket a modern kor DNS és RNS technikáival ötvözve biztosítják azt a jövőképet, hogy a 21. század állati eredetű fehérje szükséglet kielégítésére az akvakultúra szolgáltassa a megoldást.

PRESENTATIONS

UNCOVERING CHANGES IN THE ETHYLENE BIOSYNTHETIC PATHWAY OF SWEET CORN IN THE CASE OF SMALL-RNA-PRETREATMENT PRIOR TO AN MDMV-INFECTION

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Sweet corn is a significant commodity of food and other industries; thus, it can be count as one of the most important cereal crops in Hungary and worldwide. Among the biotic stressors of sweet corn, obligatory intracellular parasitic viruses, such as the Maize Dwarf Mosaic Virus (MDMV), can be highlighted. MDMV utilizes plant resources to ensure its intracellular multiplication, which contributes significantly to decreased growth and slower development of the corn. Thus, in the case of infection, plant biomass and yields can get greatly reduced, resulting in worldwide losses of up to billions of dollars.

The primary plant defence line against viral infection is based on RNA interference or RNA-based gene silencing. Through this process, the plant uses viral derived, sequence-specific small RNA (sRNA) molecules from the hereditary viral material to cleavage the viral RNA, thus preventing its reproduction. Pre-treatment with these sRNA molecules may benefit activating plant defence by mimicking infection without any real danger. Therefore, the plant may be prepared for the subsequent infection and eventually slow down or even curb viral reproduction. Plant hormones also play important roles in the control of viral infections. Most plant stress hormones could enhance plant protection against virus infection in different ways, while contradictory results have been revealed for ethylene (ET). In some cases, ET has been proved to contribute to the defence against viral infection, whereas it has only played a role in the crosstalk within the hormonal network in other plants. Furthermore, in other plant-virus interactions ET increased the susceptibility to a specific virus.

To gain an accurate insight into these issues in sweet corn, we investigated ET biosynthesis pathway during MDMV infection development. We measured ET's quantitative changes and its precursor by metabolomic studies and revealed the underlying gene expression processes. We also examined the changes of these parameters in sRNA pre-treated plants, besides monitoring plants' physiological state and the quantitative changes of the virus.

THE EFFECT OF DROUGHT AND SALICYLIC ACID ON THE RNA INTERFERENCE GENE EXPRESSION PATTERN IN MAIZE

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Maize (*Zea mays* L.) is one of the most important crops in the world. The economic use of this crop is spreading from human consumption to foraging. However, the yield depends on whether abiotic and biotic factors are in the optimal range of adaptation. Extreme weather conditions, such as drought, cause significant damages to the plants resulting in yield loss. For this purpose, researchers focus on understanding genomic, transcriptomic, molecular, and regulation-related aspects of stress response to breeding more and more stress-tolerant varieties.

Salicylic acid (SA) has a crucial role in developmental processes (germination, flowering, senescence) and biotic and abiotic stress responses. SA promotes systemic physiological processes through which stress-responsive metabolic pathways and other defense mechanisms are enhanced. Besides the classic part of the signal transduction pathways (receptors, transcription factors, phosphorylation cascades), small regulatory RNAs also play vital roles in fine-tuning almost all molecular mechanisms through RNA interference (RNAi). The stressors' perception needs to be fine-tuned; thus, in several molecular mechanisms, small RNAs play an essential role in RNA interference (RNAi). Enzymes involved in the RNAi are well known; however, we know little about their expression patterns in case of drought stress. There is even less knowledge about the effect of SA on the RNAi during optimal and stress conditions.

The strength of a stress response depends on the regulatory steps of the signal perception mechanisms. Physiological measurements are good indicators of the changes in the background of molecular mechanisms since providing information about the results of these processes. Studies about the stress-responsive small RNAs and gene expression patterns of the RNAi-enzymes help to understand the relationships between these processes. The results offer countless opportunities to enhance plant stress response and alleviate damages caused by stressors. Our study aimed to investigate the effect of exogenous salicylic acid, drought stress, and combined treatments on RNAi processes. Morphological and physiological measurements such as shoot length, fresh and dry mass, chlorophyll content, and chlorophyll-a fluorescence measurement results show that SA treatment can alleviate damages caused by drought stress. We examined gene expression changes of all RNAi-related enzymes (Dicer-like, RNA-dependent RNA polymerase, Argonaute) of maize to shed light on these genes' regulatory roles on SA-dependent and SA-independent stress defense pathways.

PRELIMINARY RESULTS ON THE TOTAL PHENOLIC CONTENT OF ALFALFA BROWN JUICE

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The constant growth of the global population and the increasing demand for sufficient protein sources are severe issues for today's agriculture. Green-biorefineries can offer high-quality protein for feedstock or even human nutrition. The process used in biorefineries usually generates large amounts of by-products, namely fiber and brown juice. The latter can take up to 50% (m/m) of the fresh biomass.

This substance is rich in sugars, micro-, macro elements, and many organic compounds. Brown juice means a severe threat to the environment if not treated properly. On the other hand, it has great potential, and a couple of studies already focused on its industrial use and showed promising results. A detailed characterization of the brown juice is still absent in the international literature. The brown juice supposedly contains bioactive compounds, and thus, it can be a potential raw material for the nutraceutical industry, as food supplements and functional foods. Our aim with this study was to examine the total phenolic content of brown juice. Phenolic compounds are excellent oxygen radical scavengers. These antioxidants show anti-inflammatory, anti-atherosclerotic, and anti-carcinogenic activities.

The samples came from our small-scale alfalfa experiments. To determine the total phenolic content of the brown juice samples, we used the spectrometric method of Singleton and Rossi (1965). According to our preliminary results, the brown juice has a potentially high amount of antioxidant compounds. 'Expressz' and 'Hunor-40' showed the highest values ($53.30 \pm 1.43 \mu\text{g ml}^{-1}$ and $51.98 \pm 11.29 \mu\text{g ml}^{-1}$) while other cultivars had lower total phenolic content. The results showed significant differences between cultivars and different periods of the year as well.

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AGRONOMIC FORTIFICATION TO ENHANCE THE ORGANIC SELENIUM CONTENT OF ALFALFA GREEN BIOMASS

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Multitudes of micronutrients are required for living organisms only in trace amounts. However, the importance of them is indisputable from the perspective of health promotion and disease prevention, as well. Selenium is such an essential microelement. The adequate selenium (Se) supply in the food chain generally reflects the soil's available Se content. The areas, where soil selenium content is low and the deficiency symptoms occur, need to elaborate strategies to alleviate it. Besides consuming or feeding salt-mineral supplements, biofortification is a possible strategy to enhance the bioavailable selenium content in edible crops or fodders.

In our experiment, an agronomic fortification was applied in an alfalfa pot experiment comparing selenate (Se⁶⁺), selenite (Se⁴⁺), and red elemental selenium (Se⁰) as inorganic selenium forms. The plants were treated with the different selenium forms in concentrations of 1, 10, and 50 ppm. During the vegetation season, the fortified alfalfa green biomass was harvested four times. Fresh biomass was processed according to the Green Biorefinery (GB) concept. The fresh shoots were squeezed using a twin-screw press, and the resulting green juice was precipitated by the patented microwave coagulation technique (MWC). The precipitated fortified leaf protein concentrate (fAPC) was isolated by filtration from the plant serum, brown juice (BJ). The quantity and quality of different fractions were analyzed to develop an effective fortification method. The protein content of fAPC varied between 35-40 m/m% DW. The total selenium content was measured by AFS technic. The dynamics of selenium uptake and accumulation in green fractions showed differences depending on the applied chemical forms. For instance, the highest selenium content (391 mg/kg DW) could be measured from 10 ppm selenate (as most photo available ionic form) treated fAPC in the second harvest. A decrease (131 mg/kg and 113 mg/kg DW) was observed in the third and fourth harvest.

ELEVATED FERTIGATION LEVEL INDUCES OXIDATIVE STRESS AND THE EXPRESSION OF A DEHYDRIN GENE IN CUCUMBER F1 HYBRIDS

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The correlation between elevated concentrations of fertigation solutions' components and the increased stress level is becoming more evident in crop plants, but little is known about the molecular mechanisms of stress triggering the effect of excessive fertigation. For understanding more about the correlation, two cucumber F1 hybrid cultivars were studied for their physiological behavior upon applying different strength of Hoagland fertigation solutions (HG). The stress-related signals and growth were monitored in young plants grown in soilless media and controlled environment. Expression of selected dehydrin (*DHN*) genes was also studied with RT-PCR and RT-qPCR. Significant differences were found among F1 hybrids treated with different fertigation levels in terms of total leaf area, level of lipid peroxidation, osmotic potential, antioxidant capacity, and the expression of the *DHN3* gene, too. Elevated HG concentrations resulted in declined leaf osmotic potential. The level of malondialdehyde, an indicator of lipid peroxidation, was significantly higher in an open field cultivar's seedling compared to a greenhouse type with an increasing trend towards elevated HG concentrations. Antioxidant capacity was increased in both hybrids when higher HG concentrations were applied. Furthermore, about 7-fold higher expression of the *DHN3* gene was recorded at the highest fertigation level (2.5× HG) in greenhouse cultivated plants compared to the open field grown type. Results presented in this report demonstrate the oxidative stress-causing potential of increased fertigation and reveal genotype-dependent stress tolerance due to differential expression of stress-responsive genes, such as *DHNs* that have been proven to play a role in plants' stress tolerance.

INVESTIGATION CAUSATIVE AGENTS OF TREE DECLINE IN APRICOT AND APPLE ORCHARDS IN HUNGARY

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There is no developed plant protection technology or authorized plant protection products against phytoplasmas and viruses; there is a growing problem of diseases caused by them. Phytoplasma-caused diseases are known today to affect more than 300 plant species. Among them, fruit tree orchards can be infected by several phytoplasmas, causing severe economic damage to fruit growers. Phytoplasmas not only destroy trees that grow for several years but can serve as a source of further infection. As fruit trees are propagated in a vegetative way, it is crucial to test the propagative material with sensitive and reliable diagnostic methods. Traditional diagnostic methods of fruit trees and viruses can only test the presence of a particular pathogen. However, with new metagenomics approaches, we can prove the presence of all of the presenting pathogens: phytoplasmas and other types of bacterial, fungal, and viral pathogens as well. *Candidatus* Phytoplasma mali and prunorum, causing Apple Proliferation and European Stone Fruit Yellows, are present in Hungary, and their presence is usually accused of causing the decline of the trees. Their detection method is based on nested PCR, amplifying part of the 16S rRNA gene. We hypothesized that although their presence can be one reason for the decline, it can coincide with other pathogens. To investigate this theory, we tested different pathogens in apricot and apple samples with different diagnostics methods.

Six trees showing symptoms, or any decline were chosen at an apple orchard in the Nyírség area and an apricot orchard in Somogy County. From the 12 trees, leaf samples were collected. First, we checked the presence of phytoplasmas using LAMP (Loop-mediated isothermal amplification). Trees showing decline showed the presence of phytoplasmas, while healthy-looking ones were free from this pathogen. As a next step, we used a more sensitive method, nested-PCR, to detect phytoplasmas. The result of this test agreed in the case of apples with the LAMP; while we could detect phytoplasmas even in 2 out of 3 healthy-looking apricot trees. To investigate the presence of other microbes in the samples, we launched a new HTS-based detection method. This “loop sequencing technology” can detect bacterial and fungal pathogens in the investigated plant. This method detected the presence of several bacterial and fungal microbes. Finally, we prepared small RNA sequencing libraries from both two sets of 12 samples and investigated the presence of viral pathogens. This survey revealed different viral pathogens in the declining and seemingly healthy trees, making the picture more complex. Our results proved the suitability of the new metagenomic methods for pathogen detection. However, to connect the microbes' presence and identify their role, the tree decline needs more in-depth and more systematic investigation.

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PROMOTERS OF SPATULA AND SPIRAL GENES IN *FRAGARIA VESCA*

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Promoters play a vital role in the regulation of gene expression; they are in upstream DNA regions of the genes overlapping the transcription start site (TSS) of target genes. Identification of the sequence patterns and activity mechanisms of promoters in the genome of model plants aids scientists have an in-depth understanding of gene expression mechanisms, thus advancing molecular genetic research.

This study aimed to characterize the *SPATULA* (*SPT*) and *SPIRAL* (*SPR*) gene promoters, which were isolated from diploid woody strawberry (*Fragaria vesca*) by finding specific motifs. The putative promoter region was identified with its algorithm for transcription factors and cis-acting regulatory elements based on the JASPAR2020 (Fornes et al., 2020) PLACE (Higo et al., 1999) promoter motif database. Moreover, agroinjection of micro tomato (*Lycopersicon esculentum* cv. Micro-Tom) and tobacco (*Nicotiana benthamiana*) with a binary vector including fusion of putative promoter region and *GFP* reporter gene was done. For proving that the putative promoter sequence has promoter function, transient expression analyses were performed using *Agrobacterium tumefaciens* strain C58C1 transformation with agroinjection in the case of *FvSPT* and *FvSPR* gene. Deletional promoters were inserted into plasmid pGWB604 containing *sGFP* as a reporter gene, followed by *Agrobacterium* transformation and injected into plant leaves and fruits. The determined transcription factors have roles in forming transcription apparatuses (Dof, RAV1, TGA1, HAT5) and their regulation under various environmental conditions (bZIP, SQUA, AP3). Cis elements were basically located on core promoter as INT, TATA-box, or specific motifs such as W-box, CAT-box, I box, and GATA-box were reported, their functions were also indicated. According to the results of *in vivo* experiments with GFP fluorescence, it can be stated that all isolated *FvSPT* and *FvSPR* upstream sequences have promoter activity, although the level of fluorescence was different.

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THE REGULATIVE EFFECTS OF IRON NUTRITION ON CHLOROPLAST FERRIC-CHELATE OXIDOREDUCTASE 7

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The iron deficiency of plants causes a decrease in plant biomass production and a decline in crop quality. Moreover, iron deficiency of plants is of great concern for human health since plant-based comestibles are important sources of iron. The majority of shoot iron content is localized in the chloroplasts of mesophyll cells. Chloroplasts operate a reduction-based iron uptake mechanism, with the help of a ferric-chelate oxidoreductase 7 (FRO7) as a key enzyme located in the inner envelope membrane. Though its importance, regulation of FRO7 is not yet revealed. FROs in the root are known to be extremely sensitive to iron deficiency, where iron shortage induces a magnitude higher expression and thus enzyme activity.

In contrast, FRO7 was previously described as not iron-regulated one. To investigate this question, we studied the expression pattern of FRO7, its ferric chelate reductase activity, and the chloroplast iron uptake in plants and leaf stages grown under altered iron nutrition conditions. *Brassica napus* (oilseed rape) was used as a model plant, a close relative of *Arabidopsis*, hence a reliable molecular and membrane isolation study model. The homolog of *Arabidopsis Fro7* was identified in the oilseed rape genome by reciprocal best homology. Expression pattern analysis of *BnFro7* was performed in leaves of various altered developmental and iron nutrition status by quantitative real-time PCR (qPCR). For an adequate normalization base, reference genes were selected using NCBI database and tested for expression. Based on the comparative gene expression data, iron deficiency decreases the expression of *BnFro7* in leaves, reaching the full area development. The saturation of the need for iron by supra-optimal iron nutrition resulted in a substantial decrease in the expression. The peak expression was found when leaves reached their full size; that peak was enhanced by iron deficiency. Ferric chelate reductase activity of chloroplast envelope membranes and the characteristics of the light-regulated iron uptake of chloroplasts gave corresponding results. Thus, we assume that the decreased iron uptake of chloroplasts originated from plants of saturated iron nutrition is an expression level regulation mainly. In contrast, the reduction-based iron uptake of the chloroplast is enhanced by developing the photosynthetic apparatus and iron shortage.

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MARKER-LABELED CHLOROPLAST FERRIC REDUCTASE ENZYME IN TRANSGENIC BRASSICA PLANTS

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Plants, like all other organisms, need microelements, in addition to the major macronutrients for the proper function of their cells. Among transition metals, iron (Fe) is vital for multiple redox enzymes, including photosynthesis and chlorophyll biosynthesis. The Ferric Reductase Oxidase (FRO) family of proteins is known to be essential for the iron and copper homeostasis of plants. Among the members of this protein family, the FRO7 is found in the inner membrane of chloroplasts. FRO7 participates in the reduction-based iron uptake of chloroplasts and reduces capacity (NADPH) from photosynthesis to reduce ferric-carboxylates.

Nevertheless, the physical connection between the enzyme and transporters contributing to the ferrous iron ion uptake is unclear. Thus, our project aims to understand the regulation of FRO7 and its protein-protein interactions. We aimed to establish marker fusion transgenic lines first, to detect FRO7 at the protein level in chloroplast envelope membrane fractions.

We apply a biotechnological approach to get answers to our questions. The model plants are Brassica taxa, such as Chinese broccoli (*Brassica oleracea* subsp. *alboglabra*, AGDH1012 dihaploid line). The T-DNA construct comprises the FRO7 protein gene of rapeseed (*Brassica napus*) accompanied by its native promoter and labeled with GFP or FLAG protein tags. The construct is introduced by classic *Agrobacterium*-mediated transformation. Following the extraction of the genomic DNA from the regenerated shoots, a successfully transformed plant containing the labeled FRO7 gene can be identified by PCR. In transgenic plants, the presence of the introduced construct is checked at RNA and protein levels. The microenvironment of the target protein is aimed to be identified by BluNative/SDS 2D native polyacrylamide gel electrophoresis. The proper characterization of the regenerated plants is in progress.

The experimental model provides a deeper understanding of the iron uptake mechanism of the chloroplast. By revealing the regulation and interactions of the FRO7 enzyme, we can improve the utilization of iron in plants, thereby enhancing the nutritional quality of edible green parts of plants for human consumption.

This work was supported by the grants financed by the National Research, Development, and Innovation Office, Hungary (NKFIH K-124159).

MODULATION OF WHITE LIGHT SPECTRUM AFFECTS DIURNAL CHANGES IN GLUTATHIONE-DEPENDENT REDOX ENVIRONMENT AND EXPRESSION OF ANTIOXIDANT GENES IN BARLEY

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Enrichment of the white incident light spectrum by red/far-red and blue components decreased and increased the glutathione-dependent redox potential, respectively, and modified its diurnal rhythm in barley. The transcription of the genes encoding cysteine and glutathione synthesis enzymes and various antioxidants was greatly induced by red/far-red light and decreased by blue light; however, its diurnal rhythm was only slightly affected. Thus, red/far-red light induces a more reducing environment and activates the antioxidants in contrast to blue light's inactivating effect. Besides, nitrate reduction, which is interconnected by sulfate reduction, was similarly affected by modulated white light.

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GROWTH PROMOTION IN PERLITE BASED CULTIVATION UPON SILICON TREATMENT IS PARALLELED BY EXPRESSION OF SILICATE TRANSPORTER GENES

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Silicon (Si) is known to have various beneficial effects on plant growth and stress resistance. Cucumber is one of the few dicots with a relatively high capacity for silicon accumulation. In this study, responses of *Cucumis sativus* cv. F1 hybrid 'Dirigent' plants were investigated under silicate (1.67 mM Si) treatment compared with control (Hoagland solution) in a perlite-based cultivation system.

Silicate transporter genes *CsLsi2*, *CsiT-1* and *CsiT-2* were selected *in silico* from the cucumber genome sequence. RNA from leaves was prepared using Trizol method, and RNeasy plant mini kit was used for root extraction. mRNA levels were measured with RT-PCR and RT-qPCR. Parallel studies were conducted to assess the silicate effect by measuring the contents of chlorophyll, carotenoid, and lipid peroxidation.

Expression of all investigated silicate transporter genes in both roots and shoots of cucumber was shown by RT-PCR and verified by RT-qPCR. Significant growth promotion by silicate treatment was investigated based on fresh weight. It was coupled to increased chlorophyll and carotenoid content of the leaves. Lipid peroxidation assay indicated lower oxidative stress with decreased malondialdehyde (MDA) content of 30.80 nmol g⁻¹ measured on the effect of silicate treatment compared with 41.05 nmol g⁻¹ of control.

POLYAMINES REGULATORY EFFECTS DURING TOBACCO POLLEN GERMINATION AND POLLEN TUBE ELONGATION

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Several signaling pathways are involved in pollen germination and pollen tube elongation regulation. Among others, exogenously applied polyamines were found to affect pollen maturation, pollen tube emergence, and elongation strongly. We aimed to demonstrate the relationship among exogenous polyamines, reactive oxygen species, and nitric oxide in the regulation of pollen germination and pollen tube elongation in tobacco plants (*Nicotiana tabacum*, SR1). We have found that putrescine had a somewhat positive effect on pollen tube emergence but negatively regulated its further elongation; spermidine enhanced both processes, while spermine had a negative effect on pollen germination but did not influence pollen tube growth. Furthermore, our data indicated that PAs regulate pollen germination primarily via regulating the ROS level, while tube elongation primarily influences the NO level. Taken together, our results further supported the involvement of PAs in the regulation of pollen germination and elongation affecting ROS and/or NO levels in a polyamine- and cellular-region-specific way.

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APPLICATION OF ANATASE AND RUTILE TITANIUM DIOXIDE NANOPARTICLES IN VINEYARD: RESPONSE OF ENZYMATIC AND NON-ENZYMATIC DEFENSE SYSTEM OF GRAPEVINES

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Photo-catalytically active titanium dioxide nanoparticles (TiO₂ NPs) induce the formation of different reactive oxygen species (ROS) if they are exposed to UV radiation. The ROS produced in the photocatalytic process can be a useful tool against plant pathogens; however, a high level of ROS results in oxidative stress and tissue damage. Therefore, besides the antimicrobial activity, the potential phytotoxic effects of TiO₂ NPs should also be investigated. In this work, we studied the enzymatic and non-enzymatic antioxidant responses of grapevine (*Vitis vinifera* L.) leaves exposed to TiO₂ NPs and solar UV. Two natural polymorphs of titania, anatase, and rutile, were synthesized and tested. TiO₂ NPs were characterized through X-ray diffraction and transmission electron microscopy. The UV-induced *in vitro* formation of ROS, such as superoxide anions and hydroxyl radicals, were confirmed. In-field experiments, the adaxial surface of “Cabernet Sauvignon” and “Kékfrankos” leaves were treated with aqueous dispersions of TiO₂ NPs (0.001, 0.01, and 0.1%). After two weeks of treatment, the level of caftaric acid and flavonol glycosides (main stress-responsive phenolics of grapevine) and superoxide dismutase (SOD) activity in grapevine leaves were measured. We found that neither anatase nor rutile treatment altered the flavonol glycoside contents. Also, the SOD activity did not change significantly. At the highest dosage (0.1%) of TiO₂ NPs, only the caftaric acid content decreased significantly. Our results show that foliar application of phase pure TiO₂ NPs with appropriate conditions can be applied on the leaves without causing strong oxidative stress. Therefore, exogenous ROS produced by TiO₂ NPs on the leaf surfaces may be exploited in plant protection for the inactivation of various pathogens.

DAYTIME-DEPENDENT REGULATION OF FLAGELLIN-INDUCED DEFENCE RESPONSES IN TOMATO PLANTS

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Recognition of microbe-associated molecular patterns (MAMP) is among the first processes to induce plant immune responses. To get an insight into the process of infection, one of the best-characterized and generally used elicitor is the flagellin22 (flg22), a conserved peptide of the bacterial flagellum. However, the daytime-dependence of flg22-induced defence responses is less known. Here we studied the effects of flg22 treatments at different daytimes on the signaling of ethylene (ET), jasmonic acid (JA), and salicylic acid (SA) in the leaves of intact tomato plants. As the first hours after flg22, perception seems to have crucial importance from the perspective of inducing many defence responses in plants, signaling events, and defence responses after flg22 treatments in the afternoon (at 5:00 p.m.) and at night (at 9:00 p.m.) were recorded one hour later. Flg22 induced the rapid closure of stomata, in a daytime-dependent manner. Production of reactive oxygen species (ROS) and nitric oxide (NO) also showed different patterns after flg22 treatments in the afternoon and night. Nor ET emission neither NO production was significant in the leaves treated in the dark phase. Significant differences were also observable in these daytimes in the accumulation of JA and SA, moreover in the expression of hormone response genes, *Pathogenesis-related 1 (PR1)*, *Ethylene response factor 1 (ERF1)*, and *Defensin (Def)* after flg22 treatments. Surprisingly, we detected a rapid systemic response of intact plants in the distal leaf levels of flg22-treated leaves. Our results confirm the importance of the defence hormones and the daytime and in the response of intact plants to bacterial elicitor treatments.

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INVESTIGATION OF THE GROWTH AND HYDROGEN PRODUCTION OF MICROALGAE STRAINS IN STARCH-CONTAINING MEDIA

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Our experiments aimed to investigate the growth and hydrogen production of *Chlamydomonas reinhardtii* cc124 and *Chlorella* sp. MACC 360 algae strains using starch-containing media and bacterial partners.

The products of starch degradation can serve as extra nutrients for photoheterotrophic algae growth. The *Bacillus amyloliquefaciens* bacterial partner completely degraded the applied amount of starch (6 mM) within 48 hours, while *Enterobacter* sp. had limited starch degradation capability. The applied bacterial strains did not produce hydrogen either in TAP (Tris-Acetate Phosphate) or starch-containing TAP.

The presence of *B. amyloliquefaciens* in starch-containing TAP stimulated the growth of *C. reinhardtii* strain, while it was unable to exert such an effect in TAP media. At the same time, *B. amyloliquefaciens* inhibited the growth of *Chlorella* sp. both in TAP and in starch-containing TAP.

The heterotrophic bacteria efficiently respire oxygen during growth, thereby creating ideal green algal hydrogen production conditions. *Chlorella* sp. could produce hydrogen both under axenic conditions and co-cultured with bacteria either in TAP or starch-containing TAP. However, *C. reinhardtii* algae did not produce any hydrogen under axenic conditions in TAP; nevertheless, algae-specific hydrogen production was observed when co-cultured with *Enterobacter* sp. bacteria. The *Enterobacter* sp. partner stimulated hydrogen production to the greatest extent in both algae strains.

The presence of the *B. amyloliquefaciens* partner stimulated the growth of *C. reinhardtii* algae. In contrast, the presence of the *Enterobacter* sp. did not influence the growth of *C. reinhardtii*, even so, *C. reinhardtii* co-cultured with *Enterobacter* sp. resulted in the highest observed algal hydrogen production.

The experiments served as a laboratory model for investigating the photoheterotrophic wastewater treatment efficiency; in our experimental system, we applied a combination of biodegradation of organic substances and concomitant biohydrogen production.

EXPRESSIONAL AND FUNCTIONAL ANALYSIS OF GIGANTEA IN POTATO (*SOLANUM TUBEROSUM* L.)

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Potato is a widely cultivated crop in the world. It has been known that the flowering and tuberization in potato are affected by several external and internal factors. Long day acclimatization and tuber bulking were essential traits for the commercialization of potato varieties. The GIGANTEA (GI) plant protein participates in various plant functions like photoperiodic responses, flowering, stress regulations, and the circadian rhythm in *Arabidopsis* and many other plant species. Recently, it has been shown that the downregulation of *GI* enhances stress adaptations and influences the tuberization pathway regulating the expression of the tuberigen *StSP6A* in *Solanum andigena*, an obligate short-day potato requiring long nights for tuberization. Our research aimed to study the function of *StGI* in *Solanum tuberosum* cultivar ‘Désirée,’ a plant cultivar permissive for tuber initiation under long days.

We found that *StGI* possesses approximately 70% identity to the well-characterized *Arabidopsis* GI at a nucleic acid level based on database search. Phylogenetic analysis revealed that *StGI* has the highest relationship to *I. batatas* GI at the protein level. A high level of *StGI* expression was detected in all *in vitro* plants' organs and was organ-specific in greenhouse-grown plants with the highest expression in flower organs. The *StGI* expression, like in other plant species, was found to be circadian regulated in ‘Désirée.’ Nevertheless, unlike in other plant species, *StGI* expression was not induced by osmotic and heat stresses, only a slight induction in its transcript level could be detected in leaves by salt and cold stresses. For functional analysis, a fragment of *StGI* was cloned in a binary vector in antisense orientation behind a strong constitutive promoter, and transgenic potato plants were generated by *Agrobacterium*-mediated transformation. Leaves of 36 putative transgenic *in vitro* plants were tested for *StGI* expression. RT-PCR identified Fourteen plants with reduced *StGI* expression. Based on qRT-PCR, three lines with 60-70% reduction in *StGI* expression were selected for further analysis. The plants were propagated *in vitro*, transferred to pots, and grown in a greenhouse. No phenotypical differences between the non-transformed and *StGI*-repressed plants were detected in the vegetative growth period of plants. No significant differences in the time of tuber initiation, tuber number, and yield were obtained either. Thus, it was concluded that a higher level of repression in *StGI* expression than 70% is needed for influencing tuberization, or the function of *StGI* may not be as important in the long-day permissive tuberizing ‘Désirée’ as it is in the obligate short-day tuberizing *Solanum andigena*.

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**SILVER NITRATE DECREASED THE EFFICIENCY OF DIRECT
ORGANOGENESIS THROUGH THE DOWNREGULATION OF
POLYAMINE BIOSYNTHESIS GENES IN *ARABIDOPSIS THALIANA***

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Plant organogenesis in vitro is one process during which de novo organs, such as buds, roots, and shoots, can be produced from cultured tissues under certain physical and chemical conditions. During this process, the establishment of apical meristems developing into shoots and roots, respectively. It can occur either directly or indirectly through callus formation.

In our laboratory, we established a direct regeneration system to induce organogenesis in *Arabidopsis*. To do this, we used a root culture-based regeneration system. In this system, first lateral root primordia (LRP) were induced by auxin. Then, the induction of LRP into shoot meristem (SM) was initiated by cytokinin. Culturing the explants for a further 2-3 weeks on cytokinin rich medium regenerated plantlets appeared on the roots' surface.

We have found that silver nitrate affected direct organogenesis by altering the expression of polyamine biosynthesis genes.

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STUDY OF GENES GOVERNING THE ANTHOCYANIN BIOSYNTHETIC PATHWAY IN PURPLE *CAPSICUM* MUTANTS

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Modulations in gene expression throughout ripening trigger color change in the fruits of *Capsicum* to a great extent. Pepper berries having naturally high anthocyanins levels could be beneficial because of their nutraceutical values that these polyphenolic molecules possess. Anthocyanin accumulation of pepper is regulated by the locus *A* encoding a MYB transcription factor, which is found to govern the expression of the pathway's late structural genes together with a WD-repeat protein and a MYC transcription factor.

RT-PCRs were carried out to determine the gene expression during 5 stages of ripening. The expression of the studied MYC transcription factor – although its level was comparable between the anthocyanin pigmented and anthocyaninless (*pax - partially anthocyaninless*) berries - in all cases decreased throughout ripening. In contrast, the expression of the WD-repeat protein remained stable in the studied phenophases indicating that the gene's basal expression might be ample for the anthocyanin biosynthesis. Besides the expression of the so called MYBa 2, putatively more positive R2R3-MYB regulators of the pathway were examined. In the mutant genotypes, their expression coincided with the immature phenophases, where the berries showed purple coloration; no expression was detected in the later phases. However, in the extreme lilac positive control, their expression was scorable in all phases. A putative repressor R2R3-MYB was also investigated; its expression corresponded to the mutants' unripe phases and positive control. However, in the white berried negative control, the expression of the repressor MYB was detectable in all phases.

Interestingly, in the case of the negative control, the positive regulators were present in each phenophases as well. Thus we suppose that other mechanisms such as post-transcriptional gene silencing may also play a role in the regulation. We are planning to involve more genes in the study that could affect the immature economically ripe and the mature biologically ripe fruit color of the berries.

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IN VITRO MICROPROPAGATION OF *STEVIA REBAUDIANA* BERTONI ON DIFFERENT MEDIA

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Stevia rebaudiana is a natural sweetener containing crop produced in large quantities around the world. The plant bears few seeds with a considerably low germination percentage, justifying an alternative propagation method. Vegetative propagation is limited, and it is not suitable to acquire genetically identical plants for experiments. To produce large amounts of clonal plants, *in vitro* micropropagation was recommended, and despite considerable efforts, there is still room to improve the existing methods. In our study, we tried to compare and improve existing *in vitro* micropropagation methods of *Stevia rebaudiana*.

Two plant explants were selected for micropropagation: 3-4 cm long apical explants and five nodes-long nodal explants. Both types of explants were surface sterilized, cut into 2 cm long segments, and deprived of mature leaves. Explants with the cut surface were put in sterile shoot inducing Murashige-Skoog media (pH 5.6) containing 1.5 mg/l 6-benzylaminopurine. Newly formed shoots were transferred to four types of sterile root inducing media containing indole-3-acetic acid (IAA) or naphthalene acetic acid (NAA). After eight weeks on root inducing media, plants were ready for acclimatizing in a non-sterile environment. Acclimatization was performed in three types of media: 1) sterile soil, 2) sterile soil:perlite (1:1), and 3) vermiculite, peat moss, and sand (1:1:1). Explants were removed from root inducing media and gently washed with tap water. Plant roots were submerged in Ridomil Gold fungicide and planted in a pot containing one of the three types of acclimatization media. Plant pots were covered in transparent polyethylene bags to ensure high humidity. After two weeks, we started gradually reducing the humidity until plants survived under normal atmospheric conditions.

Our experiments compared different micropropagation methods to evaluate the effectiveness of different phytohormones on *Stevia* regeneration. NAA-containing media was not able to induce root development; on the contrary, the IAA induction was successful. Acclimatization of *in vitro* grown plant material was a 100% success; all plants survived the standard conditions for six months, indicating that the applied methods for *in vitro* micropropagation of *Stevia rebaudiana* were effective.

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THE EFFECT OF SALT STRESS ON THE VEGETATIVE PROPAGATION, PHOTOSYNTHESIS, AND ESSENTIAL OIL COMPOSITION OF SPEARMINT

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High soil salinity is an increasingly threatening global problem that strongly affects plant growth, reproduction, photosynthesis, and agriculture. The reduction in arable lands suitable for crop production is especially problematic due to the continually increasing human population (7.8 billion people in 2020) that needs to be fed. However, the question can be raised whether medicinal or aromatic plants can be cultivated in some salt-affected areas that became unsuitable for salt-sensitive staple crops.

To answer this question, we tested adventitious root formation, analyzed the photosynthetic activity, pigment contents, organization of the chlorophyll-protein complexes of the photosynthetic apparatus and the ultrastructure of chloroplasts, as well as the essential oil composition of the leaves in cuttings of *Mentha spicata* L. var. *crispa* 'Moroccan' plants grown in water (as control), or in water supplemented with 5 mM, 25 mM and 50 mM NaCl for two weeks.

Adventitious root formation was inhibited only at 50 mM NaCl concentration, indicating that spearmint's vegetative reproduction might be hindered in soils with similar or higher salinity levels. On the other hand, no changes were observed in the leaves' 77K fluorescence spectra at any of the used salt concentrations, showing that salt stress did not affect the chlorophyll-protein complexes' organization of the photosynthetic apparatus. However, the chlorophyll a and b content of the leaves significantly decreased in plants grown in 25 and 50 mM NaCl solution, and the actual and maximal quantum efficiencies of the photosynthetic apparatus also slightly declined in the leaves of plants grown in 50 mM NaCl. Ultrastructural analyses revealed the slight swelling of the intrathylakoidal space in samples treated with 50 mM NaCl, indicating the salt-stress induced disturbance of plastid structure and function. On the other hand, solid-phase microextraction and gas chromatography-mass spectroscopy data showed no significant differences in the plants' essential oil composition grown at various salt concentrations. Our data indicate that soils with moderate salt concentration may be suitable for spearmint cultivation.

SALICYLIC ACID EXPRESSION CHANGES TO THIAMETHOXAM TREATMENT IN MYCORRHIZAL TOMATO PLANTS

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Arbuscular mycorrhiza fungi (AMF) not only increase available nutrients for plants, but they also help their symbiotic partners to overcome biotic and abiotic stresses. One pathway to achieve this tolerance increase is the activation of salicylic acid-controlled stress responses.

A pot experiment was set up in Martonvásár to study how AMF alleviates the stress caused by pesticides. Tomato plants were inoculated with AMF and treated with a plant protection product containing thiamethoxam as an active agent. The experiment consisted of four treatment groups: Control plants without any treatment; Mycorrhizal plants inoculated with AMF; Pesticide treated plants sprayed with thiamethoxam, and Mycorrhizal and Pesticide treated plants receiving both treatments.

Different plant physiological parameters like root, shoot biomass, and fruit weight was measured and compared among treatments. SA biosynthetic enzyme (isochorismate synthase - ICS) has been examined by qRT-PCR, and relative quantification of the results was done with the method of $2^{-\Delta\Delta CT}$.

According to the qRT-PCR results, ICS expression showed a significant difference in all treatments, with the highest alteration in pesticide-treated plants. This change was significantly lower in inoculated plants.

Our results indicate that the arbuscular mycorrhiza inoculation does alter how plants react to pesticides, and the expression of crucial salicylic acid biosynthetic enzyme is affected by the symbiotic interaction.

MARKER BASED GENOTYPING OF 'FURMINT' AND 'OLASZRIZLING' VARIETIES AND CLONES

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Molecular markers are potent tools to discriminate genotypes at the DNA level. In this study, three different marker techniques were used to analyze 'Furmint' and 'Olaszrizling' varieties and clones. For characterizing grape genotypes, microsatellite (SSR - Simple Sequence Repeat) markers have been applied first since they exhibit co-dominant inheritance, and the analyses based upon them are relatively simple procedures. Nine SSR primer pairs – recommended by the GrapeGen 06 European project – were used in our investigation. Neither the 12 'Olaszrizling' nor the 41 'Furmint' clones could be discriminated from each other by microsatellite analysis; they all displayed an identical pattern.

The inter-primer binding site (iPBS) marker technique has been successfully used in diversity assessment and cultivar or clone discrimination in grape. Therefore, in the case of Altering, Pink- and White-berried 'Furmint' varieties, 10 'Furmint' and 12 'Olaszrizling' clones, iPBS primers have also been applied. The iPBS amplification method's basics are the almost universal occurrence of a reverse transcriptase primer binding site in the LTR retrotransposons. Among the selected primers, iPBS 2277 showed a distinctive band in several 'Furmint clones' while in the case of 'Olaszrizling' the iPBS 2021 generated 3 polymorphic bands in 5 clones. These bands were cut out from the agarose gel, purified, and sequenced. Based on the sequence differences, clone specific markers will be designed.

Moreover, the amplified fragment length polymorphism (AFLP) technique was also applied to differentiate grapevine clones. Until now, we analyzed Variable, Pink-berried, and White 'Furmint' as well as 3 'Furmint' and 5 'Olaszrizling' clones. We are planning to sequence the potentially appearing polymorphic bands.

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ALTERNATIVE METHOD FOR THE TRANSFORMATION OF CAPSICUM SPECIES

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Capsicum species have high economic value as vegetable crops and medicinal plants in various countries, and most of these species are known to be recalcitrant to genetic transformation with *Agrobacterium tumefaciens*. The genetic improvement of plants against pathogens requires discovering resistance genes and revealing their functions and working mechanisms. The development of a new transformation method could serve this purpose. However, pepper recalcitrance to genetic transformation while using *A. tumefaciens* and its low ability to regenerate *in vitro* are significant problems for studying different genes' functions in these species. *Agrobacterium rhizogenes* mediated transformation can serve as an excellent alternative way for the transformation of *Capsicum* species, while it is also a rapid and new possibility for studying gene functions in roots. *A. rhizogenes* has a Ri (Root-inducing) plasmid carrying *rol* genes, which make the so-called „hairy root” phenotype. It also can carry a binary vector containing the gene of interest to be transferred into the host plants. The method, which does not need a regeneration step, is extensively used to produce secondary metabolites and study gene functions involved in root nodulation and plant development. It was also served to confirm the functions of genes suspected of taking part in the resistance against root-specific pathogens. Previous experiments on root transformation mostly focused on other *Solanaceae* or *Fabaceae* members or used the hypocotyls of *Capsicum frutescens* species, so we planned to introduce a highly efficient root transformation method in cultivated *Capsicum annuum* species. We already successfully validate the functionality of our method on *Lycopersicon esculentum* and *Medicago truncatula* plants. Our goal is to obtain a highly efficient transformation system that can be used to study different genes' functions in *Capsicum annuum* varieties. Our study's further goal is to validate and describe candidate genes involved in resistance against root-knot nematode species. Since these nematodes are soil-borne pathogens, *A. rhizogenes* mediated „hairy root” transformation could be a promising method to confirm resistance genes' function in *Capsicum* species.

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IS THE CELL MEMBRANE MODIFICATION A NOVEL POSSIBILITY IN THE PAIN MANAGEMENT?

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Primary sensory neurons express a wide range of nociceptive receptors, like Transient Receptor Potential Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1/A1). Natural materials [Capsaicin (CAPS), resiniferatoxin (RTX)], noxious heat, endogenous metabolites can activate TRPV1. TRPA1 can activate by mechanical stimuli, formaldehyde, noxious cold. Lipid rafts are specialized microdomains of the membrane, filled with cholesterol, sphingomyelins, and gangliosides, and constitute functional complexes with TRP channels. Sphingomyelinase (SMase) hydrolyses the sphingomyelin content, while Myriocin (Myr) blocks the *de novo* synthesis. We examined the potential antinociceptive effect of our compounds in several *in vivo* pain models in mice. We also tested some *in vitro* features with Laurdan fluorescent spectroscopy. Both had a significant effect in the experiments; however, Myr's effects were weaker than SMase's in all pain model. The fluorescent spectroscopy revealed that Myr modified the Laurdan spectra, while SMase did not. Based on our results, we assume that these interactions between the TRP channels and lipid rafts might be a promising drug target in the future.

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THE TARGETED LHRH ANALOG AEZS-108 ALTERS EXPRESSION OF GENES RELATED TO ANGIOGENESIS AND DEVELOPMENT OF METASTASIS IN UVEAL MELANOMA

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Uveal melanoma (UM) is the most common malignant tumor of the eye. Recently, we have established that 46% of UM specimens express LHRH receptors. This finding supports the idea of an LHRH receptor-targeted therapy for UM patients. Cytotoxic analog of LHRH, AEZS-108 exhibits effective anti-cancer activity in LHRH-receptor positive cancers. AEZS-108 is a hybrid molecule composed of a synthetic peptide carrier and the cytotoxic doxorubicin (DOX).

In the present study, we investigated AEZS-108 induced cytotoxicity and the altered mRNA expression profile of regulatory factors related to angiogenesis and metastasis in LHRH receptor-positive OCM3 cells.

Our results show that AEZS-108 upregulates the expression of *MASPIN/SERPINB5* tumor suppressor gene, which is downregulated in the normal uvea and UM specimens independently from the LHRH receptor-ligand interaction. AEZS-108 also substantially downregulates hypoxia-inducible factor 1 alpha (HIF1A) expression. For investigating the mechanism of the induction of *MASPIN* by AEZS-108, OCM3 cells were treated with free DOX, D-Lys⁶ LHRH analog, or AEZS-108. qRT-PCR analysis revealed in OCM3 cells that AEZS-108 is a more potent inducer of *MASPIN* than free DOX.

In conclusion, we show for the first time that AEZS-108 has a significant impact on the regulation of angiogenesis, thus plays a potential role in tumor suppression.

Our results support the development of novel therapeutic strategies for UM, focusing on LHRH receptors.

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X-RAY CRYSTALLOGRAPHY, FUNCTIONAL DYNAMICS, AND STRUCTURE-BASED ENGINEERING OF CONSERVED SUBSTRATE CHANNELS TO ALTER MIO-ENZYMES

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The MIO-containing class I lyase-like enzyme family (MIO-enzymes) constitute two functionally diverse but structurally related enzyme classes, the aromatic amino acid ammonia-lyases (ALs) and the aromatic amino acid 2,3-aminomutases (AMs). ALs catalyze the reversible ammonia elimination from histidine, phenylalanine, or tyrosine (HALs, PALs, and TALs, respectively), using a unique post-translationally formed residue, the 5-methylene-3,5-dihydro-4*H*-imidazol-4-one (MIO) as the catalytic electrophile. AMs catalyze the interconversion between α - and β -phenylalanine or α - and β -tyrosine (PAMs and TAMs, respectively). Application possibilities of MIO-enzymes are manifold, ranging from synthetic bio-transformations to direct human therapy. The native promiscuity and broad substrate scope of numerous MIO-enzymes expedite their use as biocatalysts on a laboratory and industrial scale. The enzyme substitution therapy with PAL for the treatment of phenylketonuria represents a further extension of applications. The development of such critical applications requires a comprehensive understanding of the reaction mechanism and the structure-function relationships of MIO-enzymes.

Till now, loop residues were not visualized in crystal structures of eukaryotic phenylalanine ALs (PALs), while their localization in eukaryotic phenylalanine AMs (PAMs) implicated that loop conformation dissects lyase *versus* mutase functionalities. Our latest results provide three-dimensional structures for *Petroselinum crispum* PAL (PcPAL) with clear-cut electron densities to define the catalytically competent “loop-in” conformation, enabling reliable computational modeling of Tyr-loop opening during ligand egress. Comparisons of inner-loop dynamics between PcPAL and *Taxus canadensis* PAM (TcPAM) revealed quite a similar ligand egress routes requiring only small loop motions. Substrate access/product exit paths in eukaryotic PcPAL/TcPAM seem to be conserved within the whole MIO-enzyme family.

Based on these results, site-directed mutations were investigated in PcPAL and TcPAM, altering the access paths. As a result of influencing the ligand access and egress paths, the PAL *versus* PAM activities were modified. Furthermore, the substrate- and enantio-preference of the enzymes changed as well.

GENE EXPRESSION PATTERN OF HUMAN PRIMARY ADIPOCYTES DURING BEIGE TO WHITE TRANSITION

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Obesity is one of the major risk factors for cancer, type two diabetes, and cardiovascular diseases. We distinguish three types of adipocytes: the energy-storing white and the two thermogenic classical brown and beige/brite adipocytes. Both heat-producing adipocytes have multilocular lipid droplets, increased mitochondrial mass, and express a high level of uncoupling protein 1 (UCP1). Furthermore, they can promote energy expenditure and can be potential targets for treating metabolic disorders and obesity.

This study followed up the beige to the white conversion process in primary human subcutaneous abdominal adipocytes with different FTO genotypes (rs1421085). Our future aim is to find an optimal condition to study the process with a system biology approach.

The long-term (28 days) browning differentiation, driven by the PPAR γ agonist rosiglitazone, increased UCP1 gene expression compared to the 14 days long short-term protocol. The dibutyril-cAMP treatment caused a further increase in the browning markers' gene expression levels (UCP1, CIDEA, and PGC1a). However, the cAMP stimulus did not cause changes in the CKMT1 and CKMT2 expression. The expression level of the white marker Leptin was decreased in response to the cAMP treatment. After mid-term cAMP stimuli (4 h long treatment at day 14), we could maintain or even enhance the thermogenic competency of the beige adipocytes by utilizing the browning protocol. However, when we applied the white protocol after day 14, the beige to white conversion was observed in these samples.

In the future, we intend to perform UCP1 immunostaining and laser-scanning cytometry measurements on differentiated adipocytes and to increase the sample size with primary preadipocytes from newly isolated samples. We also aim to collect cells for Western-blot analysis and do functional experiments on Seahorse oxymeter. It would enable us to find the optimal models of white, active, and masked beige adipocytes *ex vivo* for future systematic analyses.

CENTRAL SENSITIZATION AND NEUROINFLAMMATION ARE MEDIATED BY TNF- AND IL1-DRIVEN PATHWAYS IN A TRANSLATIONAL MOUSE MODEL OF COMPLEX REGIONAL PAIN SYNDROME (CRPS)

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Complex Regional Pain Syndrome (CRPS) is a chronic pain condition characterized by a continuing (spontaneous and/or evoked) regional pain and autonomic dysfunctions. CRPS is believed to be caused by damage to or malfunction of the peripheral and central nervous systems. The therapy is unsatisfactory. Therefore, there is a need to explore the pathophysiological mechanisms and identify drug targets.

Female C57Bl/6 mice were treated daily with purified serum-IgG from CRPS patients or healthy volunteers following plantar skin-muscle incision. Mechanonociceptive threshold, edema, myeloperoxidase activity (MPO), inflammatory cytokines, microglia and astrocyte markers in pain-related brain regions were analyzed. Fractalkin receptor (CX₃CR₁) gene-deficient (KO) mice were used to investigate microglia activation mechanisms. The effects of Janus kinase inhibitor tofacitinib, or the anti-tumor necrosis factor (TNF) agent etanercept were also investigated.

We detected significantly greater mechanical hyperalgesia and paw edema developed after incision in CRPS IgG-treated mice accompanied by a significant increase of astrocyte and microglia markers. MPO activity increased in the early phase, while the concentration of the inflammatory cytokines are not changed. CX₃CR₁ gene deficiency, as well as tofacitinib and etanercept, significantly reduced CRPS IgG-induced hyperalgesia and neuroinflammation. Anakinra treatment decreased myeloperoxidase activity and glia activation as well.

Our results suggest that autoantibody-induced neuroinflammation and central sensitization plays a crucial role in persistent CRPS-related pain where the critical factor is the glia activation via CX₃CR₁, TNF-signaling, and JAK pathways. Blocking these receptors or inhibiting the signal transduction may represent new therapeutic perspectives.

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NEW ENGLAND BIOLABS – „BY SCIENTISTS FOR SCIENTISTS”

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A New England Biolabs, egy laboratóriumi kutatók által az 1970-es évek közepén alapított cég, amely az élettudományi kutatásokhoz kínál olyan megoldásokat, amelyek a minden napi munka nehézségeit és kihívásait hívatottak csökkenteni. Saját tapasztalataikból és problémáikból merítkezve dolgozták ki a fejlesztéseket azért, hogy méréseik gyorsabbak, pontosabbak és könnyebbek legyenek. A családi kis cégből mára már világvezető óras vállalat fejlődött ki, amely vezető a molekuláris biológiai applikációkhoz szükséges enzimek fejlesztésében és termelésében. A gyártó mentalitása növekedés ellenére ugyan az maradt és igyekszik folyamatos segítséget nyújtani a kutatóknak, akik a laborban ugyan úgy szembesülnek nehézségekkel és fogalmaznak meg technikai kérdéseket. Számos területen biztosítanak oktatásokat ingyen, szakmai tanácsokat töltenek fel Instagram és Facebook videók formájában, így nem csak rendkívül jó termékeket állítanak elő, de segítik a Professzortól a TDK hallgatóig az összes olyan kutatót, aki tanulni szeretne.

A New England Biolabs a rekombináns és natív enzimek legnagyobb választékát kínálja a gemonikai kutatásokhoz. De a lenti módszerek fejlesztésében is jeleskedtek az elmúlt években:

- különböző új módszerek a klónozáshoz,
- DNS polimerázok változatos felhasználásokhoz
- génexpressziós vizsgálatokhoz qPCR mastermixek, reverz transzkriptázok,
- enzimek és kitek genom editáláshoz,
- új generációs szekvenáláshoz mintaelőkészítés és könyvtárkészítő kitek,
- fehérje expresszióhoz és tisztításhoz anyagok,
- RNS elemzéssel kapcsolatos termékeket

A BioTech Hungary Kft. 2019 januárjától forgalmazza a New England Biolabs teljes termékínálatát. Előadásunk során szeretnénk bemutatni a gyártó már jól ismert termékei mellett azokat az újdonságokat, amelyeket az elmúlt években fejlesztettek. Illetve szeretnénk megmutatni azokat a felületeket, ahol az ingyenes módszertani oktató videók elérhetők.

INVESTIGATION OF THE MUTATOR EFFECTS OF VARIOUS GENOTOXIC STRESSES PROVIDES INSIGHT INTO THE MECHANISM OF DRUG RESISTANCE DEVELOPMENT IN MYCOBACTERIA

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Tuberculosis is still one of the top 10 causes of death worldwide despite century-long efforts to combat it. Also, multidrug-resistant tuberculosis denotes a public health crisis. WHO estimates that there were over a half-million new cases in 2017 with resistance to rifampicin – the most effective first-line drug - of which 82% had multidrug resistance. Horizontal gene transfer is a central molecular mechanism responsible for generating genetic diversity and plays a particularly important role in driving the evolution of drug resistance in most bacteria. However, mycobacterium strains acquire antibiotic resistance merely by single point mutations. Interestingly, in contrast to the remarkable genomic diversity displayed in isolates from tuberculosis patients, mycobacteria's basal mutation rate is very low *in vitro*, suggesting that genotoxic stress and harsh conditions within the host cell may elicit transient or constitutive mutator phenotypes. We promote that the detailed understanding of the mutator effects of various genotoxic stresses would provide insight into the mechanism of drug resistance development and allow better management of current therapeutics.

To investigate the mutator effect of different environmental stress factors (*e.g.*, oxidative stress, starvation, hypoxia, alkylating agents, UV) or currently used tuberculosis drugs (first and second-line antibiotics), we analyzed the mutation rates and the spectrum of mutations by whole-genome sequencing. We also correlated the changes in the expression pattern of the DNA repair enzymes of *Mycobacterium smegmatis* upon genotoxic stress to these data. Correlating dNTP pool imbalances with a defined mutator effect and the activated DNA repair responses yields unprecedented insight into the mechanisms underlying mutagenesis by genotoxic stress.

CORRELATIONS BETWEEN THE EXPRESSION OF ANGIOGENIC FACTORS AND STEM CELL MARKERS IN HUMAN UVEAL MELANOMA

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In recent years, an exponentially growing number of studies have focused on identifying cancer stem cells (CSCs) in human uveal melanomas (UM). Their frequency in primary tumors has been correlated to angiogenesis and metastasis, and in turn, with disease prognosis. In the present study, we aimed to evaluate the expression of cancer stem cell markers and angiogenic factors in UM and investigate the relationships and correlations between the detected stem cell markers and vasculogenic mimicry patterns. We have also analyzed the correlation between CSCs markers and angiogenic factors and their contribution to patient survival.

70 uveal melanoma samples have been collected for clinical, pathological, and molecular data analysis. The expression of CSCs markers, e.g. FZD6, Nestin, NGFR, SOX10, PROM1, and major angiogenic factors, e.g., VEGFA, HIF1 α of the samples was analyzed by RT-PCR, Western blot, and immunohistochemistry using tissue microarray technique.

Spearman statistical analysis showed a statistically significant, strong correlation between the CSCs marker FZD6 and the angiogenic factor VEGFA expression in UM samples. However, their expression did not show a significant association with patient survival.

Taken together, the significant correlation between FZD6 and VEGFA we assume the presence of special cancer cells with a primitive neural/ectodermal stem cell-like phenotype that is in association with the vascularization and spread of the primary tumors.

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EXAMINATION OF LONGITUDINAL CORRELATION OF MULTI-WALLED CARBON NANOTUBES (MWCNTS), OBSERVATION OF THEIR GENO- AND CYTOTOXIC EFFECTS DURING CORNEAL REGENERATION

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Carbon nanotubes are ultrafine particles in size range of 1-100 nm and organized graphene sheets consisting of one or more layers. Due to their unique structural, physical, and chemical properties, their application in industry is significant. At the same time, they offer promising medical and biological applications, but their safe use in these areas requires reliable toxicological studies.

Industrial grade multiwalled carbon nanotubes (MWCNTs) were used in our experiments. Nanoparticles of this type are present in the combustion products of fuels and carbon fiber components used to reinforce some vehicles' plastic elements. Our study aimed to investigate eye injuries caused by car accidents in the presence of carbon nanotubes, using a long-term *in vitro* regeneration scratch model, where different concentrations (5-100 $\mu\text{g ml}^{-1}$), diameter (10-50 nm), and length (0.5 - 30 μm) MWCNTs were tested for toxic effects.

Our research was carried out on human limbal (HuLi) transient amplifying cells. Near-infrared time-lapse video microscopy was used to monitor the effect of nanotubes on the regeneration process. The images thus obtained were quantified by digital image analysis. Examination of chromatin condensation intermediates provides information on genotoxicity. The examination of the chromosome structures is made possible by fluorescence staining using a microscope.

Our studies have confirmed that these nanoparticles can enter the wound during exposure to ocular injuries where, depending on the concentration, they can cause poor healing of the cornea. By their physical presence, they can induce cell lawn assembly and partial inhibition of cell proliferation; and extend the time of division compared to control samples. In most cases, MWCNTs prevented the formation of final metaphase chromosomes during nuclear condensation.

The prolonged regeneration and slower, scarred healing of the injured cell lawn and the disruption of the chromatin condensation process may be further enhanced by the aggregation phenomenon of carbon nanotube length. In our experience, the length of carbon nanotubes is proportional to the size of their micro-aggregates. However, increased aggregate sizes (300-9000 μm^2) showed less genotoxic and cytotoxic effects than their shorter variants, where aggregates fell in the range of 250-350 μm^2 .

EFFECT OF SHIKONIN ON THE EXPRESSION OF ONCOGENIC miRNAs IN HUMAN RENAL CANCER CELL LINES

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Renal cell carcinoma (RCC) is the sixth most frequently diagnosed cancer in men and the 10th in women, accounting for 5% and 3% of all oncological diseases. Tyrosine kinase inhibitors, including sunitinib, have been introduced in RCC treatment because of their anti-angiogenic properties. However, in recent years, sunitinib use has been less effective, especially in patients with metastatic renal cancers. Therefore, searching for new therapeutic options and investigating this malignancy's underlying molecular mechanisms are of great importance.

The objective of the present study was to analyze the cytotoxic effect of Chinese plant extract, shikonin (5,8-dihydroxy-2-[(1R)-1-hydroxy-4-methylpent-3-enyl] naphthalene-1,4-dione) on CAKI-2 and A-498 human renal carcinoma cell lines. Moreover, we aimed to compare the efficacy of shikonin with sunitinib. Also, we studied whether shikonin exerts an effect on the expression of some specific oncogenic miRNAs (miRNA-21, miRNA-155, miRNA-223) and their specific targets (e.g., PTEN, Bcl-2) after 6 and 24 h treatment. For *in vitro* assay, shikonin was used at concentrations of 1-40 µM. The Cell Titer-Blue cell proliferation test (Promega) was used to detect the compound's cytotoxic activity. The effect of shikonin on miRNA expression was tested with specific stem-loop primers by qRT-PCR. Shikonin showed a dose-dependent effect on both human renal cancer cell lines examined.

In contrast to sunitinib, shikonin treatment resulted in significantly more potent inhibition of cell proliferation *in vitro* at 2,5 µM concentration. Given the miRNAs we tested, compared to untreated control samples, there was a slight increase in miRNA-21 and miRNA-223 expression in the CAKI-2 cell line, whereas miRNA-155 showed no expression change. In the A-498 cell line, a slight increase in the expression of miRNA-21, miR-155, and miRNA-223 was found compared to untreated control cells. Based on our results, we might suppose that shikonin-induced miRNAs may inhibit cell proliferation by affecting signaling pathways through their specific targets. miRNAs may also influence the therapeutical sensitivity of the cells.

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CHARACTERIZATION OF P120-GAP MUTANTS THAT ACCELERATE THE IMPAIRED GTPASE ACTIVITY OF ONCOGENIC KRAS MUTANTS

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KRAS is a guanine binding signaling protein, which works as a molecular switch in controlling cell growth, differentiation, and proliferation. GTP-bound KRAS is an active conformation that can interact with the downstream effectors, like Raf-kinase, while in the GDP-bound state, the signaling decays (Milburn et al., 1990). Specific mutants of KRAS are locked in the active state, causing permanent signaling, which results in uncontrolled cell division and cell growth, leading to oncogenic transformation. Mutant KRAS proteins are amongst the most prominent oncogenes and play a significant role in almost 25 % of all human cancers. Those tumorous malignancies caused by KRAS mutations, such as pancreatic and lung cancer, are generally difficult to treat (Prior et al., 2012).

KRAS has an intrinsic GTPase activity, but it is relatively low ($k_{cat} = 68 \times 10^{-5} \text{ s}^{-1}$); under physiological circumstances, GAP proteins are responsible for hydrolyzing GTP bound to KRAS ($k_{cat} = 4300 \times 10^{-5} \text{ s}^{-1}$ in case of p120-GAP activated hydrolysis). Based on the crystal-structure of RAS-GAP complex, the R789 “arginine-finger” of p120-GAP aligns the residues of KRAS in a conformation that is optimal for GTP-hydrolysis (Scheffzek et al., 1996). Mutations at the G12 residue of KRAS sterically collide with the “arginine finger,” which results in a 1000-fold decrease in hydrolysis rate (Hunter et al., 2015) but only a five-fold decrease in the affinity of KRAS for GAP (Gideon et al., 1992).

Based on QM/MM screenings, in which the hydrolytic reaction's energy profile was calculated in the presence of different GAP mutants, our collaborators at the King's College London identified several point-mutants of p120-GAP that could potentially counteract the decrease of activity caused by the steric collision of R789. To confirm their results, we aim to characterize the most promising GAP mutants by measuring their affinity for KRAS and their effect on GTP hydrolysis. To define the affinity between proteins, we used isothermal titrating calorimetry, and to observe GTPase function, we measured P_i release in a time-dependent manner. Based on our result, we plan to crystallize the most potent KRAS-GAP complexes, get a structural insight into the active center, and present structural proof of the *in silico* results.

ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN CELL DEATH CONTROL

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In a multicellular organism, apoptosis is a normal and essential process. Apoptosis means genetically programmed cell death. Apoptotic regulation has been implicated in many human diseases, including cancer, autoimmune disease, inflammation, and neurodegradation. In the past few years, several cell death pathways have been reported, including necroptosis. One of the key features of various tumors is to avoid apoptotic cell death. Naturally, apoptosis inhibits necroptosis. Consequently, necroptosis is a good process for destroying tumors cells, as inhibition of apoptosis facilitates the activation of necrotic cellular pathways. The influence of immunological chemistry (tolerogenic, inflammatory, immunogenic) on cell lines may have a significant clinical benefit in reducing the tissue destruction of inflammatory processes, enhancing tumor immune response, neurodegenerative diseases, or tumors.

Aromatic amino acids play several specific roles in biology. Although Phe, Tyr, and Trp represent less than 9% of the amino acids, they are over-represented at the binding sites. One reason for this is the cation- π interaction. In addition to hydrophobic activity, hydrogen bonding, and ionic bonding (salt bridges), the cation- π interaction is the fourth key force contributing to macromolecular structure and molecular recognition in biology. Four cation- π interactions were identified in the Caspase 9 and AurkA interaction assays.

Our results may help understand the regulatory mechanism of different cellular processes, thereby providing an opportunity to influence cell death and immunology output in different pathologies.

EVALUATION OF CYTOTOXIC AND GENOTOXIC EFFECTS OF FLUORESCENT RETINOID DERIVATIVES ON CHO CELLS

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Retinoids, such as tretinoin (all-*trans*-retinoic acid, ATRA) are endogenous signaling molecules that influence a wide range of cellular processes by affecting transcriptional events. Endogenous retinoids have been used as therapeutics for some types of cancers and skin conditions, but their central role in differentiation could open the potential for widespread therapeutic use. To investigate the complex signaling pathways controlled by retinoids, a stable ATRA analog, EC23, its strongly fluorescent analog DC360, and a longer, non-retinoid, fluorescent derivative DC324 were designed and synthesized by the Whiting group. The effects of these fluorescent retinoic acid analogs, compared to the non-retinoid system, were examined using viability and genotoxicity methods.

Chinese hamster ovary (CHO) cells were cultured under standard conditions (37°C, 5% CO₂). Subsequent treatment with all compounds was carried out for 24 hours at 1 μM and 10 μM concentrations compared to the solvent. Cell viability was quantified by the CellTiter-Glo (Promega Corp, Madison, WI) ATP measurement system based on the manufacturer's protocol. Also, DNA damage was determined by three parameters (tail length, DNA%, and tail moment) using the Comet assay.

ATRA, EC23, and DC360 did not influence cell viability, but 10 μM DC324 induced a statistically significant cytotoxic effect. On the other hand, all the tested compounds increased DNA percentage in the tail, showing the same, or slightly higher, genotoxic profile than ATRA.

All fluorescent retinoids showed similar safety profiles to ATRA, both regarding cytotoxicity and genotoxicity. These stable synthetic mimics of ATRA present an outstanding potential for understanding the diverse biological activities and complex signaling pathways affected by endogenous retinoids.

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PEPTAIBOL PROFILES OF THE CLINICALLY RELEVANT SECTION *LONGIBRACHIATUM* AND THE BIOACTIVITY OF *TRICHODERMA REESEI* PEPTAIBOLS

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Trichoderma's filamentous fungal genus includes five clades, one of which is the phylogenetically separated monophyletic section *Longibrachiatum*. Numerous *Trichoderma* strains can be used as biocontrol agents. The members of the *Longibrachiatum* section mainly have an important role in agriculture and biotechnology because of their ability to produce different antibiotics and enzymes and to degrade various xenobiotic substances. Certain species are widely found cosmopolitan fungi living in various natural and agricultural soils, while other members of the section can be found only in restricted areas.

Peptaibols are linear, bioactive oligopeptides with 6-20 amino acid residues. They are secondary metabolites produced by numerous *Trichoderma* species. This study aimed to analyze and compare peptaibol production in the case of various members of *Trichoderma* section *Longibrachiatum*. Analytical experiments were performed with the HPLC-ESI-MS technique on crude extracts of 22 strains belonging to 17 species of the section (*T. aethiopicum*, *T. andinense*, *T. capillare*, *T. citrinoviride*, *T. effusum*, *T. flagellatum*, *T. ghanense*, *T. konilangbra*, *T. longibrachiatum*, *T. novae-zelandiae*, *T. pinnatum*, *T. parareesei*, *T. pseudokoningii*, *T. reesei*, *T. saturnisporum*, *T. sinensis*, and *T. orientale*). All peptaibol sequences were determined and classified into subgroups. In total, 79 already known and 64 yet unknown, new peptaibol compounds could be identified. However, mostly 20-residue long peptaibols are produced by *Trichoderma* section *Longibrachiatum*; eight new 19-residue long peptaibol sequences were also discovered.

The peptaibols' bioactivity was also studied against bacteria, yeasts, filamentous fungi, *Arabidopsis*, and tomato plants. The tests were carried out using the purified peptaibol extract of the biotechnologically relevant, hyper-cellulolytic *T. reesei* species in different concentrations. During the tests on *Arabidopsis* and tomato plants, the primary roots' growth, the biomass, the surface of the leaves, and photosynthetic pigment contents were measured. Based on the results of the tests carried out on *Arabidopsis* plants, the 0.1 mg ml⁻¹ peptaibol extract proved optimal to increase plant growth; furthermore, this concentration was successfully used to inhibit several plants' growth, human pathogen bacteria, yeasts, and filamentous fungi. The 0.1 mg ml⁻¹ peptaibol concentration similarly showed positive effects on tomato plants and suggested that peptaibol extracts may have practical application potential in agriculture and crop production.

CYCLODEXTRIN-MEDIATED QUORUM QUENCHING IN *ALIIVIBRIO FISCHERI* MODEL SYSTEM

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According to our knowledge, most prokaryotic microorganisms use cell-to-cell bacterial communication (quorum sensing, QS) to better adapt to the current environmental conditions through phenotypic changes. QS accomplished by the production and detection of signaling molecules, which also work as autoinducer molecules. Bacterial communication in *Aliivibrio fischeri* is controlled by unique protein systems, which build up the *N*-acyl-homoserine-lactone (AHL) signal molecules that diffuse through the cell membrane and binds to the appropriate receptors. As the number of signal-receptor complexes reaches a threshold concentration that activates several target genes' transcription, the bioluminescent light emission will be encoded. Cyclodextrins-mediated decrease of *quorum sensing* (*quorum quenching*, QQ) is an innovative approach, and the available information about their effects is very scarce. These cyclic oligosaccharides with a hydrophilic external surface and a hydrophobic internal cavity are known to form inclusion complexes with various organic compounds. Three native cyclodextrins (α -, β -, γ -cyclodextrins), their epichlorohydrin-crosslinked polymers and derivatives are well studied and widely used in drug formulation, cosmetic and food industry, agriculture, and the environment. The cavities of ACD and BCDs can accept AHL molecules' acyl chains, and AHL-CD inclusion complexes can be formed in aqueous media that prevents the signal molecules from binding the receptors.

A systematic study was performed with the derivatives of native cyclodextrins to determine their concentration- and time-dependent bioluminescence inhibitory effect in the *A. fischeri* model system. The highest QQ effect was found for ACD, but BCD and HPBCD seem to be effective in the bioluminescence inhibition. Experiments with the co-administration of ACD and AHL, the most common signaling molecule of *A. fischeri* were also confirmed, and we observed that ACD was able to compensate for the stimulating effect of supplemented AHL signal molecule.

According to our results, the QS mechanism was significantly inhibited by the cyclodextrins, which presumably prevented the signaling molecules from reaching its receptor, thus inhibiting the signal-receptor interaction. The efficiency of each CD was influenced by several parameters: the size of the interior cavity, the structure, the concentration, and the contact time. Based on these results, we conclude that cyclodextrin-based traps' application for the complexation of signal molecules may be a promising method for fighting against bacterial pathogens.

TRANSCRIPTOMIC ANALYSIS OF CARBON LIMITATION STRESS RESPONSES IN *ASPERGILLUS NIDULANS*

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Carbon stress is one of the most common stress types for microorganisms, both in nature and in the industry. The quality and availability of carbon sources are critical external factors influencing the growth, differentiation, pathogenesis, and extracellular hydrolase production and secondary metabolism of filamentous fungi. In this study, carbon limitation and carbon starvation stress responses of *Aspergillus nidulans* were recorded by RNA sequencing. Stress conditions were induced by transferring mycelia from growing cultures into carbon source free (carbon starvation), lactose, or arabinogalactan containing (carbon limitation) media. For reference cultures, glucose-containing media were used. Carbon stresses significantly affected the transcription of 3000–4000 genes in each kind of stress treatment. The overlap among these genes was remarkable: 1192 and 921 genes showed up- or down-regulation, respectively, in all the three types of carbon stress treatments. These genes included up-regulated secondary metabolism and carbohydrate-active enzyme genes and down-regulated glucose utilization, respiration, and oxidative phosphorylation genes. Up-regulation of autophagy-related genes was recorded only in carbon starved cultures, while up-regulation of genes encoding hemicellulose and pectin hydrolyzing enzymes were characteristic for cultures utilizing arabinogalactan. Up-regulation of the D-galactose oxidoreductive pathway genes was characteristic for lactose growing cultures; however, up-regulation of lactose permease and β -galactosidase genes were observed in the stressed cultures. Our results support the view that fungi secrete several hydrolyzing enzymes at the beginning of carbon stress to “sense” their environment. During the stress adaptation process, the compounds liberated by them can up-regulate genes needed only to utilize the “discovered” nutrients. This mechanism can also be suitable for adapting fungi to grow on compounds - like lactose - that are not typical carbon sources in their environment.

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INTERACTIONS BETWEEN PROBIOTIC AND ORAL PATHOGENIC STRAINS

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More than 6 billion bacteria and other microorganisms live in the adult oral cavity in a fragile balance. As a result of any deleterious damage to this relative equilibrium, the number of the disease-causing microorganisms can increase, which triggers pathogenic processes like caries, halitosis, gingivitis, and periodontitis. The consequences of these conditions are widely known because nowadays, oral infections are among the most frequent diseases globally. The suffering patients' quality of life deteriorates notably, while the treatment is often unpleasant, expensive, and irreversible, i.e., losing the patients' teeth.

Is there an alternative solution, which can be used to restore the normal microbial balance? Could we prevent the development of immense trouble with the help of the naturally flourishing protective probiotic microbes?

In the experiments testing this possibility at the “proof of concept” level, we investigated eight individual pathogenic interactions with eight probiotic strains and examined a mixed probiotic product, which is commercially available. Almost all eight pathogens, namely *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus gordonii*, *Enterococcus faecalis* and *Prevotella buccae* are regarded as important pathogens in the oral cavity. The used probiotic strains were *Lactobacillus plantarum*, *L. rhamnosus*, *L. casei*, *L. acidophilus*, *L. delbrueckii*, *Bifidobacterium thermophilum* and two *Streptococcus dentisani*. Using a modified agar diffusion test system, we investigated if the probiotic bacteria could prevent the pathogens' growth to identify those that can be used selectively for treatment in the future.

The results indicated successful bacteriocin production, i.e., growth inhibition, against every pathogenic bacterium by at least five probiotic strains. Therefore, after future detailed optimization, we can develop probiotic mixtures to control the most pathogenic individual oral bacteria's growth and diverse communities.

INTESTINAL MICROBIOTA OF WILD BOARS AS POTENTIAL SOURCE OF BENEFICIAL MICROORGANISMS

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Balanced microbiota with substantial diversity is a critical component of the living organisms' healthy life within the animal kingdom. Disruption of this equilibrium results in dysbiosis, which leads to the development of different diseases. However, beneficial microorganisms with the ability to prevent or alleviate the symptoms of microbiota disruptions have also been found; some were able to promote livestock production growth and yield.

Four different parts of the gastrointestinal tract (ileum, caecum, colon, and rectum) of free-living wild boars (*Sus scrofa*) were sampled to isolate and characterize lactic acid bacteria (LAB). Determination of taxonomic identity and *in vitro* antimicrobial activity, bile salt, and acid tolerance have been planned to find potential candidates for efficient feed additives in the swine industry.

Specific culture media have been used to isolate LAB strains, which were screened using genus- and species-specific PCR primers, and their taxonomic identity was confirmed by sequencing their 16S rRNA gene. The antimicrobial activity of the selected LAB isolates was assayed by the agar-well diffusion method. Most of the examined isolates had antibacterial activity against at least one of the used indicator strains from the *Escherichia*, *Staphylococcus*, *Salmonella*, and *Streptococcus* genera. Bile salt and acid tolerance characterization are in progress using specific culture media for evaluation, supplemented with bile salt and HCl, respectively.

Isolated *Lactobacillus mucosae*, *Leuconostoc mesenteroides* strains with a high bile salt, and acid tolerance may have beneficial features. The whole genome of one LAB isolate has been sequenced and analyzed to ensure that it does not carry known acquired antibiotic resistance genes. Our results demonstrate that some LAB strains isolated from the gastrointestinal tract of wild boars can inhibit potential pathogenic bacteria's growth. Further investigations are planned to include animal feeding studies and developing suitable feed additives to prevent *in vivo* gastrointestinal infections and promote higher yields in the swine industry.

IDENTIFICATION OF *PRD-2* GENE THAT LINKS CIRCADIAN RHYTHM AND NONSENSE-MEDIATED DECAY IN *NEUROSPORA CRASSA*

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Circadian rhythm presents in all phyla of organisms and helps to respond to the alternating environmental conditions during the day. Disruption of circadian rhythm is associated with various diseases; thus, it is important to understand the circadian clock's complex genetics. *Neurospora crassa* fungus is a model organism for studying the molecular mechanism of the circadian rhythm. The clock is driven by transcription/translation feedback loops with negative and positive feedback controls. FREQUENCY (FRQ) protein acts as the negative element, and WHITE-COLLAR 1 (WC-1), WHITE-COLLAR 2 (WC-2) are the positive elements. FRQ protein has two isoforms (short (sFRQs) or long (lFRQs)) because of temperature-modulated alternative splicing of the *frq* transcript (*sfrq*, *lfrq*). The ratio of sFRQ/lFRQ and FRQ phosphorylation plays an essential role in period determination. Many other factors are also involved in clock regulation, including the components (UPF1, UPF2, UPF3) of the Nonsense-mediated decay system (NMD). NMD is a eukaryotic surveillance mechanism that degrades faulty transcripts to avoid the accumulation of harmful aberrant peptides. Various circadian rhythm mutant (called *period* (*prd*)) strains are available, but only a few of them are thoroughly investigated. The *prd-2* mutant shows a lengthened period and has a genetic relationship with the core NMD factor *upf1*. The *prd-2* gene is unknown; thus, we aimed to identify and place it in the circadian clock system. We demonstrated that the genetic relationship between *prd-2* and other NMD factors appears as well. We found that *frq* mRNA level increased in *prd-2* mutant resulting in an altered ratio of *sfrq/lfrq*, in parallel with decreased transcript level of the *casein kinase 1* (*ck1*) circadian rhythm regulator. These results might explain the altered period of *prd-2* strain at the molecular level. The *prd-2* was previously mapped to a 100 kb region. To identify it, we have sequenced the mutant's genome and look for mutations in the mapped region. As transcripts of NCU03775 are absent in *prd-2* mutant, we hypothesize that *prd-2* gene is NCU03775, a hypothetical protein with unknown function. To prove that *prd-2* encodes NCU03775, we try to complement the rhythm defects of the *prd-2* mutant with the wild-type gene.

THE EFFECTS OF GENOTOXIC STRESS FACTORS ON DNA REPAIR SYSTEM IN MYCOBACTERIA

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Tuberculosis is still among the most challenging global health problems. One of the main reasons for that is the quick and efficient adaptation of the pathogen *Mycobacterium tuberculosis* to the variety of harsh conditions in the host. Mycobacteria uncommonly develop resistance only by chromosomal mutations, single-nucleotide polymorphisms. A significant mutation rate could be expected in the bacteria based on mycobacteria's remarkable genetic diversity in isolates from tuberculosis patients. Although, *in vitro* studies show an exceptionally low basal mutation rate in mycobacteria ($\sim 10^{-10}$). These contradictory results suggest that genomic stress factors like the immune pressure and antituberculous drugs within the patients may provoke increased mutational rates in the bacteria. Cells provide genome stability and low mutation rates by presenting countless DNA surveillance and correction processes. Mycobacteria has a unique yet not entirely understood DNA repair system with several redundant enzymes, absent canonical mismatch repair pathway, and proteins with special functionality.

We aim to study the activation pattern of the mycobacterial DNA repair system upon *in vitro* genotoxic stress with the help of qPCR. Our experiments use the non-pathogen *Mycobacterium smegmatis*, which shares the same metabolic and repair routes as the medicinally relevant strains.

Our results will hopefully contribute to a better understanding of the adaptive events resulting in mycobacteria's resistance.

EXAMINATION OF THE ROLE OF MYCOBACTERIAL dUTPASE IN HOMOLOGUES RECOMBINATION

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dUTPase is a nucleotidase enzyme that catalyzes the $dUTP \rightarrow dUMP + PPi + H^+$ reaction. Uracil is not a conventional DNA base, but most polymerases can pair it with adenine during the replication. Normally, this kind of error is corrected by the base excision repair system, but if the dUTP/dTTP ratio is high, uracils can be incorporated into the genomic DNA, and mutations can be significantly increased.

The mycobacterial dUTPase has a genus-specific loop motif. Interestingly, the absence of this loop motif is lethal but has only a minor influence on the enzymatic function. Therefore, we hypothesized that this loop might be essential for protein-protein interactions.

To prove this hypothesis, we carried out a yeast two-hybrid assay. With this assay, we could detect AdnA as a promising interactional partner. AdnA is a bacterial helicase/nuclease which has an important role in the homologous recombination in *Mycobacteria*.

To investigate the interaction between the mycobacterial dUTPase specific loop motif and AdnA *in vivo*, we designed a mutant *Mycobacterium smegmatis* strain encoding the specific loop motif deleted version of dUTPase (Dloop) besides the wild type enzyme. Then we examined the recombination efficiency in these strains and found that the Dloop variant has a negative effect on recombination efficiency.

We also tried to express AdnA as a recombinant protein. As we could not express the full-length AdnA, which otherwise exists as a heterodimer with AdnB, we designed three smaller protein fragments of different lengths considering the crucial parts of interaction and domain structure. With these fragments, we would like to investigate the dUTPase-AdnA interaction *in vitro* as well.

USE OF ENSILED GREEN WILLOW BIOMASS IN BIOGAS FERMENTERS

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The biggest challenges of our era include climate change and the associated global fossil energy shortage. Extensive utilization of renewable energy most certainly could be part of the solution for both these problems. Biogas, produced from biomass, is a versatile renewable energy carrier that can potentially substitute fossil fuels, at least partially. The most frequently utilized substrate for the anaerobic digestion process (AD) is maize silage, but there is an increasing demand for second-generation energy plants, which are cheaper and interfere with the cultivation of food cereals much less. According to our experimental results, green biomass from short rotation coppice willow (green willow biomass= GWB), a novel potential biogas substrate, may be a promising alternative. However, to fully exploit this potential biogas substrate and ensure a sufficient feedstock quantity all year round, a preservation method must be developed. With this aim in mind, we attempted to ensilage the willow biomass before AD bioreactors. We used mixtures of lactic acid bacteria to inoculate the substrate in anaerobic jars for 60 days. During the ensiling, we carried out HPLC analytical examinations to follow the build-up of fermentation products. We also determined the pH of silage samples at the beginning and at the end of the ensiling procedure to establish the efficiency of lactic acid production and the resulting pH decrease. AD fermentations were assembled from the ensilaged biomass, and the methane production was measured for 56 days. The results suggested that the total methane yields of the ensilaged biomass were 8%-15% higher than that of the control green biomass, and methane production rates were also improved in some cases. Finally, community total DNA isolation and 16S rDNA amplicon sequencing was carried out on three selected silage samples and their corresponding AD reactors to explore the developed microbial community's composition. We compared microbiome composition results with that of the analytical examinations to determine the ensiling's efficiency and contribution to the AD processes. Our findings suggest that ensiling is an excellent preservation method for willow biomass and stimulates biogas production. These findings contribute to the utilization GWB, a promising second-generation biogas-substrate.

ENHANCING BIOGAS PRODUCTION FROM AGROINDUSTRIAL WASTE PRE-TREATED WITH FILAMENTOUS FUNGI

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Our society's energy consumption tends to exceed fossil energy resources. Hence, the most challenging challenge today is to exploit the vast resources in the utilization of renewable energies. One of them is biogas, the product of anaerobic digestion (AD) of organic waste and is one of the most valuable natural renewable energy carriers. Plant biomass represents the most abundant eco-friendly energy reservoir on Earth. Moreover, agriculture and households generate tremendous amounts of organic waste daily that may fuel biogas production. However, the lignocellulose-rich substrates' tenacious and heterogeneous structure makes it difficult for the involved microbes to digest the recalcitrant substrates. Fortunately, we can promote the degradation process and enhance the biogas production yield by appropriate pre-treatment of these materials.

Filamentous fungi were previously reported as colonizers of fibrous plant tissues and identified as producers of exceptionally rich and diverse hydrolytic enzymes. While their rhizoid system can penetrate even the most lignified plant residues, their various assortments of extracellular enzymes contribute to the decomposition of cell wall polysaccharides.

Considering these promising properties, we aimed to use filamentous fungi as a pre-treatment method to improve the biogas production and organic waste degradation simultaneously. Our pre-treated substrates (corn stover, wheat straw, and willow chips) are rich in lignocellulose, making their direct AD slow and incomplete. We tested *Aspergillus nidulans*, *Trichoderma reesei*, *Rhizomucor miehei*, and *Gilbertella persicaria* filamentous fungi strains for pre-treatment. During the pre-treatment phase, the β -glucosidase and endoglucanase activities were measured spectrophotometrically. In the AD step, methane production was monitored by gas chromatography.

Our preliminary results showed that the applied strains were highly effective enzyme synthesizers, which might explain the highly improved AD results. Pre-treatment with the above-mentioned filamentous fungi strains positively affected the biogas production. Depending on the used substrates and the pre-treating strain, methane yields exceeding two-fold that of the controls were observed. Meanwhile, the amount of degraded organic total solid matter increased by 20 % in certain cases.

Therefore, rationally designed and practically attainable biotechnological intervention may facilitate the utilization of the available plant biomass waste for efficient and economically feasible renewable energy production.

INVESTIGATION OF AFLATOXIN M1 BINDING CAPACITY OF PROBIOTIC BACTERIAL CULTURES AND THEIR PREPARATIONS

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Mammals consuming feed contaminated with aflatoxin B1 mycotoxin excrete aflatoxin M1 into their milk, mostly bound to milk proteins. The presence of aflatoxin M1 in milk and dairy products is a global problem as dairy products are regularly consumed. Numerous studies have shown that lactic acid bacteria reduce molds' growth and the production of aflatoxin B1 and can reduce aflatoxin M1 content. We aimed to investigate the tolerance against and binding capacity of aflatoxin M1 of some probiotic cultures. We considered that the toxin binding on the bacterial cell wall is of great importance; therefore, besides cell cultures, heat-killed and variously treated (trichloroacetic acid, hydrochloric acid, SDS) *Lactococcus lactis* ssp. *lactis* R703, *Bifidobacterium animalis* ssp. *lactis* BB12, *Lactobacillus sakei*, and *Lactobacillus casei* bacterial preparations were also tested. Aflatoxin M1 inhibited the growth of the R703 strain, whereas no growth difference occurred with the BB12 strain. The amount of aflatoxin M1 removed by the R703 and BB12 cells was lower than that of the heat-killed cell preparations. Experiments were also carried out using pure aflatoxin M1 toxin without milk proteins, which proved that trichloroacetic acid-treated, heat-killed R703 and BB12 preparations have a better affinity for aflatoxin M1 than the viable cells, and the affinity did not depend on milk protein bindings. Aflatoxin M1 binding ability of *L. casei* and *L. sakei* strains was low, and the different treatments did not eliminate the mycotoxin. The peptidoglycan purified with TCA treatment of the cultures removed aflatoxin M1 at a higher amount from the naturally contaminated milk than the live cells. Differences in cell wall structures are considered to affect binding ability.

THE SCREENING OF ENDOPHYTES FROM AGRICULTURAL AND NON-AGRICULTURAL CROPS IN NORTHERN KAZAKHSTAN

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Endophytes are microorganisms that interact with the plant and colonize plants' roots without causing any visible symptoms on the plant. In Northern Kazakhstan, little is known about endophytic fungi isolated from agricultural and non-agricultural crops. Screening the variety of endophytic fungi in this area may serve important findings for applied approaches because endophytes can help protect host plants from diseases, pests, and drought.

This research aimed to isolate and identify the root colonizing endophytic fungi of the *gramineous* plant in the Akmola region's agricultural and non-agricultural areas, Northern Kazakhstan. Roots of various *species of the Poaceae*; wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), wild barley (*Hordeum jubatum*), oat (*Avena sativa*), and *Stipa capillata* were collected from the field to isolate endophytic fungi. For molecular identification, total DNA was isolated from the isolates gained, and the ITS (internal transcribed spacer) region of the nrDNA was amplified and sequenced. In representative *Fusarium* isolates, the TEF-1 α (translation elongation factor 1-alpha) region was also obtained.

The results indicate the large number of endophytic fungi isolated from roots of the different plant species: roots of barley, wheat, oat, *Stipa*, and wild barley. Most of the isolated fungi are presented from agricultural and non-agricultural plants, and many belong to *Fusarium* species. Therefore, representative *Fusarium* isolates were chosen to study their functional role and screen their secondary metabolite production.

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ESTABLISHMENT OF A MODEL SYSTEM FOR STUDYING THE EFFECT OF BACTERIOPHAGE dUTPASE ACTIVITY ON HORIZONTAL GENE TRANSFER IN *STAPHYLOCOCCUS AUREUS*

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Approximately 20% of the genomes of *Staphylococcus aureus* strains are made up of mobile genetic elements that are densely exchanged. These elements often carry pathogenicity factors and genes responsible for antibiotic resistance; thus, they contribute to bacterial infectivity and spread of the virulence and antibiotic resistance factors. It is also common for them to encode factors that influence uracil DNA repair, such as the dUTPase enzyme of bacteriophages. The function of dUTPase is to maintain the proper ratio of dUTP / dTTP to prevent uracil incorporation into the DNA and the resulting mutagenic changes. This gene was previously considered essential, but it is not encoded by the core *S. aureus* genome itself (the part free of mobile genetic elements). Thus, the question arises on how the bacterium survives without dUTPase, and if the bacterium does not need this gene, why the lysogenic phages carry it. We hypothesize that the genetic material of mobile genetic elements is more sensitive to the uracil content of DNA; hence, dUTPase promotes the spread of mobile genetic elements, in this case, lysogenic phages.

The research work's main objective was to develop a model system capable of investigating the role of *S. aureus* bacteriophage dUTPase enzyme activity in bacteriophage replication. That is most easily assayed by inhibiting the dUTPase of a given bacteriophage during phage replication. To study how dUTPase influences phage spread, a lysogenic strain containing only one kind of bacteriophage was required, one whose activity could be inhibited by the available Stl protein inhibitor. Most strains contain 2 to 3 phages that all encode dUTPases; not all these dUTPases can be inhibited by Stl protein.

For this purpose, the $\phi 11$ lysogenic phage was selected, which has got a known trimeric dUTPase that is affected by the inhibition of the Stl protein. To this end, we activated the prophages in a strain containing several bacteriophages, then infected sensitive strains, and attempted to isolate the lysogenic *S. aureus* strain containing $\phi 11$ prophage exclusively. A suitable screening method was also developed for this purpose. The generated lysogens were also tested by next-generation sequencing. After that, the Stl protein was cloned in a *S. aureus-E. coli* shuttle vector to express the inhibitor protein in the strain to be tested. We also tested whether the lysogen strain we isolated was capable of uptaking *S. aureus-E. coli* shuttle expression vectors.

We will study how dUTPase enzyme activity influences phage propagation and horizontal gene transfer with the established and tested model system.

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SECONDARY METABOLITES OF *FLAVOMYCES FULOPHAZII*, A ROOT ENDOPHYTE OF SEMIARID SANDY GRASSLANDS

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Endophytic fungi, colonizing plants internally and asymptotically, are valuable sources of diverse natural products since they interact with other organisms, at least partially by their secondary metabolites (SMs). During investigations on root endophytic fungal communities of semiarid sandy grasslands of Hungary and Mongolia, we frequently isolated the recently described *Flavomyces fulophazii* (Pleosporales). Since its isolates secrete significant amounts of yellow pigments into their media, based on which the name „flavo” was given, we aimed to identify these SMs.

Metabolite composition of culture media was analyzed using high-performance liquid chromatography hyphenated with diode array and high-resolution mass spectrometry detection (HPLC-DAD-HRMS). The detected compounds' chemical structures were determined by HRMS and nuclear magnetic resonance spectroscopy (NMR). The *in vitro* cytostatic activity of some of these compounds was also tested.

As a result, for the first time, we identified tetramic acid- and azaphilone-type metabolites in the extracts of *F. fulophazii*. Except for the tetramic acid derivative vermelhotin, these compounds were determined to be new natural products. Among the compounds tested, vermelhotin exhibited *in vitro* cytostatic activity against all the twelve cancer cell lines included. Further experiments are in progress to test whether these SMs might have a role in interacting with this fungus with other microorganisms.

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DETECTION OF PULCHERRIMIN GENE CLUSTER IN THE *METSCHNIKOWIA FRUCTICOLA* TYPE STRAIN (CBS 8853^T)

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In the case of countless organisms, it is observed that they produce secondary metabolites that fundamentally determine their relationship with the environment and other organisms. Fungi are well known for their secondary metabolites, including siderophores (chelators that bind extracellular iron). Specific yeasts, such as *Metschnikowia* spp. and *Kluyveromyces* spp., can produce red pulcherrimin pigment (a special siderophore). Numerous studies have highlighted the antagonistic effect of pulcherrimin on nonproducing yeasts, filamentous fungi, and bacteria as being of ecological significance.

The genes encoding the proteins responsible for pulcherrimin production occur in gene clusters; such a cluster contained four genes that were previously identified in *K. lactis*.

Our research aimed to identify the suspected genes responsible for the biosynthesis of pulcherrimin in *M. fructicola* type strain (CBS 8853^T), furthermore designing primers and developing PCR protocols for successful amplification of *PUL* genes.

Amino acid sequences of the *PUL1-4* genes from *K. lactis* (NC_006039.1) were used as query sequences to search Saccharomycotina BLAST database (SequenceServer; y1000plus.org/blast) for homologs of these proteins. We found significant hits ($e < 0.001$) in the genomes of *M. fructicola* (ANFW01000000) on the ANFW01000757.1 contig. We applied the progressive Mauve algorithm (<http://asap.ahabs.wisc.edu/software/>) to align this nucleotide sequence with the new whole genome sequence of *M. fructicola* type strain (ANFW02000000), where we detected the *PUL* gene cluster on the ANFW02000036.1 contig.

Primers specific for these sequences were designed using the SnapGene molecular biology software. These were used to amplify the four *PUL* genes from the genomic DNA of the *M. fructicola* type strain by PCR. For accurate results, protocols providing optimal annealing temperatures for primers were needed. *In vitro* PCR analysis revealed that the optimal annealing temperature is 58°C for the primer pairs designed for *PUL1*, *PUL2*, and *PUL4* genes, while the optimal T_m is 54°C for the primer pair of *PUL3* gene.

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OENOLOGICAL AND MOLECULAR ANALYSIS OF TWO INDIGENOUS *SACCHAROMYCES* POTENTIAL HYBRID STRAINS FROM BADACSONY WINE AREA

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In the modern winery, commercially available starter yeasts are used for winemaking. The organoleptic parameters of wine are dependent on the inoculated yeasts as well. *Saccharomyces* species are usually applied in wine fermentation as a starter due to achieving the final products' appropriate ethanol concentration. The sensory character can develop with a co-inoculation strategy when different starter yeasts are applied together. Members of the *Saccharomyces* genus can form interspecific hybrids, potentially combining desirable features of the parental strains. Several hybrids can be characterized by beneficial technical parameters for winemaking. Inoculation of the must by hybrid strains can enrich the aroma complexity of wine avoid the competition among two or more applied starter yeasts.

In this study, two *Saccharomyces* isolates from fermented grape juice were investigated. Species-specific PCR and PCR-RFLP methods were used for identification. The molecular test results showed that one isolate belonged to the *S. cerevisiae* species and the other isolate was *S. cerevisiae* x *S. kudriavzevii* natural interspecific hybrid. Physiological properties of two isolates and representative reference strains (*S. kudriavzevii* C950, *S. cerevisiae* CBS 1171 type strains, and *S. cerevisiae* starter) were analyzed. Glucose tolerance, ethanol tolerance, killer toxin production, and glucose fermentation capacity of the strains were tested based on microbiological assays. We have founded that the investigated strains could grow in the presence of 30 % of glucose and 30% of ethanol at 30 °C after two days; however, *S. kudriavzevii* C950 type strain showed less growth ability than the others. Glucose fermentation of strains was monitored at 16 °C and 24 °C for one week using Durham tubes. Our isolates and *S. kudriavzevii* C950 type strain could ferment faster than two control *S. cerevisiae* strains at 24 °C. At 16 °C, all strains showed a slower fermentation rate, but the less effective strain was *S. cerevisiae* CBS 1171 type strain. In this study, the newly isolated *S. cerevisiae* strain was a killer toxin producer only.

Further investigations will determine the different sensory profiles of wines generated with these two different strains.

HABITAT ADAPTATION OF FUNGAL ROOT ENDOPHYTES — LINEAGES IN GRAMINEOUS CROPS ON AGRICULTURAL AREAS

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One of the various groups of microorganisms that are living in symbiosis with plants is endophytic fungi. They are found within internal tissues of living plants without any immediate, overtly adverse effects. Fungal root endophytes (so-called dark septate endophytes, DSE) may have various effects on plant survival under different stress conditions such as drought, which was the main factor behind lower harvests of key crops for many regions of central and northern Europe in 2018. The effect of a DSE species enormously varies, and these functional differences may be derived from a certain degree of habitat adaptation of an isolate to the host or the conditions of the sampling ‘home’ area. We have information on the main grass-associated DSEs of natural grasslands; however, our knowledge of the DSEs of agriculturally important gramineous crops is limited.

To investigate the habitat adaptation of DSE fungi, we aimed to gain conspecific DSE isolates from agricultural areas, where monocultures of certain crops are grown for decades, enabling the proposed adaptation of the fungi to the host. In the present work, our goals were to screen the DSE community of gramineous crops and to isolate endophytic fungi, which are also present in natural grasses and can be used for *in vitro* inoculation experiments.

Root samples were collected from experimental monoculture plots of Agricultural Institute, Centre for Agricultural Research, HAS, at Martonvásár, in which parcels only wheat (*Triticum aestivum*) or maize (*Zea mays*) have been cultivated since 1960. For molecular identification of the isolates gained from the surface-sterilized root sections, total DNA was extracted, and the internal transcribed spacer (ITS) region of the nrDNA was amplified. In *Fusarium* isolates, the TEF1 (translation elongation factor 1 alpha) region was also sequenced.

Altogether 400 isolates were collected from the two plants. The isolates represented several fungal taxa dominated by ascomycetes, including several lineages, which have been detected previously in grassroots world-wide. Representatives of these clades are chosen for inoculation experiments to test the hypothesized effects on the ‘home’ conditions or the original hosts. These results may provide crucial information on the inoculum production for better survival of agronomically important gramineous plants.

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THE COMBINED EFFECTS OF THE PROTEIN PHOSPHATASE Z1 GENE DELETION AND OXIDATIVE STRESS ON THE GENE EXPRESSION OF THE GLUCOCORTICOID TREATED *CANDIDA ALBICANS*

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The opportunistic pathogen *Candida albicans* adapted to the oral, gastrointestinal, and genital mucosa of healthy individuals. Several factors have contributed to the recent increase in the incidence of *Candida* infection. Corticosteroid therapy has emerged as an important risk factor for candidemia because of its anti-inflammatory and immunosuppressive effects. Our observations suggest that applying a high dose of glucocorticoid betamethasone (BM) may predispose patients to *C. albicans* infections by stimulating the hypha formation of *C. albicans* and affects the interactions between *C. albicans* and the epithelium cells. Interestingly, BM exposure may render yeast cells more vulnerable to oxidants like the superoxide-generating agent menadione (MSB).

Furthermore, the fungus specific protein phosphatase Z1 (CaPpz1) enzyme has a pivotal role in the pathogenicity and the oxidative stress response of *C. albicans*, it can be considered as a potential drug target for the treatment of mucosal infections. This study aimed to investigate the combined effects of the *CaPPZI* gene deletion, and the MSB induced oxidative stress on the gene expression patterns of the BM treated *C. albicans*. The expression of oxidation-reduction, membrane transport and metabolism-related genes was revealed by RNA sequencing and was confirmed by RT-qPCR assays. We found that the deletion of the phosphatase and/or the glucocorticoid treatment alone had slight effects, while the combination of MSB treatment and the lack of *CaPPZI* gene resulted in robust alterations in expression patterns. The genes involved in iron ion transport (*CFL4*, *FTR1*) and gluconeogenesis process (*PCK1*) were downregulated, while genes related to the fatty acid metabolism (*PXP2*), oxidative stress response (*CAT1*, *GLR1*, *SOD1*, *TRR1*), drug resistance (*CDR1*, *MDR1*), transmembrane transport (*PMA1*) *HGT17*) were upregulated in the MBS and BM treated deletion mutant cells. In summary, the synergism between the fungus specific serine/threonine protein phosphatase and MBS induced oxidative stress can be utilized to develop a new topical antifungal therapy for preventing surface mycoses in BM treated dermatology patients.

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CONSTRUCTION AND CHARACTERIZATION OF THE BZIP- TYPE TRANSCRIPTION FACTORS ATFA AND ATFB OVEREXPRESSION MUTANTS IN *ASPERGILLUS NIDULANS*

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All organisms have developed effective defense and adaptation systems against varying stress exposures in their environment. For example, filamentous fungi, being mainly aerobic organisms, often encounter oxidative stress. The b-ZIP type transcription factors play a central role in the regulation of oxidative stress. For example, AtfA is one of the key elements of the stress defense system of filamentous fungi. It also takes part in the production of secondary metabolites and fungal development, for instance, in *Aspergillus nidulans*, *Neurospora crassa*, and *Fusarium graminearum*, or even in fungal virulence in *Aspergillus fumigatus*. In *A. nidulans* another b-ZIP type transcription factor, the AtfB may be responsible for the survival of conidiospores. These two transcription factors, most likely by forming a heterodimer, can be involved in the stress defense of *A. nidulans*. To confirm this hypothesis, we constructed overexpression mutants under the control of a nitrate-inducible promoter in the following combinations: *atfAOE*, *atfBOE*, Δ *atfAatfBOE*, *atfAOE* Δ *atfB*, and tested the mutants in the presence of oxidative stress. The applied stressors were H₂O₂, *t*BOOH (responsible for lipid peroxidation), and menadione sodium bisulfite (raises the cells' peroxide level). The following phenotypes were observed in the oxidative stress exposed cultures: Compared to the control strain, the *atfB* overexpression mutant grew significantly better in the presence of H₂O₂. However, the *atfB* overexpression in the Δ *atfA* strain was not able to compensate for the absence of *atfA*; no growth was observed in the presence of H₂O₂. The *atfA* overexpression in the Δ *atfB* mutant increased the tolerance against H₂O₂. Examining the mutants' *t*BOOH sensitivity, neither the *atfA* nor the *atfB* overexpression compensated the effect of *t*BOOH in the deletion mutants. However, the overexpression of *atfB* protected the fungus against *t*BOOH stress, but this effect was not significant. Neither *atfA* nor *atfB* overexpression enhanced the fungus's tolerance to menadione sodium bisulfite, but *atfB* overexpression alone resulted in similar menadione sodium bisulfite sensitivity as in the control strain.

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EFFECTS OF ZINC OXIDE AND TITANIUM DIOXIDE NANOPARTICLES ON *PSEUDOMONAS AERUGINOSA* AND *SERRATIA MARCESCENS* BIOFILM FORMATION

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Bacterial communication is a quorum-mediated process, which depends on different signal molecules or autoinducers. These signal molecules are low molecular weight compounds, produced and detected by bacterial cells, and can induce several bacterial processes, such as bioluminescence, toxin and pigment production, or biofilm formation.

Biofilm formation is responsible for about 80% of all microbial infections. On the other hand, bacterial biofilm is useful and necessary in our life; for example, it enhances the crop yield of leguminous plants and enhances bioremediation and wastewater treatment efficiency. However, the current research targets in this field aim to influence and control biofilm formation. Nanoparticles (NPs) have commonly been used nowadays for this purpose. Zinc-oxide (ZnO) and titanium dioxide (TiO₂) nanoparticles are widespread in various applications, including the production of personal care products.

This study's fundamental aim was to investigate the influence of these NPs on bacterial biofilm formation by *Pseudomonas aeruginosa* and *Serratia marcescens* within the 1-250 mg l⁻¹ concentration range.

Biofilm formation was quantified by a modified microtiter-plate test method. This technique involves staining the bacterial film with 0.1% crystal violet and releasing the bound dye with 30% acetic acid, then the solution optical density (OD) is measured by an immunosorbent assay reader at 544 nm. Pigment production of bacteria was measured by using an acetic acid treatment. Bacterial density and enzymatic activity in the microplate wells were measured at 630 and 490 nm to observe cell proliferation, respectively.

Our results show a concentration-dependent effect of the applied NPs on biofilm formation. Interestingly, these NPs can inhibit or stimulate biofilm production in the case of both bacteria, depending on the concentration and duration of exposure, although there is no significant effect on cell proliferation. The highest stimulation (147%) was achieved in *Pseudomonas aeruginosa* biofilm formation at 250 mg l⁻¹ nZnO for 24 hours, while at the same concentration, 65% inhibition was detected already after 3 hours of incubation. For understanding this phenomenon, further studies are needed. Otherwise, the biofilm production by *Serratia marcescens* was also more intensive in the presence of NPs; however, its pigment (prodigiosin) production was ~30% lower at 250 mg l⁻¹ nZnO compared to the control.

These results suggest an excellent opportunity to influence bacterial communication, especially biofilm formation and pigment production with nanoparticles.

SELECTIVE IMMOBILIZATION OF A RECOMBINANT PHENYLALANINE AMMONIA-LYASE FROM FERMENTATION MEDIA

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This work aimed to develop magnetic biocatalyst using a combined method for one-step enzyme purification and immobilization based on the immobilized metal ion affinity chromatography (IMAC).

The magnetic nanoparticles (MNPs) were synthesized according to the solvothermal method, and amino groups were created by proper grafting of the surface. In this work, bisepoxides as cross-linkers and ethylenediaminetetraacetic acid (EDTA)-derived chelating functions were applied to prepare Covalent-IMAC-MNPs, and recombinant phenylalanine ammonia-lyase (*PcPAL*) was immobilized as a model enzyme. During optimization of the process leading to the bifunctional surface, neopentylglycol diglycidyl ether (NPDGE) and EDTA-anhydride were used in different ratios. The surface grafting was considered optimal when the highest enzymatic activity of *PcPAL* could be achieved. Presumably, at the optimal surface grafting ratio, the amount of the EDTA-derived chelator functions could express their selectivity towards the His-tagged target protein. In contrast, the amounts of epoxy groups were sufficient to anchor the retained *PcPAL* covalently to the surface.

The results clearly showed that for the effective immobilization of the His-tagged target enzyme directly from the crude fermentation media, both epoxy and metal-chelate groups were required in a proper ratio at the surface. Modification of the surface with bisepoxides other than NPDGE offers a further possibility to tailor the spacer arm for optimal binding of the target protein.

The bifunctional Covalent-IMAC-MNPs presented in this study provide a comfortable, economical, and environmentally friendly way for immobilization of His-tagged enzymes directly from crude cell-lysate without the need for extensive purification.

URACIL-DNA REPAIR INFLUENCING GENES OF MOBILE GENETIC ELEMENTS DO NOT COUNTERBALANCE THE LACK OF GENOMIC dUTPASE IN *STAPHYLOCOCCUS AUREUS*

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The core genome of the biomedically relevant *Staphylococcus aureus* (*S. aureus*) bacterium lacks the usually essential deoxyuracil triphosphate pyrophosphatase (dUTPase) enzyme, which is responsible for preventing uracil incorporation into the genome. In the lack of dUTPase, the DNA's elevated uracil content may overload uracil-DNA repair, increasing mutational rate and double-strand breaks. Interestingly, the bacterium's mobile genetic elements that encode antibiotic resistance and/or pathogenicity factors carry dUTPases, or the SaUGI (*S. aureus* Uracil-DNA Glycosylase Inhibitor) protein, which protects from overloading uracil-DNA repair. Mobile genetic elements of *S. aureus* are mostly responsible for the spread of antibiotic resistance and virulence factors. Processes that are important for horizontal gene transfer may be targets for slowing down these factors' spread. Therefore, we would like to determine whether the uracil –DNA repair modifying factors are advantageous for the bacterium itself or the horizontal gene transfer of mobile genetic elements. As a first step, we aimed to investigate whether mobile genetic elements integrated into the bacterium's genome have any role in counterbalancing the lack of genomic dUTPase.

For this purpose, we measured the uracil level, and the deoxynucleoside-triphosphate (dNTP) pools of *S. aureus* strains with a different genetic background (+/- mobile genetics). Although we found that all investigated *S. aureus* strains have an elevated uracil-DNA level, we could neither see a remarkable difference in the uracil content of the genomic DNA nor in the dNTP pools dUTP concentration of the different strains.

To see whether different stress conditions resulting in elevated uracil level of the DNA affect the survival of *S. aureus* strains +/- mobile genetic elements, we treated the strains with ciprofloxacin, oxacillin, trimethoprim/sulfamethoxazole combination, methotrexate, 5-fluorouridine, and hydroxyurea. The 5-fluorouridine was extremely sensitive to all strains (MIC values were tested), but there was no difference in sensitivity between the different strains, neither for 5-fluorouridine nor other drugs.

Based on the present result, we assume that *S. aureus* cannot take advantage directly of the dUTPase and the SauGI proteins carried by integrated mobile genetic elements. Our results suggest that uracil-DNA repair modifying enzymes carried by mobile genetic elements are not expressed, even under the stress factors affecting the uracilization of DNA, suggesting that these factors are advantageous for the mobile genetic elements and not for the bacterium.

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ADAPTATION OF THE CRISPR GENOME EDITING SYSTEM FOR SYNTHETIC MICROBIOLOGY

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Synthetic microbiology uses living microorganisms as chassis to biosynthesize de novo products (proteins or small molecules) or modify existing products. Synthetic microbiology can manufacture existing medicines more easily and cheaply or discover and develop novel bioactive agents. Genome engineering is necessary to optimize the chassis for synthetic microbiology. For such optimization, targeted methods such as CRISPR/Cas9 is rapidly replacing random mutagenesis. Our goal here was to adopt and apply CRISPR/Cas9 genome engineering to optimize *Saccharomyces cerevisiae* for two synthetic microbiology applications. First, we considered that glycosylation is an outstanding strategy to optimize drug-like small-molecule scaffolds' pharmacokinetics properties. Thus, we used CRISPR/Cas9 to knock out two genes whose products may reduce glycosylation efficiency in a *S. cerevisiae* host. EXG1 is the major exo-1,3-beta-glucanase involved in cell wall β -glucan assembly, while SPR1 is an EXG1 paralog that arose during whole-genome duplication. We successfully made partial and complete deletions of these genes using CRISPR/Cas9, and the resulting chassis strains are under evaluation for their utility to glycosylate various small molecules. Second, we focused on a more complicated genome engineering project. In this work, we hope to develop a yeast synthetic microbiology chassis that is more efficient in sulfonating small molecules. For this application, we are deleting the YOL164W sulfatase gene and replacing it with gene cassettes that may improve the synthesis of the active sulfate donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS). The PAPS-producing enzymes will include the human PAPSS1 or PAPSS2 genes, and the CV_1639 gene from *Chromobacterium violaceum*. To introduce these cassettes into *S. cerevisiae*, we combine CRISPR/Cas9 with the high-fidelity homology directed repair (HDR) pathway and insert codon-optimized synthetic genes into the DNA repair template. To make these repair templates, we merge two homology regions targeting YOL164W, and insert two promoters, two ORFs, and two terminators. Instead of fusion PCR, we use the Golden Gate reaction and the MoClo yeast tool kit. It allows us to merge the required fragments in one-step reactions combining digestion and ligation. With this method, the complicated repair templates can be generated in just a couple of weeks. Following *S. cerevisiae* transformation and the knockout and cassette insertion validation, we will test the genome-edited strains for small molecule sulfonation. Meanwhile, we show that CRISPR methods can be used very efficiently in *S. cerevisiae* genome editing for synthetic microbiological applications.

EFFECTS OF T2 MYCOTOXIN TREATMENT ON RABBIT EMBRYO DEVELOPMENT IN VITRO

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Among mycotoxins, fusariotoxins are widely distributed in the European food chain, with emerging problems accompanying global warming. Tolerable daily intake (TDI) is usually not reached regarding the general population. However, in special populations like young children, ingestion exceeds TDI in the case of fusariotoxins, especially T2 and its toxic metabolite HT-2 toxin. T-2 toxin can cross the placenta, having a possible effect on the developing embryo and fetus. Observation, morphological characterization, and molecular study of *in vitro* cultured rabbit embryos allow us to study the effects of mycotoxin exposure on preimplantation embryo development.

In vitro cultured preimplantation embryos were exposed to low concentrations of T2 mycotoxin (5, 2.5, 0 ng ml⁻¹) in the culture media in the range that could be encountered in blood circulation with maternal ingestion of toxin contaminated feed. The morphological observation of embryos characterized the effect of T2 mycotoxin. 4-8 cell embryo and blastocyst developmental rates were monitored after 27 and 81 hours of T2 toxin treatment accordingly.

Our first experiment found no significant difference among treatment groups of 1, 2, and 2.5 ng ml⁻¹ T2 toxin exposure on blastocyst developmental rate. However, as a tendency, a developmental delay was observed with increasing concentrations of T2 toxin during treatments. Our recent findings prove the adverse effect of T2 treatment on preimplantation rabbit embryo development in connection with blastocyst developmental rate. 5ng ml⁻¹ T2 toxin decreased blastocyst developmental rate (0 ng ml⁻¹: 87%, 2.5 ng ml⁻¹: 63%, 5 ng ml⁻¹: 56%). Also, in another parameter, namely 4-8 cell-developmental rate, the difference between groups of T2 treated embryos was observed.

Changes in fine molecular pathways are proposed because of T2 treatment in preimplantation embryos. Besides the morphological evaluation of embryos, gene expression studies (qPCR) are under the way to show how mycotoxin exposure affects molecular pathways playing a role in developing non-communicable diseases (for example, cardiovascular and neurological diseases) later in life.

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DETECTION OF ATYPICAL PORCINE PESTIVIRUS IN THE TESTICLES OF SWINE

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Pestiviruses of the *Flaviviridae* family are highly variable RNA viruses with a high economic impact worldwide, affecting cloven-hoofed animals as well as bats and rats. They are often characterized by their detrimental effect on the embryo or the fetus, causing neuronal degeneration and subsequent neurological dysfunction, developmental anomalies, like cerebellar hypoplasia or even stillbirth. The neurological dysfunction may result in congenital tremor (CT), characterized by tremors of newborn piglets' head and limbs. Atypical Porcine Pestivirus (APPV, Pestivirus K) is a newly discovered RNA virus of the *Pestivirus* genus whose presence since its' discovery was reported from various European countries, America, and Asia. Experimental infectious trials with APPV verified that the inoculation of pregnant sows with tissue homogenates of viremic animals induces CT in most of their offsprings.

Interestingly, almost all of the clinically unaffected piglets born to inoculated sows were PCR-positive. Although the shaking itself does not directly cause death, the tremor can prevent the piglets from finding a teat to suckle, which can subsequently cause severe growth retardation or death by starvation. Based on observations, an acute outbreak of an APPV epidemic on a farm can be responsible for 10–30% of piglet mortality. Apart from various tissues, APPV was also detected in the semen of boars, which suggests that the male tract could act as a viral reservoir as previously has been observed for other pestiviruses such as Classical Swine Fever Virus (CSFV) and Bovine Viral Diarrhoea Virus (BVDV) contributing to the persistence and spread of the virus.

So far, the presence of APPV in the testicles of CT affected piglets has not been described; therefore, our research aims to identify the cell type(s) infected in the testis of newborn piglets by (i) immunohistochemistry and (ii) *in situ* hybridization (RNAscope) in order to gain a better understanding of the still unknown pathogenesis of APPV.

Controversary to previous reports of pestiviruses where mainly the Sertoli cells (BVDV) or the macrophages (CSFV) were affected, we observed that APPV was localized in the Leydig cells and spindle-shaped mesenchymal cells around the seminiferous tubuli. On consecutive sections, most of these cells appeared positive for factor VIII antigen, suggesting that they are endothelial cells. The infection of these cells by APPV has already been confirmed for other organs in previous studies. Persistent infection of the testicles and subsequent shedding of the virus via semen can be a continuous source of infection for all the female reproductive herd both in the case of locally kept animals and boar studs.

GENOME WIDE STUDY OF WILD EUROPEAN RABBIT (*ORYCTOLAGUS CUNICULUS*) POPULATIONS IN HUNGARY

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The European rabbit (*Oryctolagus cuniculus*) is a species widely spread across Europe and the world. The rabbit population of Hungary is ecologically important, but its genome has not been thoroughly investigated before. The ecological significance of rabbits in Hungary comes from their ability to influence vegetation composition (Kertész et al., 1993; Markó et al. 2011). Moderate grazing can reduce the spread of fire in grassland areas (Ónodi et al., 2008) and can be used to control the spread of invasive plant species (Dúcs et al., 2015). However, in the 1980-1990 years, myxomatosis and rabbit viral hemorrhagic disease caused a dramatic population decrease, while nowadays, hybridization with released or escaped domestic rabbits is likely leading to a genetically contaminated population.

Whole-genome sequencing (WGS) is a reliable and precise source of genomic information. We sampled six wild *O. cuniculus* individuals from Hungary, and ten subjects from four selected domestic breeds for WGS, while WGS data of wild rabbits from the Iberian Peninsula and France is publicly available (Carneiro et al. 2014).

WGS data was quality controlled, aligned to the reference genome, and all polymorphisms were identified. Preliminary data suggest that Central-European rabbit populations belong to a different haplogroup from those of Iberian origin. We are investigating the relatedness of Hungarian samples to Western-European ones, looking for signs of selection, potential admixture, and introgression events. On the other hand, we use the domestic samples to test for any recent gene flow between wild and domestic populations to investigate whether the wild population was contaminated. We check genetic diversity looking for signs of inbreeding by examining if runs of homozygosity are present.

COMPARISONS OF GENE EXPRESSION PROFILES IN HEALTHY AND AFFECTED POPULATIONS BY THE LEFT SIDED DISPLACEMENT OF THE ABOMASUM IN DAIRY CATTLE

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Displacement of the abomasum is a frequent condition of dairy cattle that occurs due to abomasal hypomotility and gas accumulation. In most cases, the abomasum is replaced to the left side, although right displaced abomasum (RDA) also exists. Left displaced abomasum (LDA) is a typical dairy cattle disease occurring in 1-7% of the Holstein-Friesian population and many other breeds. The LDA has commonly associated high yielding and intensively fed dairy cows in early or late lactation, but the disease can be found in beef cattle populations. The effective prediction would be needed as LDA increases dairy farms' veterinary costs and decreases life expectancy with restricted milk production.

The LDA's multifactorial cause is presumably based on genetic factors affecting the initiation and maintenance of the Migrating Motor Complex (MMC). Earlier studies have shown mutations in the coding regions of some gastrointestinal hormones. Mostly the gene coding for motilin hormone associated with the presence of the disease. The estimated heritability, mostly measured in Holsteins, fluctuates between 0.2 and 0.5. For examining the differences between the LDA affected and healthy animals at the RNA level, abomasum tissue samples from nine Holstein cows were collected during veterinary surgery. Three additional animals as controls were used. Five of the sampled animals were diseased. RNA Sequencing using an Illumina NextSeq500 analyzer system was performed, and the differences in gene expression between the healthy and the LDA affected samples were studied. We found differences in gene expression levels that coded gastrointestinal hormones or related to the gastrointestinal tract's motility. These genes may play a role in the development of the LDA. For evaluation of the importance of the mentioned genes, further investigations are required.

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GOLDEN JACKAL'S WHITE FUR IS CAUSED BY MC1R MUTATION FOUND IN DOGS

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Two genes control white coat color in dogs – one is MITF, microphthalmia inducing transcription factor, where a SINE insertion is causing the so-called piebald spotting, which extreme case results in the absence of melanocytes on the whole body, besides some minor spotting. The second is the MC1R, melanocortin-1 receptor, in which variations are causing loss of function results in the exclusive production of pheomelanin by melanocytes.

In dogs, three mutations of MC1R cause white fur: the most widespread and present in almost all breeds is R306Ter, where instead of arginine coding CGA, a STOP codon, TGA is found. The same mutation was also found in North American coyotes and wolves with white fur, but a melanocortin-1 receptor mutation causes white fur in American black bears, called Kermode bears, and MC1R mutations are responsible for several color variations in foxes.

With the emerging population of golden jackals in Hungary, several color variations showed up; the most striking is the white-coated one. We tested them via microsatellite markers for possible hybridization, and when that resulted in them being non-hybrids, we tested them for coat color mutations.

As it was described before, an early hybridization was possible between dogs and jackals, so our candidate gene and mutation for the white coat was the R306Ter mutation in MC1R.

We obtained samples from dogs bearing phenotypical results of MC1R mutations (white/golden coat, masked face), sequenced them, and aligned their MC1R sequence to the white jackals' sequence, which showed the same R306Ter mutation in homozygous form in both the white and golden coated dogs and the white jackals. They were also carrying the same synonym mutation at codon 380, a glutamic acid, where the first position of the codon is G, instead of C. These results support the hypothesis that either the R306Ter mutation happened earlier than the different *Canidae* species diverse or an early hybridization event between dogs and golden jackals; however, further research is required.

ESTABLISHMENT OF AN RNA INTERFERENCE BASED GENE SILENCING SYSTEM IN A SOMATICALLY TRANSGENIC MOUSE MODEL

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RNA interference (RNAi) is a biological process in which small non-coding RNA molecules silence gene expression transcriptionally or post-transcriptionally. In the past decade, this cell physiological phenomenon has become a powerful tool to analyze loss-of-function phenotypes, allowing analysis of gene function when null-mutant alleles are not available. Among different model systems, mice can mimic the human physiological processes most faithfully. Therefore, it is essential to optimize RNAi based gene silencing in somatically transgenic mouse models. Additionally, equalized co-expression of a gene of interest, a marker gene, and a miRNA can also open new possibilities to understand the role of different mammals' genes.

Our main aims were to optimize miRNA-based gene silencing in a somatically transgenic mouse model and characterize endogenous bidirectional promoters to find one what can initiate well balanced transcriptional activity.

For these purposes, we tested several endogenous promoters in 3T3 mouse embryonic fibroblast cells. In our *in vitro* experimental system, we monitored the sense and antisense transcription using mCherry and GFP marker genes. RT-qPCR measurements were carried out for the quantification of marker gene expression. Then we chose the well-balanced one, and we started to utilize it in our somatically transgenic mouse model. Our somatically transgenic mouse model is the animal model of tyrosinemia type I. In our model, the hepatocytes can be genetically altered using the *Fah* therapeutic gene as a selectable marker gene in the liver of *Fah*^{-/-} recipient mice.

For this reason, we created a transposon plasmid construct bearing with the GFP marker gene, the *Fah* selection marker gene, and the mCherry marker gene with an intronic sequence. After that, we built an amiR element against GFP in the intron of mCherry. The endogenous bidirectional promoter drove balanced transcription of mCherry, *Fah*, and GFP marker genes. A hydrodynamic injection of each transposon plasmid mixed with transposase coding helper plasmid was performed into the tail vein of *Fah*^{-/-} mice. Five months following the injection, the mCherry and GFP signals were monitored by stereomicroscopy of the treated animals' liver. Levels of transgene expression in livers were determined with RT-qPCR. The efficiency of GFP knockdown was also tested in Western Blot.

Data generated by RT-qPCR suggested that the bidirectional promoter can initiate balanced bidirectional transcription both *in vitro* and *in vivo*. Microscopic investigations also supported these findings. So far, we managed to measure GFP's efficient knockdown *in vivo* by RT-qPCR and Western Blot.

Our preliminary results revealed that we successfully identified a new bidirectional promoter, which is more balanced than any other promoter in the current biotechnological toolkit. We established an *in vivo* experimental system suitable for equalized co-expression of genes of interest and robust miRNA-based gene silencing, as well.

DEVELOPMENT AN EFFECTIVE METHOD TO PREVENT THE NEGATIVE EFFECTS OF HEAT STRESS BY STUDYING THE MRNA AND MIRNA EXPRESSION PROFILE SUBSEQUENT A POST-HATCH HEAT-TREATMENT

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It is now well established that animal adaptation will be one of the key elements for the maintenance and development of future agriculture. The heat stress is one of the numerous aspects of these changes, and poultry, due to its extensive use in the EU and worldwide, must face these coming and changing farming conditions. The ability of animals to sense and respond to elevated temperatures is essential for survival. Transcriptional control of the heat stress response has been much studied, whereas its post-transcriptional regulation by microRNAs (miRNAs) is not well understood. We planned to analyze the miRNA response to heat stress in 2-day-old chickens and tried to find a thermoregulated subset of miRNAs.

Fertilized eggs of Transylvanian Naked Neck chicken were collected in the National Centre for Biodiversity and Gene Conservation, HGI, Gödöllő, Hungary. Then eggs were incubated at 38°C and 60% humidity. The hatched chicks were divided into four groups. The first was the control group, in which 2-day-old chicks (2day-C) were grown up under normal conditions without exposure to any heat treatment. The second group (2day-HT) was subjected to heat treatment (38.5°C) at two days for 12 hours. The third (23week-C) and fourth group (23week-HT) were the control chicks and heat-treated chicks which were reared until 23 weeks of age.

The most frequently studied HSP family member in chicken is HSP70. Our analysis revealed that the heat-treated group had a higher HSP70 expression in the examined organs (liver, brain, muscle, gonads) in 2-day-old chickens than in the control group. These findings confirm the role of HSP70 in the folding of newly synthesized proteins by controlling the protein quality and turnover during both normal and stress conditions. We found a lower expression level of miR-138 in brain tissues of heat-treated groups compared to the control. These findings are indicating a cross-talk between HSP70 and miR-138. We found that the heat-treated group had a significantly higher tolerance to heat stress comparing the developmental potential of treated and non-treated chicken progenies. In the future, we plan to find other heat-related miRNAs using micro-RNA microarray analysis.

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OPU (OVUM PICK-UP) AND ICSI (INTRACYTOPLASMIC SPERM INJECTION) TECHNIQUES IN MARES

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Assisted reproductive techniques (ART) developed for cattle in the last 30 years, like ovum pick up (OPU), intracytoplasmic sperm injection (ICSI) has been transferred and adapted to horses. However, the development of assisted reproductive technologies in horses has been relatively slow compared to other domestic species, specifically ruminants and pigs. The scarce availability of abattoir ovaries and the lack of interest from horse breeders have been the main reasons for this delay. Although work in the horse can present many obstacles, individual animals' high economic value justifies the labor and expense needed to obtain foals using ART. Equine oocytes are primarily collected through transvaginal, ultrasound-guided follicle aspiration (OPU). The performance of ovum pick-up in the horse is similar in concept to that performed in cattle, but it differs in several aspects. Reported oocyte recovery rates on the aspiration of immature follicles in the mare have typically been low, repeatedly 25% or less. It appears to be due to the strong attachment of the equine oocyte to the follicle wall. Investigations of the equine follicle show that the cumulus hillock has a broader attachment to the mural granulosa than seen in cattle. Besides, there are projections from the cumulus cells into the underlying theca in the horse, essentially anchoring the oocyte-cumulus complex (COC) to the follicle wall. Therefore, the oocyte collection requires an incision of follicles and scraping of the follicle wall with a curette and extensive flushing to detach the cumulus-oocyte complexes in horses. There are two main approaches to obtaining oocytes from the donor mare: aspiration of the one dominant, stimulated follicle to recover an in vivo-matured oocyte just before it ovulates, or aspiration of all the immature follicles on the ovaries, without ovarian stimulation, to recover immature oocytes that must then be matured in vitro. During one OPU session, 6-7 oocytes can be collected on average. Intracytoplasmic sperm injection is a method for in vitro fertilization in which one sperm is injected into the cytoplasm of a mature oocyte to achieve fertilization. In Europe presently, there is only one ICSI laboratory in Italy. OPU and ICSI methods can be used to produce foals from mares that cannot become pregnant or provide an embryo under standard reproductive management. Indications for ICSI in stallions include old age, in which stallions may no longer produce quantity or quality of semen needed for standard insemination, or death of the stallion or other situations where frozen semen stores are the only existing source of semen. Among Hungarian horse breeders, a growing interest in these assisted reproductive techniques can be observed recently.

GENETIC DIVERSITY OF FIVE HUNGARIAN COMMON CARP (*CYPRINUS CARPIO* L.) LANDRACES BASED ON MITOCHONDRIAL DNA – INITIAL RESULTS

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The demand for aquaculture products is projected to increase in the future. Therefore, the maintenance and breeding of the genetic landraces of the carp (*Cyprinus carpio* L.), the most important species for Hungarian fish farmers, is of great importance. It is essential to understand better and preserve the genetic basis of these landraces developed over the last centuries. Therefore, we aimed to assess the genetic diversity of 5 common carp landraces kept in Hungary. In this study, we presented the mitochondrial DNA (mtDNA) results based on the analysis of the D-loop's 728 bp region. The following breeds were studied: the Böszörmény mirror (n = 5), the Hortobágy mirror (n = 5), the Hortobágy scaled (n = 5), the Szeged mirror (n = 5), and the Szeged scaled (n = 5) and we used the Amur wild carp (n = 5) as outgroup. Genetic diversity indices, haplotype diversity (Hd), number of polymorphisms, and nucleotide diversity (π) were calculated. The genetic relationship between haplotypes of landraces was visualized in a phylogenetic tree constructed using the Neighbour-Joining clustering method. Twenty haplotypes were identified in the studied populations. The haplotype diversity (Hd) of the populations varied between 0.800 +/- 0.164 (Hortobágy mirror) and 1.000 +/- 0.127 (Böszörmény mirror, Szeged mirror). The nucleotide diversity value (π) was between 0.003 +/- 0.002 (Hortobágy mirror) and 0.018 +/- 0.012 (Böszörmény mirror). In both cases, these values are relatively high compared to the literature. The phylogenetic tree showed that the haplotypes of the Hungarian landraces do not mix with haplotypes of other foreign landraces. Our results help to reveal the genetic uniformity and the differentiation of the studied landraces.

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ADAPTATION OF SHEEP BREEDS TO SEASONAL HEAT STRESS IN HUNGARY

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The most direct impact of global climate change is extreme weather causing significant temperature fluctuations. Environmental heat stress potentially affects biochemical pathways, regulation of inflammatory processes, and animals' proteomic and physiological characteristics, thereby in the case of animal livestock resulting in reduced production and affecting the quality of animal products. Livestock heat stress tolerance is typically breed-specific. Therefore, we presume that breeds produced in different climates adapt to their environment in different ways. Changes in heat tolerance related gene expression levels characterize the animal's response to environmental heat stress and their ability to adapt. Mammalian cells respond to high-temperature environmental stress with the production of molecular chaperones influencing the production of key proteins involved in physiological processes and maintaining and establishing their constitutive structure. These are referred to collectively as heat shock proteins (HSPs). As a result of environmental heat stress, reactive oxygen species (ROS) are formed. While ROS are important signal molecules in physiological conditions, their elevated levels adversely affect the function of antioxidant enzyme systems and cause oxidative stress, which plays an important role in many pathological processes, including inflammation. Toll-like receptor transmembrane proteins (TLRs) and the family of interleukin proteins (ILs) are important regulators of inflammatory processes.

We investigated three sheep breeds' environmental adaptability in Hungary, Debrecen, under natural heat stress conditions in August 2019. The cold-tolerant cigaja, the warm-tolerant dorper, and an intermediate breed, merino, is involved in the study. Relative gene expression pattern of HSP70 IL10, TLR2, TLR4, and TLR 8 genes were examined using GAPDH as the housekeeping gene. Further studies are to be performed to analyze seasonal changes in the adaptation process.

We aimed to explore breed-specific differences in climate adaptation, which can be used to improve sheep breeding under changing climatic conditions.

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SORCS2 GENOTYPE IS ASSOCIATED WITH PLUMAGE TRAITS IN LAYING CHICKEN HYBRIDS

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Increasing consumer pressure to further improve animal welfare is a major challenge in modern animal production. Besides welfare issues, aggressive behavior in intensively farmed animal populations poses potential risks of economic loss. In this preliminary study, a single nucleotide polymorphism of *SORCS2* (sortilin related VPS10 domain containing receptor 2) was genotyped in different chicken hybrid populations (e.g. Bábolna C1, C2, Tint) as a potential marker for aggressive behavior. A polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method was developed to identify *SORCS2* genotypes. Required DNA was isolated from feather samples, and agarose gel electrophoresis was applied for visual allele discrimination. Allele and genotype frequencies were calculated, and population differences were analyzed. In genotype-trait association studies, allele effects were investigated on several traits (e.g., general plumage condition at week 30 and 60, the occurrence of molting at week 60, frequency of behavioral types). The CC genotype was most frequent in the Tint group, and the least frequent in the C1 group was 77.8% and 52.8% ($p < 0.05$), respectively. Temperament scores did not differ significantly ($p > 0.05$) between birds with CC or CT genotypes. Results of plumage bonitation at 60 weeks of age differed significantly ($p < 0.05$) between genotypes; on average, CC animals scored 32.6 ± 3.8 points, whereas the mean of CT hens was 34.3 ± 2.6 . At 60 weeks of age, molting was observed at 29.7% of CC, whereas at 12.2% of CT birds ($p < 0.05$). The mortality rate of homozygous C chickens was also significantly ($p < 0.05$) higher compared to heterozygous (CT) chickens (18.2 and 6.8, respectively). Previously, *SORCS2* genotypes have not been associated with chicken plumage and molting traits or mortality rate; however, genotype-trait associations may develop through a potential linkage disequilibrium between the analyzed polymorphism and the relevant candidate loci; further *in silico* analyses are required to discover nearby markers with physiological relevance to the observed trait differences.

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NEW PHENOTYPE OF MYOSTATIN MUTANT RABBIT CREATED BY CRISPR/CAS9 SYSTEM

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Myostatin (growth and differentiation factor 8 or GDF8) was identified as a negative regulator factor of muscle mass more than 20 years ago. Several studies indicated multiple functions of the myostatin protein, which has been supported by our investigation. It has an essential role in inhibiting myoblast proliferation, downregulation of IGF-Akt pathway, and increasing ubiquitin-proteasomal activity. Myostatin plays a role in adipogenesis, glucose metabolism, and cardiomyocyte homeostasis. Several animal breeds were described with natural myostatin mutation causing super-muscléd phenotype in bovine, sheep, or dog, and numerous myostatin knock-out animals have been created by genome editing technologies to investigate the function of the protein.

We aimed to analyze and compare the phenotypic alterations in two different myostatin deficient rabbit breeds. We efficiently created myostatin mutations by CRISPR/Cas9 method. These mutations caused incorrect gene function, which resulted in lower myostatin expression in heterozygote animals. Although the well-recognized double-muscléd phenotype was not observed, decreased fat ratio, fertility problems, and movement dysfunction were noticed in both breeds. The lower fat ratio may cause the fertility problems found in females, which confirms myostatin's role in adipogenesis and its effect on reproduction. The degeneration of nervus tibialis caused the disorder of locomotion. This observation reveals the critical role of myostatin in the peripheral nervous system, which requires further study.

A FIELD-BASED DIAGNOSTIC DEVICE FOR SWINE VIRAL DISEASES: THE SWINOSTICS H2020 PROJECT

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The SWINOSTICS project aims to develop a novel diagnostic device using advanced bio-sensing and photonics technologies to identify selected emerging and endemic viral diseases of pigs on the field. The SWINOSTICS approach is based on the combination of photonic integrated circuit (PIC) and bio-sensing technology to detect the following viruses: African swine fever virus (ASFV), classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), porcine circovirus 2 (PCV2), and swine influenza virus (SIV). The aim of the present work was the detection of PCV2. Our biosensor PIC comprises three main building blocks: sensing ring resonators, light coupling block (grating couplers), and optical power distribution block. The ring resonators are used as sensor elements (using one resonant ring for each targeted virus), the light coupling section allows to introduce and extract the optical signals into/out of the PIC while the power distribution block feeds all the resonator rings of the PIC with a standard laser source input. Several photonic sensor PICs were functionalized to detect PCV2. On top of the surface, commercial rabbit polyclonal antibodies against PCV2 (Thermo Fisher Scientific #PA5-34969) were covalently immobilized in an oriented manner (by their carboxy-terminal of the Fc part). PCV2b cell culture supernatant (qPCR C_t:10) was used in serial PBS dilutions to test the functionalized PIC. The functionalized rings showed a remarkable shift in their resonance compared to the non-functionalized rings, even up to 1/5000 dilutions of the original sample. One of the fabricated PICs was used in a preliminary experiment for the detection of PCV2. After the rings' functionalization with specific antibodies and attaching a microfluidic layer on the PIC, positive detection of several virus concentrations was achieved. The use of clinical samples and the detection of other viruses are aimed at the project's future.

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