Fiatal Biotechnológusok

Országos Konferenciája

Abstract Book

„FIBOK 2018”

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Szerkesztette

Tamás László
Zelenyánszki Helga

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Eötvös Loránd Tudományegyetem, Természettudományi Kar

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Fiatal Biotechnológusok Országos Konferenciája 2018

2018.03.28. - SZERDA

9:00 - 10:00 **REGISZTRÁCIÓ, POSZTEREK ELHELYEZÉSE**

10:00 - 10:15 **MEGNYITÓ**
Fehér Attila, Tamás László

10:15 - 10:45 **PLENÁRIS ELŐADÁS - PLE1**
Bősze Zsuzsanna: Haszonállat modellek a transzlációs medicina szolgálatában

10:45 - 11:45 **ELŐADÁSOK**

Elnök: Fehér Attila

10:45 - 11:00 Roland TÓTH et al. **ÁE4**
Isolation, characterization and long-term culture of GFP-expressing chifcken PGCs

11:00 - 11:15 Szilárd KOVÁCS et al. **NE4**
A newly identified symbiotic gene is required for the rhizobial infection of *Medicago truncatula*

11:15 - 11:30 Anita KURILLA et al. **ME3**
Translation termination factor 1 is regulated by readthrough and NMD through 3'UTR intron in *N. crassa*

11:30 - 11:45 Hajnalka Laura PÁLINKÁS et al. **GE4**
Early embryonic lethality in mice caused by targeted gene knockout of dUTPase using CRISPR/Cas9 genome editing technology

11:45 - 12:15 **KÁVÉSZÜNET**

12:15 - 13:15 **ELŐADÁSOK**

Elnök: Kocsy Gábor

12:15 - 12:30 Csaba ÉVA et al. **NE2**
Histone acetylation and promoter-binding bZIP transcription factors ensure tissue-specificity of HMW GS genes in wheat

12:30 - 12:45 Mátyás PAJKOS et al. **BE2**
A novel motif centric protein alignment method

12:45 - 13:00 Fanni TEMESVÁRY-KIS et al. **ME6**
Investigation of the interaction between *E. coli* deoxyuridine-triphosphatase and Stl bacterial repressor protein

13:00 - 13:15 Henrietta VADÁSZI et al. **GE6**
Synaptic pruning-associated complement protein cloning: from DNA constructs to functional experiments.

13:15 - 14:00 **EBÉD**

14:00 – 14.30 **PLENÁRIS ELŐADÁS – PLE2**

Ifj. Bogsch Erik: Biotechnológiai Gyógyszerek jelene és jövője, közép európai fejlesztési perspektívából

14:30 - 15:30 **ELŐADÁSOK**

Elnök: Márialigeti Károly

14:30 - 14:45 Perczel András
Bemutatkozik az ELTE – BME 2018 őszén induló Gyógyszer-biotechnológiai mesterképzés

14:45 - 15:00 Richard IZRAEL et al. **GE3**
Characterization of an antimalarial drugtarget in mammalian cell lines

15:00 - 15:15 Zoltán PATAKI et al. **BE3**
Development of analytical method to improve detection of extracellular transmembrane protein segments

15:15 - 15:30 Gábor HARAMI et al. **ME2**
Aiming at the Achilles heel of bacterial growth

15:30 - 16:00 **KÁVÉSZÜNET**

16:00 - 17:30 **ELŐADÁSOK**

Elnök: Maráz Anna

16:00 - 16:15 Bernadett PATAKI et al. **ÁE3**
Short-term effects of DON and T-2 toxin contaminated feed on carp juveniles

16:15 - 16:30 Dóra FARAGÓ et al. **NE3**
PlantSize: an affordable, non-destructive method to measure plant size and color *in vitro*

16:30 - 16:45 Kornélia BODÓ et al. **GE1**
Characterization of novel antimicrobial molecules in *Eisenia andrei* earthworms and their involvement in immunity, development and regeneration

16:45 - 17:00 Liliána TÓTH et al. **ME9**
A *Penicillium chrysogenum*-based expression system for bulk production of *Neosartorya fischeri* antifungal protein 2 (NFAP2)

17:00 - 17:15 Valéria NAGY et al. **NE6**
Establishment of fully phototrophic, efficient and sustainable H_2 production by the green alga *Chlamydomonas reinhardtii*

17:15 - 17:30 Melinda TURÁNI et al. **GE5**
The effect of the different matrix materials on the *in vitro* corneal wound healing

17:30 - 19:30 **POSZTEREK MEGTEKINTÉSE**

19:30 **– KONFERENCIA VACSORA**

Fiatal Biotechnológusok Országos Konferenciája 2018

2018.03.29. - CSÜTÖRTÖK

08:00 - 08:30 **REGISZTRÁCIÓ**

08:30 - 09:00 **PLENÁRIS ELŐADÁS – PLE3**
Vass Imre: A fotoszintetikus napenergia hasznosítás mechanizmusa és biotechnológiai lehetőségei

09:00 - 10:00 **ELŐADÁSOK PLE3**

Elnök: Papp István

09:00 - 09:15 Roland Wirth et al. **ME10**
Cultivation of algal-bacterial biomass for renewable energy production

09:15 - 09:30 Tímea TÓTH et al. **BE4**
Show me your neighbours, and I'll tell you what you are – cellular microenvironment matters

09:30 - 09:45 Emese DEMIÁN et al. **NE1**
GLPV - a 30-year-old new virus

09:45 - 10:00 Lilla DÉNES et al. **ÁE2**
Identification of atypical porcine pestivirus in Hungarian herds

10:00 - 10:30 **KÁVÉSZÜNET**

10:30 - 12:00 **ELŐADÁSOK**

Elnök: Vass Imre

10:30 - 10:45 Flóra SZENTGYÖRGYI et al. **ME5**
Identification and characterization of biofilm bacteria from BTEX-contaminated groundwater biofilm

10:45 - 11:00 Fanni TÓTH et al. **ME8**
Searching for potentially mycotoxin degrading bacteria during *Agaricus bisporus* mushroom compost production and beginning the exploration of the compost microbial community

11:00- 11:15 Szabolcs Cselgő KOVÁCS **BE1**
Biosynthesis of industrially relevant compounds by underground enzyme reactions

11:15 - 11:30 Judit TAJTI et al. **NE9**
Differences in the mode of action of various polyamine pre-treatments during cadmium stress in wheat

11:30 - 11:45 Henrik Mihály SZAKER et al. **NE8**
The roles of a *Brassicaceae*-specific miRNA in heat stress response

11:45 - 12:00 Adrienn TÓTH et al. **ME7**
Effects of combined HHP and heat treatment on viscosity and microbiological safety of liquid egg yolk

12:00 - 12:45 **EBÉD**

12:45 - 14:15 **ELŐADÁSOK**

Elnök: Galiba Gábor

12:45 - 13:00 Pál CSUKA et al. **ME1**
***Pseudomonas fluorescens* strain R124 encodes three different MIO enzymes**

13:00 - 13:15 Edit NÉMET et al. **NE7**
Binding of manganese to chloroplast glutamine synthetase and its effect on enzyme activity in wheat

13:15 - 13:30 Gergő GYARMATHY et al. **GE2**
Secret life of a transmembrane, heparan sulfate proteoglycan

13:30 - 13:45 Dalma BÖRCSÖK et al. **ÁE1**
***In vitro* lipidation assay for the investigation of autophagy regulatory factors**

13:45 - 14:00 Zsófia NAGY et al. **ME4**
Cell growth patterns and size control in cell cycle mutants of *Schizosaccharomyces pombe*

14:00 - 14:15 Andrea LENYKÓ-THEGZE et al. **NE5**
Production of wheat/barley translocation lines

14:15 - 14:45 **KÁVÉSZÜNET**

14:45 - 16:00 **POSZTER ELŐADÁSOK**

Elnök: Gócza Elen, Várallyai Éva

16:00 - 16:30 **POSZTEREK MEGTEKINTÉSE**

16:30 - 17:00 **DÍJÁTADÁS**

Plenáris előadások összefoglalói

PLE1

HASZONÁLLAT MODELLEK A TRANSZLÁCIÓS MEDICINA SZOLGÁLATÁBAN

Bősze Zsuzsanna (bosze.zsuzsanna@abc.naik.hu)[1]

NAIK MBK, Tudományos tanácsadó

Az új génszerkesztési módszerek korábban elképzelhetetlen távlatokat nyitottak meg humán betegségek modelljeinek létrehozásában. A DNS ollók lehetővé tették, hogy haszonállat modellek genomjában is létrehozzunk hely-specifikus mutációkat. A még kezdeti stádiumban lévő technológiától távlatokban azt várjuk, hogy a gyógyszerek fejlesztésében nélkülözhetetlen, a preklinikai kutatásokban hasznosuló állatmodelleket és általuk új terápiás lehetőségeket eredményeznek.

Előadásomban összefoglalom a DNS ollók működési mechanizmusát és bemutatok néhány már létező betegségmodellt.

Munkánkat az NVKP_16-1- 2016-0039 projekt támogatja.

PLE2

BIOTECHNOLÓGIAI GYÓGYSZEREK JELENE ÉS JÖVŐJE, KÖZÉP EURÓPAI FEJLESZTÉSI PERSPEKTÍVÁBÓL.

Bogsch Erik

(Richter Gedeon Nyrt., Biotechnológiai K+F Főosztály vezetője)

Az elmúlt két évtizedben a biológiai eredetű gyógyszerek (ún. biológikumok) térhódítása a világban erőteljes volt, a globális piacnak már 20%-at teszik ki. A biológiai gyógyszerek szinte kivétel nélkül nagyméretű molekulák, szerkezeti bonyolultságuk mértéke ezzel arányban nő. Többségében rekombináns sejt fermentációs technológiával állítják elő a biológiai hatóanyagokat. Jelentőségük különösen markáns az immunológia és az onkológia területén. Terápiás fontosságuk nem ok nélkül való, bizonyos súlyos betegségek esetén az egyetlen terápiát nyújtják a tünetek jelentős enyhítésére és/vagy az élet meghosszabbítására. Terápiás tulajdonságaik mellett ezek a gyógyszerek rendkívül drágák és fejlesztésük és gyártásuk is rendkívül tőke igényes. Ez jelentős finanszírozási problémaként jelentkezik minden ország egészségügyi költségvetésében, aminek eredményeképp sok beteg nem kap hozzáférést ezekhez a készítményekhez. A terápiás fehérjék pontos másolatának, azaz generikumának előállítása a molekula mérete és bonyolult szerkezete miatt szinte lehetetlen. Ennek megfelelően jött létre az ún. biohasonló (bioszimiláris) termékkategória és az ehhez tartozó törzskönyvezési szabályozás. Biohasonló készítmények terápiás használata jelentős megtakarítást hozhat a gyógyszerkasszák számára és sokkal nagyobb elérhetőséget biztosíthat a betegek számára ezen gyógyszerek esetében.

PLE3

A FOTOSZINTETIKUS NAPENERGIA HASZNOSÍTÁS MECHANIZMUSA ÉS BIOTECHNOLÓGIAI LEHETŐSÉGEI

Vass Imre

MTA, SZBK, Növénybiológiai Intézet, Intézetigazgató

Földünkön a legnagyobb potenciálú megújuló energiaforrás a Napból származó fényenergia, ami a Föld egészére vonatkozóan éves átlagban 150 000 TW teljesítményt jelent. Így az emberiség jelenlegi éves igényeit potenciálisan kielégítő energiamennyiség kevesebb, mint egy óra alatt érkezik a Napból a Föld felszínére. A napenergia fontos tulajdonsága, hogy a Földön lényegében mindenütt, a sarkvidéki régióktól eltekintve, jórészt egyenletesen, mindenki számára rendelkezésre áll.

A legnagyobb napenergia-átalakító rendszert a természetes fotoszintézis képviseli. Ennek során a fotoszintetikus baktériumok, algák és zöld növények a Nap fényenergiáját a légköri CO_2 megkötésére és szerves anyagokba történő beépítésére használják fel a vízből vagy más szervetlen forrásból származó elektronok felhasználásával. A fotoszintetizált vegyületek kémiai kötéseiben tárolt napenergia globálisan 125 TW/év-re becsülhető, ami csaknem tízszeresen haladja meg az emberiség jelenlegi energiaigényét. A fotoszintézis mint az evolúció évmilliárdok során optimalizált energiaátalakító és -tároló folyamatának sikere elsősorban abból fakad, hogy a hozzá szükséges nyersanyagok (víz és CO_2), valamint a hajtóerő (napfény) gyakorlatilag kimeríthetetlen mennyiségben állnak rendelkezésre, a szerves vegyületek kötéseiben pedig akár százmillió éveken keresztül tárolható energia.

A fotoszintetikus rendszerek által átalakított és tárolt napenergia felhasználásának a légköri szén-dioxid mennyiségét tartósan nem növelő módszerei a növényekből származó biomassza elégetése vagy bioüzemanyaggá (bioetanol, biodízel) való alakítása. Mivel az energiacélú és élelmiszercélú növénytermelés ugyanazokért a mezőgazdaságra alkalmas földterületekért verseng, e módszerek alkalmazása globális szinten csak átmeneti megoldást jelenthet. Ezért a fotoszintetikus napenergia-hasznosításra irányuló kutatások elsődleges célja tárolható üzemanyagok (folyékony vagy illékony szénhidrogének, hidrogén) előállítása mezőgazdasági tevékenységre nem alkalmas területeken energiatermelésre optimalizált természetes vagy mesterséges rendszerekkel. A fotoszintetikus fényenergia-átalakítás mechanizmusának megértésében az utóbbi években elért szerkezet-funkcióalapú áttörés, a szintetikus kémia, szintetikus biológia, anyagtudományok és nanotechnológia területein bekövetkezett rohamos fejlődéssel együtt jó alapot szolgáltat arra, hogy a biológiai rendszerek által évmilliárdok óta sikerrel használt fényenergia-átalakítási mechanizmusok alkalmazhatók legyenek az emberiség energiaigényeinek kielégítésére.

Előadások összefoglalói

ÁE1

IN VITRO LIPIDATION ASSAY FOR THE INVESTIGATION OF AUTOPHAGY REGULATORY FACTORS

***Dalma BÖRCSÖK* (borcsok.dalma@brc.mta.hu) – Gábor HORVÁTH – Hajnalka LACZKÓ-DOBOS – Gábor JUHÁSZ**

Institute of Genetics, Biological Research Centre, HAS, Temesvári körút 62., 6726 Szeged, Hungary

The term 'autophagy', derived from the Greek meaning 'eating of self', is an essential degradation pathway of the eukaryotic cells. Autophagy plays a pivotal role in cellular homeostasis, as it is involved in the elimination of dysfunctional proteins, damaged organelles and pathogens.

During this process a double membrane structure (autophagosome) is formed in the cytoplasm which sequesters the cytosolic components. The autophagosome is able to fuse with lysosomes, which contain acidic hydrolases necessary for degradation.

The ubiquitin-like Atg8 protein's lipidation is the key for the formation of the autophagosome (in *Drosophila melanogaster* Atg8 has a and b paralogs, their mammalian homologs are the LC3 and GABARAP proteins). Atg8 binds covalently to the head group of phosphatidylethanolamine (PE) lipid molecule in a complex reaction cascade.

Several proteins are needed for the lipidation of Atg8, like protease Atg4; E1-like Atg3; E2-like Atg7 and a heterotrimeric protein complex (Atg12-Atg5-Atg16).

Our aim is to create an *in vitro* lipidation system for the investigation of *D. melanogaster*'s Atg8a and b proteins and determine whether the two Atg8 paralogs can be lipidated in a similar manner and rate?

Liposomes with high PE content (lipid sources) were prepared from *E. coli* total lipid extracts. Atg8a, b and Atg3 recombinant proteins were expressed in *E.coli* cells. Despite our attempts the Atg7 showed rapid degradation in the chosen host strains. In order to circumvent this, Atg7 is planned to be expressed in yeast cells.

Based on these results, we are on the way to successfully create an *in vitro* lipidation system for the investigation of *Drosophila* Atg8 lipidation, provided that the problematic Atg7 protein can be purified in the near future.

The system's applicability goes beyond answering the above question. We would like to use this i*n vitro* system to investigate many other regulators and inhibitors of autophagy, such as pathogen-derived proteins, which are able to hinder the Atg8 lipidation.

ÁE2

IDENTIFICATION OF ATYPICAL PORCINE PESTIVIRUS IN HUNGARIAN HERDS

***Lilla DÉNES* (denes.lilla@univet.hu)[1] – Imre BIKSI (biksi.imre@univet.hu)[2] – Mihály ALBERT (mihaly.albert@ceva.com)[3] – Gyula BALKA (balka.gyula@univet.hu)[1]**

[1]*Department of Pathology, University of Veterinary Medicine, István Str. 2., 1078 Budapest, Hungary*
[2]*Department and Clinic of Food Animal Medicine, University of Veterinary Medicine, Dóra major, 2225 Üllő, Hungary*
[3]*CEVA-Phylaxia Vaccine Producer Inc. (Ceva Sante Animale), Szállás Str. 5., 1107 Budapest, Hungary*

Pestiviruses are highly variable RNA viruses within the Flaviviridae family causing economically relevant diseases of swine, cattle, sheep and goat. Several novel pestiviruses have been described in the last few years, such as the atypical porcine pestivirus (APPV), which was identified in 2015 in the USA. Since then, the presence of APPV has been also reported in Europe, Asia and South-America. Previously published experiments confirmed that intrauterine APPV infection can induce A-II type congenital tremor (CT) in the newborn piglets, which is characterized by shaking of the head and the limbs.

The aim of our research were (i) to identify the virus in Hungary, (ii) to characterize the strains and compare them to each other and to previously published sequences, (iii) to localize and visualize the virus in the cerebellum and cerebrum.

We performed RT-PCR assays to detect the virus in the brain and lymphoid tissue of CT affected newborn piglets. In the case of positive samples, we sequenced the partial NS2-NS3 protein coding region. Our sequences supplemented with APPV sequences deposited in the GenBank were assembled using E-INS-i method of the online software mafft version 7. Maximum-Likelihood analyses were carried out with RAxML implemented in raxml-GUI v. 1.3 and the tree was plotted using MEGA 7. Visualization of the virus in the brain was carried out by RNAscope method with target probes complementary to the region obtained.

Here, we present the first report of APPV in Hungarian swine farms. To date, we have identified the virus in 24 samples originating from six Hungarian farms. The NS2-NS3 region of eight APPV were sequenced and phylogenetic analyzes were carried out. The Hungarian strains were clustered into three distinct lineages. One of them showed exceptional similarity with a strain from Austria. We found a maximum of 11.5% genetic distance among the Hungarian strains, which is similar to previous findings on APPV strains in other countries.

Using a novel RNA based *in situ* hybridization method (RNAscope), the virus was detected not only in the inner granular layer of the cerebellum, which is reported to be responsible for the clinical signs, but in ependymal and subependymal cells of the cerebrum too. Our findings on APPV might help in the better understanding of this novel and poorly described virus.

ÁE3

SHORT-TERM EFFECTS OF DON AND T-2 TOXIN CONTAMINATED FEED ON CARP JUVENILES

Bernadett PATAKI (bebe.betti@gmail.com)[1] – Csilla PELYHE (pelyhe.csilla@gmail.com)[2] – Erika ZÁNDOKI (zandoki.erika@gmail.com)[2] – Miklós MÉZES (mezes.miklos@mkk.szie.hu)[1] – Krisztián BALOGH (balogh.krisztian@mkk.szie.hu)[1]

[1]Department of Nutrition, Szent István University, Páter K. u. 1. 2100 Gödöllő, Hungary
[2]Mycotoxins in the Food Chain" Research Group, Hungarian Academy of Sciences, MTA-KE, Kaposvár University, Guba S. u. 40. 7400 Kaposvár, Hungary

Fusarium species, as field moulds may produce different mycotoxins, e.g. trichothecenes. T-2 toxin is a 'type A', while deoxynivalenol (DON) is a 'type B' trichothecene mycotoxin. Both are known as powerful inhibitors of protein synthesis, which can cause failures in the immune- and antioxidant defence system and may reduce productivity.

The purpose of present study was to evaluate the short-term effects of different combined doses of DON and T-2 toxin on lipid peroxidation processes and on the amount/activity of glutathione redox system in common carp juveniles.

A total of 112 one-year-old (50.42±16.72 g b.w.) common carps were used. After a week adaptation period, 4 groups were formed: a control ('C') and 3 treated groups, which were as follows: low ('T0.5D1' – 0.51 mg T-2 toxin and 1.01 mg DON/kg b.w.), medium ('T1D2' – 1.01 mg T-2 toxin and 2.01 mg DON/kg b.w.), and high combined doses of T-2 toxin and DON ('T2D4' – 1.97 mg T-2 toxin and 3.97 mg DON/kg b.w.). The treated carps received single oral dose of mycotoxin contaminated complete feed by gavage. Hepatopancreas samples were taken at the start of the experiment (as absolute control) and at every 8th hour (n=6/group) during the 24h long experimental period, where activity of glutathione-peroxidase (GPx) and reduced glutathione (GSH) concentration were measured. To investigate the lipid peroxidation processes, the amount of conjugated dienes (CDs) and -trienes (CTs) and malondialdehyde (MDA) concentration were determined.

The highest applied doses increased the mortality rate rapidly; 10% of the animals died between the 8th and 16th hours after the mycotoxin exposure.

CTs was significantly higher in 'T2D4' group than in 'T0.5D1' at 8h, showing a rapid prooxidant effect of the applied trichothecenes. GSH concentration significantly increased in 'T1D2' group as compared to 'T0.5D1' group at 16h. In the same time also elevated GPx activity were found in 'T1D2' and 'T2D4' groups as compared to the 'T0.5D1' group.

The results show that the single oral doses of mycotoxins caused rapid changes in the lipid peroxidation processes in the hepatopancreas of common carp. The emerging free radical burden quickly activated the glutathione redox system which was able to reduce and eliminate the prooxidant effect of the investigated mycotoxins. In this case, the end product of lipid peroxidation processes (MDA) did not increase significantly.

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ÁE4

ISOLATION, CHARACTERIZATION AND LONG-TERM CULTURE OF GFP-EXPRESSING CHICKEN PGCs

***Roland TÓTH* (toth.toland.imre@abc.naik.hu)[1,2] – *Bence LÁZÁR* (lazar.bence@abc.naik.hu)[2,3] – *Mahek ANAND* (sweet_anand2@yahoo.com)[1] – *Eszter PATAKINÉ VÁRKONYI* (varkonyi.eszter@hagk.hu)[3] – *Krisztina LIPTÓI* (liptoi.krisztina@hagk.hu)[3] – *Elen GÓCZA* (gocza.elen@abc.naik.hu)[2]**

[1]*Doctoral School of Animal Husbandry Science; SZIU, Páter K. str. 1., 2100 Gödöllő, Hungary*
[2]*Department of Animal Biotechnology; ABC, NARIC, Szent-Györgyi A. str. 4., 2100 Gödöllő, Hungary*
[3]*Research Centre for Farm Animal Gene Conservation; HáGK, Isaszegi road 200., 2100 Gödöllő, Hungary*

Primordial germ cells (PGCs) are the precursors of germinal cells, the progenitors of eggs and spermatozoa, and provides an alternative way to preserve both male and female genetic materials in poultry. These cells are isolated from the blood of 2.5–3 days old chicken embryo. In this period, the number of PGCs has a peak in the blood vessels and it is possible to isolate them easily from the embryo. Cryopreserved the PGC lines, we are able to save endangered poultry species. The main aim of our research work is to preserve the genetic background of seven Old Hungarian Chicken Breeds. The cryopreserved PGCs are stored in liquid nitrogen. In that case, if we need that endangered breed, we defreeze the PG cells and reinject them to a recipient embryo. At the end of 2016, our group established 9 GFP-expressing PGC lines. These PGC lines were characterized by using RT-PCR analysis and immunostaining. We determined the stem cell- and germ cell specific marker expressions *(CVH, cNANOG, cPOUV, cDAZL)*. Simultaneously, the sex of the PGC lines were determined using P2-P8 primer-pairs. The female cell lines contained Z and W chromosomes whereas the male cell lines have only Z.
These GFP-expressing PGCs were injected back into two different recipient breeds (White Hungarian and Partridge colour Hungarian). The integration efficiency, when the male PGCs (4ZP line) were injected back to the White Hungarian breed was 31.6%, when 4ZP cells were injected back to the Partridge colour Hungarian breed, was 22.4%. When the female PGCs (5ZP) were injected back to White Hungarian breed, the integration rate was 25% in the case of White Hungarian breed, but in case of Partridge colour Hungarian breed it was just 14.3%.
In conclusion, we could not find significant difference between the male and female cell lines compared the stem cell- and germ cell specific marker expression. We demonstrated that both male and female PGCs can integrate into the gonads of both recipient chicken line.

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BE1

BIOSYNTHESIS OF INDUSTRIALLY RELEVANT COMPOUNDS BY UNDERGROUND ENZYME REACTIONS

***Szabolcs Cselgő KOVÁCS* (kovszasz@gmail.com)**

Department of Biochemistry, MTA BRC, Temesvári krt. 62., 6726 Szeged, Hungary

Nowadays sustainable development is one of the most challenging global issues. The production of industrially relevant compounds by microorganisms contributes to the sustainable development. Microorganisms often require optimization in order to increase yield which is frequently achieved by engineering heterologous pathways. Heterologous pathways are artificially designed biochemical routes containing transgenic metabolic steps. However, inserting transgenic reactions into the metabolic network of the host organism often causes unexpected side-effects like producing cytotoxic intermediers, changing the distribution of cofactors, or incompatibility with the metabolism of the host cell.

Here, we suggest the use of underground enzyme activities as a new approach of metabolic engineering. Underground activities are side-reactions the enzymes can catalyze, albeit at low rates, due to their limited substrate specificity. Although the physiological effect of underground reactions is mostly negligible their activities can be enhanced by well-established molecular genetics methods making them an ideal surrogate to heterologous metabolic steps.

We used metabolic modeling to investigate the potential of underground reactions to aid the synthesis of industrially relevant compounds. We extended the genome-scale metabolic reconstruction of *Escherichia coli* with 572 underground reactions and simulated the production of several hundred industrially relevant compounds. In several cases introducing a single underground reaction extended the ability of the native metabolic network to produce certain compounds, thus suggesting that underground enzyme activities can be utilized in industrial strain development.

BE2

A NOVEL MOTIF CENTRIC PROTEIN ALIGNMENT METHOD

***Mátyás PAJKOS* (matyaspajkos@caesar.elte.hu) – Zsuzsanna DOSZTÁNYI (dosztanyi@caesar.elte.hu)**

Department of Biochemistry, ELTE Eötvös Loránd University; Pázmány Péter sétány 1/C 1117 Budapest, Hungary

SLIMs (Short Linear Motifs) are common interaction modules that play critical roles in diverse biological pathways. SLIMs usually reside in disordered regions and their short length and weak phenotype makes their experimental discovery challenging. As a result, SLIM mediated interactions are highly underrepresented in current protein networks. This underlines the importance of computational approaches for the discovery of functional de-novo motifs.

Currently, there are two main approaches for de-novo motif discovery. Alignment free methods seek to find enriched motif sequences in a group of related sequences. Alignment based methods, like SLIMPrints[1], exploit the specific evolutionary conservation of SLIMs. As functional SLIM sites show stronger evolutionary constraints compared to their disordered sequential neighborhood, this gives the appearance of island like conservation in multiple alignment of orthologues.

However, evolutionary approaches rely heavily on good quality sequence alignments covering larger evolutionary distances. Such alignments are often not available for disordered protein segments, which harbor most SLIMs. In order to overcome this major limitation of evolutionary based de-novo motif discovery methods, we propose a novel SLIM specific alignment method. In this approach, the starting scoring is based on motif enrichments within homologous, and alignments are not forced in regions that have no evolutionary conservation. This enables a more accurate detection of evolutionary conservation over larger distances even within disordered segments, making it feasible to detect functional SLIMs within any homologous, from vertebrate to plants.

[1]Davey *et al*. *Nucleic Acids Research. 2012 40:10628-10641*

BE3

DEVELOPMENT OF ANALYTICAL METHOD TO IMPROVE DETECTION OF EXTRACELLULAR TRANSMEMBRANE PROTEIN SEGMENTS

Zoltán PATAKI* (zoltang.pataki@gmail.com)[1] – Tamás LANGÓ (lango.tamas@ttk.mta.hu)[1] – Lilla TURIÁK (turiak.lilla@ttk.mta.hu)[3] – Júlia VARGA (varga.julia@ttk.mta.hu)[1] – László DOBSON (dobson.laszlo@ttk.mta.hu)[1] – Beáta G. VÉRTESSY (vertessy.beata@ttk.mta.hu)[1,2] – Gábor E. TUSNÁDY (tusnady.gabor@ttk.mta.hu)[1]

[1]*Hungarian Academy of Sciences, RCNS, Institute of Enzymology, Magyar Tudósok krt 2, 1117 Budapest, Hungary*
[2]*Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Sciences, Szent Gellért tér 4, 1111Budapest , Hungary*
[3]*Hungarian Academy of Sciences, RCNS, Institute of Organic Chemistry, Magyar Tudósok krt 2, 1117 Budapest, Hungary*

Transmembrane proteins (TMP) play crucial role in living systems, they participate in metabolic-, energy- and information transport processes. Amount of TMPs are encoded by human genome about 6-8 thousand, although in structure databank the ratio of known structures of TMPs are only 2%. One of the reasons that makes exceedingly difficult to determine their structures by traditional structure examination methods like NMR or X-ray crystallography is their disadvantageous physical-chemical properties.
Due to the difficult experimental approaches, the bioinformatical methods and mathematical models in TMPs structure examination have major role recently. Synergy of theoretical and experimental methods is highly efficient approach to assign the low resolution structure/topology as well as detailed 3D structure of TMPs. Our research group developed a Constrained Consensus TOPology (CCTOP) *in silico* prediction algorithm and a high throughput topology assay to produce and predict topology data for more than hundreds of proteins at the same time with more reliability and accuracy. Our recent work focuses on modification and insertion of few steps in the previously published (Langó *et al*., 2017, SciRep.) experimental method in order to increase identified extracellular TMP segments in HL60 cell lines. Main steps of the process are labeling of cell surface proteins with membrane impermeable Sulfo-NHS-SS-biotin, lysis of cells and then membrane preparation and digestion with proteolytic enzymes, enrichment and isolation of labeled peptides, finally purification to prepare samples for tandem mass spectrometry. We inserted a partial proteolysis of cell surface proteins as a new step, used trypsin and chymotrypsin serine proteases with different concentration and digestion time in order to observe, how change the yield of TMPs and their labeled peptides. We use the tandem mass spectrometry identified labeled peptide pattern and protein database to determine several labeled peptide sequences of TMPs, moreover, we can identify the regular site of labeling. The identified labeled positions can be used as constraints in the CCTOP algorithm that help to increase reliability and accuracy of topology prediction of TMPs.

BE4

SHOW ME YOUR NEIGHBOURS, AND I'LL TELL YOU WHAT YOU ARE – CELLULAR MICROENVIRONMENT MATTERS

Tímea TÓTH* (totht@brc.hu)[1] – Tamás BALASSA (balassa.tamas@brc.mta.hu)[1] – Norbert BARA (baran@brc.hu)[1,2] – Ferenc KOVÁCS (fkovacs@brc.hu)[2] – András KRISTON (kristona@brc.hu)[1,2] – Csaba MOLNÁR (molnar.csaba@brc.mta.hu)[1] – Lajos HARACSKA (haracska.lajos@brc.mta.hu)[1] – Farkas SUKOSD (sukosd.farkas@med.u-szeged.hu)[3] – Péter HORVÁTH(horvath.peter@brc.mta.hu)[1,4]

[1]Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary.
[2]Single-Cell Technologies Ltd, Szeged, Hungary.
[3]University of Szeged, Szeged, Hungary.
[4]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland.

To answer major questions of cell biology, it is essential to understand cellular complexity. Modern automated microscopes produce vast amounts of images routinely, making manual analysis nearly impossible. Due to their efficiency, machine learning-based analysis software have become essential tools to perform single-cell-level phenotypic analysis of large imaging datasets. However, an important limitation of such methods is that they do not use the information gained from the cellular micro- and macroenvironment: the algorithmic decision is based solely on the local properties of the cell of interest. Here, we present how various microenvironmental features contribute to identifying a cell and how such additional information can improve single-cell-level phenotypic image analysis. The proposed methodology was tested for different sizes of Euclidean and nearest neighbour-based cellular environments both on tissue sections and cell cultures. Our experimental data verify that the microenvironment of a cell largely determines its entity. This effect was found to be especially strong for established tissues, while it was somewhat weaker in the case of cell cultures. Our analysis shows that combining local cellular features with the properties of the cell's microenvironment significantly improves the accuracy of machine learning-based phenotyping.

GE1

CHARACTERIZATION OF NOVEL ANTIMICROBIAL MOLECULES IN *EISENIA ANDREI* EARTHWORMS AND THEIR INVOLVEMENT IN IMMUNITY, DEVELOPMENT AND REGENERATION

***Kornélia BODÓ* (bodo.kornelia@pte.hu)[1] – *Ákos BOROS* (boros.akos@ddr.antsz.hu)[2] – *Éva RUMPLER* (rumpler.eva@gmail.com)[3] – *Péter NÉMETH* (nemeth.peter@pte.hu)[1] – *Péter ENGELMANN* (engelmann.peter@pte.hu)[1]**

[1]*Department of Immunology and Biotechnology, Clinical Center, Medical School, University of Pécs; Szigeti u. 12 , 7643.Pécs, Hungary*
[2]*Regional Laboratory of Virology, ANTSZ, Baranya County Institute of State Public Health Service; Szabadság út 7., 7623 Pécs, Hungary*
[3]*Department of Comparative Anatomy and Developmental Biology, Faculty of Sciences, University of Pécs; Ifjúság út 6., 7624Pécs, Hungary*

Invertebrate organisms operate with distinct innate cellular and humoral immune components to maintain their self-integrity. Antimicrobial peptides (AMPs) are ancient molecules that are structurally conserved during the evolution. Until now, only one earthworm AMP the lumbricin and its orthologues were described in annelids. Our aims were to isolate the potential homologue of lumbricin in *Eisenia andrei* earthworms and characterize its expression pattern.
In order to isolate the potential lumbricin mRNA in *E. andrei*, conventional RT-PCR, 5' and 3' RACE PCRs were performed. Q-PCR analyses were executed to evaluate the expression levels of lumbricin in various tissues of earthworms, during development as well as antero-posterior regeneration process. To estimate the temporal expression pattern of this peptide upon pathogen exposure Q-PCR measurements were performed. We identified and characterized two novel homologues of lumbricin AMP from *E. andrei* earthworms. The cloned cDNA of *Ea-lumbricin* and *Ea-lumbricin-related peptide* consist of 189 and 177 bp open reading frames, respectively. The predicted molecular weights of the proline-rich lumbricin and the related peptide are 7413.35 and 7066.84 Da. Phylogenetic analysis revealed strong sequence similarity among annelids. Q-PCR analysis evidenced that the highest expression of both AMPs mRNA was observed in whole foregut (pharynx, gaster), while other tested tissues had moderate or basal expression. Both of AMPs were expressed in all developmental stages. Their expression revealed steady elevation up to the fourth developmental stage (E4). During anterior and posterior regeneration the patterning mechanisms of *Ea-lumbricin* and its related peptide were rather consistent. In coelomocytes, both of AMPs expression was significantly increased after 48 hrs of *in vivo* Gram-positive *Staphylococcus aureus* stimulation.
In conclusion, our unique data further supports the conservation of lumbricin and the existence of multiple isoforms of these proteins within annelids.

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GE2

SECRET LIFE OF A TRANSMEMBRANE, HEPARAN SULFATE PROTEOGLYCAN

Gergő GYARMATHY (gyarigeri12@gmail.com)[1] – Bettina UGHY (ughy.bettina@brc.mta.hu)[1] – Ivan RANDELOVIC (ivan.randel@gmail.com)[2] – Anett HUDÁK (anett.hudak@pharmacoidea.eu)[3] – Tamás LETOHA (tamas.letoha@pharmacoidea.eu)[3] – László SZILÁK (laszlo.szilak@gmail.com)[4]

[1]Institute of Plantbiology, Biological Research Centre of HAS, Temesvari krt 62., 6726 Szeged, Hungary
[2]Department of the Experimental Pharmacology, National Institute of Oncology, 1122 Budapest, Hungary
[3]PharmacoIdea Ltd., Derkovits fasor 7-11., 6726 Szeged, Hungary
[4]Insititute of Biology, SEK ELTE, Karolyi G. ter 4., 9700 Szombathely, Hungary

The syndecan family belongs to the type I transmembrane proteins, bearing heparan sulfate (HS) and chondroitin sulfate on the core proteins. It comprises four members syndecan-1–4 (SDCs) in mammals. The expression of SDC1–3 is tissue specific, meanwhile that of SDC4 is universal. The state-of-the-art emphasizes their primary role being co-receptors next to the main receptors. They can bind and enrich growth factors, cytokines and chemokines in the cell surface. However, SDC4 can be considered as a real receptor because it can transmit signals solely, regulating the activity of Rho family of small GTPases. The signal transduction can occur upon phosphorylation of the cytoplasmic domain of syndecan-4. The phospho-syndecan-4 and its phosphomimetic mutant can reduce the Rac1 activity 3-fold (EU2365984) triggering Rac1-RhoA-Cdc42 dependent signals. Further SDC4 can influence the mitosis and cytokinesis in phosphorylation dependent way. Its phosphomimetic form can cause delayed cytokinesis with elongated intercellular bridges and the non-phosphorylatable mutation induces aneuploidity. During the cell cycle the phospho-syndecan-4 can escape from the membrane and distributed with the mitotic spindles[1]. The phospho-form can enter the nuclei and enriched in the nuclear speckles (EU2078195). SDCs promote endocytosis and the endocytosed SDC4 follows a retrograde route concentrating in the cis-Golgi network. Most of the known cell penetrating peptides (CPP) employ syndecans for the cell entry[2,3].

The CPPs form heterogeneous groups of peptides having the ability to internalize into cells and can deliver various attached cargoes. They are considered as usually short (5 – 30 amino acids), water-soluble and partly hydrophobic, and/or polybasic peptides with a net positive charge at physiological pH. Few sequence similarities has been found between different classes of CPPs. Here we present using different syndecan-4 mutants how the different segments (HS, core protein) of syndecan-4 can contribute to CPP uptake.

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EU2365984 SYNDECAN-4 IS A REGULATOR OF RAC1
EU2078195 INTRACELLULAR TARGETING OF MOLECULES

GE3

CHARACTERIZATION OF AN ANTIMALARIAL DRUGTARGET IN MAMMALIAN CELL LINES

***Richard IZRAEL* (izrael.richard@ttk.mta.hu)[1,2] – *Lívia MARTON* (marton.livia@ttk.mta.hu)[2,3] – *Beáta G. VÉRTESSY* (vertessy@mail.bme.hu)[1,2]**

[1]*Institute of Enzimology, Research Centre of Natural Sciences, Hungarian Academy of Science, Magyar tudósok körútja 2. 1117 Budapest, Hungary*
[2]*Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Szent Gellért tér 4., 1111 Budapest, Hungary*
[3]*Doctoral School of Multidisciplinary Medical Sciences, Dóm tér 9.. University of Szeged, 6720, Szeged, Hungary*

In spite of the extended and active research, malaria is still a major threat amongst vector-borne diseases. The prevalence of multidrug-resistant Plasmodium strains urges the identification of new potential drug targets and development of new antimalarials against them. The parasite depends crucially on membranogenesis during the intraerythrocytic stage. Phosphatidyl-choline (PC) is the major contributor of eukaryotic membranes[1]. The main biosynthetic pathway of *de novo* PC biosynthesis is the Kennedy-pathway[2]. The rate-limiting step of the procedure is catalysed by the CTP:phosphocholine cytydilyltransferase (CCT) enzyme, which turns the phosphocholine (ChoP) into cytidine diphosphate choline (CDPCho). As a key enzyme of the pathway, it has been validated as a potential antimalarial drug target[3].
CHO-MT58, the conditional mutant Chinese Hamster Ovary (CHO) cell line strain was generated with random mutagenesis from the wild type CHO-K1. In the CHO-MT58 cell line a point mutation turned the endogenous CCT into a thermosensitive phenotype. At the permissive temperature 37 °C, the cell line can maintain its membranogenesis, however at the restrictive temperature 40 °C the CCT degradation increases and the loss of PC content leads to apoptosis of the cells[4]. Nevertheless, the heterologously expressed CCT is able to rescue the CHO-MT58 cells, thus it is an appropriate tool for analysis of CCT specific elements of the orthologue *Plasmodium falciparum* and mammalian CCTs.
Here we demonstrate the rescue potential of the two catalytic domains of the pseudoheterodimer *Plasmodium falciparum* CCT (*Pf*CCT). We expanded our research *in silico* to look for *Pf*CCT specific structural elements compared to its mammalian orthologue rat CCT (*Rattus norvegicus, Rn*CCT), which might serve as potential drug development target. Furthermore, we analysed the localisation of the different protein constructs in African Green Monkey kidney cell line (COS-7).

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GE4

EARLY EMBRYONIC LETHALITY IN MICE CAUSED BY TARGETED GENE KNOCKOUT OF DUTPASE USING CRISPR/CAS9 GENOME EDITING TECHNOLOGY

Hajnalka Laura PÁLINKÁS* (palinkas.hajnalka@ttk.mta.hu)[1,2] – Gergely RÁCZ (racz.gergely@ttk.mta.hu)[1,3] – Zoltán GÁL (zoltan.gal89@gmail.com)[4] – Gergely TIHANYI (tihanyi.gergely@ttk.mta.hu)[1,3] – Elen GÓCZA (gocza.elen@abc.naik.hu)[4] – László HIRIPI (hiripi.laszlo@abc.naik.hu)[4] – Beáta G. VÉRTESSY (vertessy@mail.bme.hu)[1,2,3]

[1]*Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt. 2., 1117 Budapest, Hungary*
[2]*Doctoral School of Multidisciplinary Medical Science, University of Szeged, Dóm tér 9., 6720 Szeged, Hungary*
[3]*Department of Applied Biotechnology and Food Sciences, Budapest University of Technology and Economics, Szent Gellért tér 4., 1111 Budapest, Hungary*
[4]*Agricultural Biotechnology Institute, Department of Animal Biotechnology, Szent-Györgyi Albert u. 4., 2100 Gödöllő, Hungary*

Multiple knock-out mouse models can provide key novel insights into dynamics of genome integrity. The object of our studies, dUTPase, is a key enzyme in genome maintenance. It catalyzes the hydrolysis of dUTP into pyrophosphate and dUMP, supporting low cellular dUTP/dTTP ratio thus prevents genomic uracil accumulation. The molecular mechanism of thymine-less cell death, induced by the lack of dUTPase, is poorly understood although several routinely applied chemotherapeutic drugs in the clinic (fluoropyrimidines, methotrexate and its derivatives); interfere with the *de novo* thymidylate biosynthetic pathway. Previous reports showed that overexpression of dUTPase causes partial resistance against fluoropyrimidines, while its deficiency sensitizes tumor cells. Therefore a better knowledge of dUTPase role and function is particularly important in medicine. To investigate the physiological role of dUTPase *in vivo*, we established mice lacking dUTPase using CRISPR/Cas9-mediated genome engineering. So far two transgenic mouse models (*dut* +/-) were gained with 6 or 47 base pairs deletion in the coding gene of dUTPase. After crossing heterozygous mutants no viable homozygous pups were born indicating a lethal mutation. dUTPase homozygous mutant embryos were identified among blastocysts, which exhibited a normal appearance, but homozygous embryos were never found by E8.5, suggesting that mutant embryos die immediately after implantation. Single blastocysts are being undertook real-time PCR analysis to investigate potential maternal contribution of dUTPase transcripts at pre-implantation stages. We also performed Western blot studies on tissues derived from wild type and *dut* +/- heterozygote tissues in order to quantify dUTPase protein expression level and immunohistochemical staining on mouse sections to investigate cellular localization pattern. Our results establish for the first time that dUTPase is indispensable for proper post-implantation embryogenesis in mouse. However, the interesting unexpected aspect of this finding is that *dut*-/- biallelic KO individuals seem to survive the first replicative stages of pre-implantation and lethality sets in only after implantation.

GE5

THE EFFECT OF THE DIFFERENT MATRIX MATERIALS ON THE *IN VITRO* CORNEAL WOUND HEALING

Melinda TURÁNI (femystra@gmail.com) – Zsuzsanna BELÁNY (belany.zsuzsi@gmail.com) – Viktoria BAKSA (viktoriabaksa@gmail.com) – Gábor NAGY (bigdegu@gmail.com)

Department of Microbial Biotechnology and Cell Biology, University of Debrecen; Debrecen Egyetem Square 1, 4010 Debrecen, Hungary

In vitro monolayer scratch model was established to examine the wound regeneration of the corneal surface. The process of the monolayer regeneration of human limbal stem cells was based on time lapse microscopy and the evaluation of the results was performed by using digital image analysis. In this experimental setup the purpose of the study was to observe the physiological effect of the different surfaces (glass, polystyrene), and matrix materials (collagen, hyaluronic acid, gelatin) on wound healing. The regeneration profile was determined by using specifically adapted dynamical image analysis. The analysis of the time lapse image sequence shows the cellular changes of the monolayer minute-by minute for an extended time period.
The healing of the monolayer can be described by the motility and regeneration curves. All of the regeneration curves consisted four phases:
I.) The expanding and reattachment phase. An initial delay in the regrowth, caused by the cell-reattachment to the edges of the scratch.
II.) The exponential growth phase. Repopulating the disturbed area with cell migration and division.
III.) Transition phase.
IV.) Stationary phase. Attaining the complete confluency
Motility curve shows the movement of scratched edges of monolayer minute-by-minute which can be trended to the inward or outward of the disturbed area. The positive oscillation shows the inward movements of the cells (migration, proliferation, reattachment). The negative oscillation describes the outward movements of the cells (detachment, apoptosis of the damaged cells, transient or permanent cellular shrinkage)
The regenerations on the different surfaces were compared and the spreading of the monolayers were calculated in a defined region of interest (ROI) selection of the image sequence.
Furthermore, our further aim to monitor the adhesion of filamentous fungi on these matrix components to get information about the pathogenicity of *Aspergillus fumigatus* during the corneal wound healing.

GE6

SYNAPTIC PRUNING-ASSOCIATED COMPLEMENT PROTEIN CLONING: FROM DNA CONSTRUCTS TO FUNCTIONAL EXPERIMENTS

***Henrietta VADÁSZI* (vadaszi heni@caesar.elte.hu) – *András MICSONAI* (micsonai@ttk.elte.hu) – *Réka KOVÁCS* (koveszreka@gmail.com) – *Éva BULYÁKI* (bulyakie@ttk.elte.hu) – *Judit KUN* (juditkun.bc@gmail.com) – *Balázs GYÖRFFY* (gyorffy.balazs88@gmail.com) – *József KARDOS* (kardos@elte.hu)**

Department of Biochemistry, Institute of Biology, ELTE Eötvös Loránd University; Pázmány Péter sétány 1/c, 1117 Budapest, Hungary

Synaptic network of the central nervous system is highly dynamic with an estimated lifetime in the order of hours. Synaptic pruning controls the number of synapses by eliminating the non-functional ones in the healthy brain. In neurodegenerative diseases, such as Alzheimer's, this process is shifted to the aberrant levels of synapse loss. The molecular mechanisms of synaptic pruning has not been known in details. Components of the complement system have been shown to play a significant role in synapse elimination, however, the molecular background and the interacting protein partners have not been characterized yet. Our group investigates the proteins that label the synapses for elimination or modulate the process. C1q is a molecule that labels the synapses for microglial removal. According to our results pentraxins are interaction partners of C1q and also participate in synapse elimination. Complement components are often huge multimer complexes kept together by disulphide bonds and have extensive posttranslational modifications like glycosylation. The effective production of these extracellular proteins is challenging and the most promising way is to express them in eukaryotic expression systems. We managed to optimise the efficient mammalian expression resulting functional protein complexes. For this purpose we constructed our own eukaryotic and prokaryotic expression vectors containing exchangeable affinity tags. C1q is an oligomeric protein consisting of six heterotrimer globular heads and collagen-like tail regions. The C1q head is responsible for recognition and the tail exhibits effector functions. We created a single chain C1q head construct (scC1qh) which might be a useful tool to investigate the *in vivo* functions of C1q by lacking its effector functions. Our aim is to characterize the interactions between scC1qh and its potential binding partners such as the pentraxins *in vitro* and *in vivo*. ScC1qh might be also used as an inhibitor of aberrant synapse loss by competing with functional C1q and can serve as a possible pharmaceutical targe

ME1

PSEUDOMONAS FLUORESCENS STRAIN R124 ENCODES THREE DIFFERENT MIO ENZYMES

Pál CSUKA (csuka.pal@mail.bme.hu)[1] – Vivien JUHÁSZ (j32vivien@gmail.com)[1] – Szabolcs KOHÁRI (szabolcs.kohari@fermentia.hu)[2] – Alina FILIP (alina.filip@chem.ubbcluj.ro)[3] – Andrea VARGA (andrea.varga@chem.ubbcluj.ro)[3] – Péter SÁTORHELYI (peter.satorhelyi@fermentia.hu)[2] – László Csaba BENCZE (cslbencze@chem.ubbcluj.ro)[3] – Hazel BARTON (bartonh@uakron.edu)[4] – Csaba PAIZS (paizs@chem.ubbcluj.ro)[3] – László POPPE (poppe@mail.bme.hu)[1,3,5]

[1]*Department of Organic Chemistry and Technology Budapest University of Technology and Economics, Műegyetem rkp. 3, H-1111 Budapest, Hungary*
[2]*Fermentia Microbiological Ltd, Berlini út 47–49, 1049 Budapest, Hungary*
[3]*Biocatalysis and Biotransformation Research Center Faculty of Chemistry and Chemical Engineering Babes-Bolyai University of Cluj-Napoca, Arany János str. 11, 400028 Cluj-Napoca, Romania*
[4]*Department of Biology, The University of Akron ASEC, West Tower 178, OH-44325 Akron, USA*
[5]*SynBiocat Ltd, Szilasliget u. 3, 1172 Budapest, Hungary*

The enzymes in this study belong to enzyme classes playing a major role in nitrogen metabolism related to aromatic amino acids in microbes, fungi, plants, and animals. Aromatic amino acid ammonia-lyases acting on L-histidine, L-phenylalanine, or L-tyrosine as natural substrates are closely related and share high similarity to aromatic 2,3-aminomutases acting on L-phenylalanine or L-tyrosine as natural substrates. All members of this enzyme superfamily, involving aromatic ammonia lases and 2,3-aminomutases, contain the unique 3,5-dihydro-5-methylidene-4H-imidazol-4-one (MIO) catalytic moiety, which forms post-translationally from an XSG triad (X is A in most cases, but may also be T, S, or C).

The *Pseudomonas fluorescens* R124 strain was isolated from a nutrient limited orthoquartzite sandstone cave. Nutrient limitation puts environmental pressure on the living organisms to use novel metabolic pathways. As part of our efforts to obtain novel MIO enzymes from organisms living under extreme conditions and locations, herein we describe the identification and characterization of a histidine ammonia lyase, a tyrosine/phenylalanine/histidine ammonia lyase, and a phenylalanine-2,3-aminomutase from *Pseudomonas fluorescens* R124

In addition to their important roles in nature, the MIO enzymes - due to their tolerance towards unnatural amino acids as substrates and the reversibility of their reaction – constitute a natural biocatalysis toolbox for syntheses of enantiopure α and β-amino acids.

ME2

AIMING AT THE ACHILLES HEEL OF BACTERIAL GROWTH

***Gábor HARAMI* (harami@caesar.elte.hu) – Anna BUDAI (94.budai.anna@gmail.com) – Mihály KOVÁCS (kovacsm@elte.hu)**

Department of Biochemistry, ELTE Eötvös Loránd University, Pázmány P. stny. 1/C, 1117 Budapest, Hungary

Resistance to antibacterial drugs emerges as one of the most serious threats to human health and economy. The discovery of antibiotics with novel mechanisms has severely slowed down due to overmining of existing drug sources and lack of novel targets. These facts highlight the need for increased efforts in search for new lead molecules and drug targets.

Currently we aim to explore the biological roles and biomedical potential of the bacterial single-stranded (ss) DNA binding (SSB) protein's multilateral interactions. These interactions are ideal but unconventional drug target candidates. SSB proteins are present in all organisms and sequester nascent ssDNA generated during DNA metabolism to protect it from damage that could lead to harmful genetic defects. Moreover, SSB proteins serve an equally crucial additional role: they interact with more than a dozen DNA-modifying enzymes involved in DNA replication and repair, mainly to organize multiprotein complexes on ssDNA. Bacterial SSBs possess a conserved short C-terminal peptide (CTP) segment, which is their main protein interaction platform. The roles of CTP interactions are not fully understood, however our recent studies indicate that CTP interactions are required to provide access to ssDNA coated by SSB to DNA-modifying enzymes. In line with these results CTP interactions appear to be essential for bacterial viability and importantly such interactions are absent in eukaryotes. These properties along with earlier studies highlight that molecular agents capable of blocking CTP interactions would potentially suppress the growth of bacterial cells, but not that of eukaryotes.

To explore the precise role of CTP interactions and to identify interaction blocking agents we have devised a fundamentally novel high-throughput *in vivo* screening approach that will allow us to probe a large number of modified CTP peptide variants for their antibacterial potential brought about by intracellular competition with natural CTP-protein interactions.

We would like to use our potential CTP hits to investigate the role of CTP interactions in DNA metabolism. Moreover, we hope that our potential CTP hits can serve as starting points in development of peptide-based broad spectrum antibiotics and the explored structural/chemicophysical information can be used in designing and/or screening of novel small molecule leads, to which emergence of resistance has a presumably decreased likelihood.

ME3

TRANSLATION TERMINATION FACTOR 1 IS REGULATED BY READTHROUGH AND NMD THROUGH 3'UTR INTRON IN *N. CRASSA*

***Anita KURILLA* (kurilla.anita@abc.naik.hu) – *Dániel SILHAVY* (silhavy.daniel@abc. hu)**

Agricultural Biotechnology Centre, Szent-Györgyi Albert str. 4., 2100 Gödöllő, Hungary

Translation termination factor (eRF1) has a crucial role in protein synthesis through stimulating both peptide release and ribosome recycling. The level of such factor should be strictly controlled, both over and under expression of eRF1 lead to strong phenotypes. Previously our group has shown that eRF1 protein level is controlled by a complex autoregulatory circuit in plants. We hypothesize that a similar regulatory system functions in filamentous fungi. Thus I study the regulation of eRF1 in *Neurospora crassa.* The results have an evolutionary relevance, because the eRF1 autoregulation circuit in plant and fungi may supports our understanding about the origin of this mechanism.

Aberrant transcripts containing intronic 3'UTR can induce NMD (Nonsense-mediated RNA decay), an eukaryotic surveillance system which degrades faulty transcripts to avoid the accumulation of harmful peptides or to balance the optimal protein level. Plant *eRF1* mRNA has a unique structure, this transcript has an intronic 3'UTR and its stop codon is present in a readthrough stimulating context. *eRF1* transcript level is decreased by NMD through its intronic 3'UTR, while the readthrough stop context can partially protect the mRNA from NMD. Increased eRF1 protein level decreases the frequency of readthrough, so the *eRF1* mRNA will be degraded by NMD more efficiently. These attributions make the eRF1 autoregulated by readthrough and NMD. The very special structure of *eRF1* transcript also appears in *Neurospora*. We demonstrated that the readthrough stop context of *eRF1* can also protect the *eRF1* mRNA from NMD in *Neurospora.* The results suggest that autoregulatory circuit of eRF1 also exists in filamentous fungi, so it has evolved in the common ancestor. Our further plan is to study the effect of the overexpression of eRF1 and make an allelspecific gene replacement of *eRF1* by CRISPR system to clarify the physiological relevance of the specific structure of *eRF1* mRNA.

ME4

CELL GROWTH PATTERNS AND SIZE CONTROL IN CELL CYCLE MUTANTS OF *SCHIZOSACCHAROMYCES POMBE*

***Zsófia NAGY* (zsofianagy992@gmail.com) – Anna MEDGYES-HORVÁTH (horvath.anna11@gmail.com) – Eszter VÖRÖS (eszter210@gmail.com) – Ákos SVEICZER (asveiczer@mail.bme.hu)**

Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics; Műegyetem rkp. 4., 1111 Budapest, Hungary

The fission yeast *Schizosaccharomyces pombe* is a widely used model organism. Its rod-shaped cells grow only at their tips with constant diameter so its length is proportional to its volume. The newly born cells grow only at their old end (the one that was a growing end even in the previous cycle). Later, the NETO (new end take-off) event happens at a critical size and growth changes from unipolar to bipolar.

Microscopic photographs were taken of several steady state and induction synchronous cultures of different cell cycle mutants of fission yeast. Measurements of cell length were performed of 55–174 cells per culture on each photo taken from cell birth to division. The measured rough data was smoothened using the *rsmooth* function of MiniTab. For the growth patterns of individual cells, three functions (linear, bilinear or exponential) were fitted by the *Solver* extension of MS Excel. The most adequate function was determined by using statistical criteria (like Akaike Information Criteria, AIC) on the fitted models. This was necessary because the models contained different numbers of parameters.

The cultures showed heterogeneous growth patterns and we investigated if there is some correlation between the growth pattern and either birth length or cycle time. In cultures with wide size distribution, our observation was that cells with larger birth length tend to grow linearly, while the smaller cells tend to grow bilinearly (only a very small amount of cells showed exponential growth pattern). Our data demonstrated that the RCP (rate change point) in the bilinear pattern occurs earlier in the cell cycle of larger cells. This indicates that the transition from bilinear to linear takes place through an RCP shifting near to the beginning of the cycle. The RCP is thought to be caused by the NETO event, which promotes a higher cell extension rate. Cells with larger birth length might exceed the critical size for NETO, so they may grow bipolar during almost the whole cycle, and show no rate change.

In all of the steady state cultures studied (*cdc2-33*, *cdc2-M35* and *cdc2-33 wee1-6* mutants), a cell size control mechanism can be observed. Studying artificially enlarged cells of induction synchronous cultures (*cdc2-33* and *cdc10-129* mutants) we could determine the cell size where the G2 size control becomes cryptic. By similar analyses, the critical maximal birth size for the G1 size control was also determined in a *cdc2-33 wee1-6* mutant's induction synchronous culture.

ME5

IDENTIFICATION AND CHARACTERIZATION OF BIOFILM BACTERIA FROM BTEX-CONTAMINATED GROUNDWATER BIOFILM

Flóra SZENTGYÖRGYI (floraszentgyorgyi5@gmail.com)[1] – Tibor BENEDEK (benedek.tibor@fh.szie.hu)[2] – Balázs KRISZT (kriszt.balázs@mkk.szie.hu)[1] – András TÁNCSICS (tancsics.andras@fh.szie.hu)[2]

[1]Department of Environmental Safety and Ecotoxicology, Institute of Aquaculture and Environmental Safety, Szent István University, Páter Károly str 1., 2100 Gödöll,ő Hungary
[2]Regional University Centre of Excellence, Szent István University, Páter Károly str1., 2100 Gödöllő, Hungary

We face serious environmental problems, when we deal with contaminations caused by oil and its derivatives (e.g. Exxon Waldez - 1989; Deepwater Horizon - 2010). Removal of these petroleum hydrocarbon pollutants from soil and groundwater is important because hydrocarbons may have carcinogenic, mutagenic and teratogenic effects on living organisms. In Hungary, environmental pollutions with petroleum hydrocarbons mainly occur during extraction, storage and transportation. Still nowadays, to eliminate hydrocarbons from the environment, the most environmentally friendly and cost-effective method is bioremediation. Therefore, the aim of the research was to obtain a strain collection of good BTEX-degrading (benzene, toluene, ethyl-benzene, ortho-, meta-, para-xylene) and biofilm forming bacteria applicable in the near future for alternative biofilm-based *in situ* bioremediation systems (e.g. biofilm-based permeable reactive barriers).

For strain collection, bacterial isolates were obtained after enrichment from a biofilm developed in a BTEX contaminated groundwater in central Hungary. Bacteria were enriched under aerobic and microaerobic conditions. A molecular fingerprinting method (T-RFLP) was used to investigate the effect of selective enrichments on the initial biofilm bacterial community throughout of the enrichment period. The obtained isolates were characterized regarding their biofilm forming and BTEX degrading abilities.

BTEX degradation ability of isolates was assessed by using a spectrophotometric assay based on the redox indicator resazurin. Bacterial strains showing the highest activity in resazurin amended test solutions were re-tested by using a more accurate approach (GC-MS). Biofilm forming ability of strains was assessed by using crystal-violet and 96-well microplates.

As a result of aerobic and microaerobic enrichments a total 37 bacterial strains were isolated. Isolates belonged mainly to Beta and Gammaproteobacteria. Representatives of genera *Pseudomonas* and *Rhizobium* exhibited extreme biofilm forming abilities. The most promising BTEX degraders belonged to the genera *Variovorax*, *Nocardioides*, *Pseudomonas* and *Rhodococcus*.

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ME6

INVESTIGATION OF THE INTERACTION BETWEEN E. COLI DEOXYURUDINE-TRIPHOSPHATASE AND STL BACTERIAL REPRESSOR PROTEIN

***Fanni TEMESVÁRY-KIS* (f.temesvary@gmail.com)[1,2] – András BENEDEK (benedek.andras@ttk.mta.hu)[1,2] – Beáta G. VÉRTESSY (vertessy@mail.bme.hu)[1,2]**

[1]*Department of Applied Biotechnology and Food Sciences, Budapest University of Technology and Economics; Műegyetem rkp. 3, 1111 Budapest, Hungary*
[2]*Laboratory of Genome Metabolism and Repair, Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok körútja 2, 1117 Budapest, Hungary*

The enzyme dUTPase plays a key role in excluding uracil from DNA via hydrolysis of dUTP to dUMP and pyrophosphate. Deficiency of dUTPase results in a high level of dUMP incorporation into DNA. Under high dUTP/ dTTP ratio the uracil-excision repair transforms into a hyperactive futile cycle, which finally leads to cell death via double-stranded DNA breaks (thymine-less cell death)[1]. Inhibition of dUTPase is a promising novel approach to significantly enhance the efficiency of thymidylate synthase targeted chemotherapeutic agents[2]. A naturally occurring interaction partner and inhibitor of the Φ11 phage dUTPase has recently been reported, namely the Stl repressor protein from *Staphylococcus aureus*[3]. Further inhibitory tests in our laboratory have proven Stl's inhibitory effect on some other dUTPases including the mycobacterial, the human and the *Drosophila melanogaster* enzyme. Interestingly, for the *Escherichia coli* and *Yersinia enterocolitica* dUTPases no inhibiton was found, but we have verified that at least the *E. coli* enzyme and Stl specifically interact with each other. At this point, our direct aim is to understand which amino acid residues on the dUTPase side of these interactions are responsible for dUTPase inhibition beyond complex formation. During my research I prepared recombinant dUTPase enzymes, containing point mutations near the active site, to explore the interaction sites between the two proteins. I characterized the interaction between the wild type dUTPase and Stl, and between the recombinant dUTPases and Stl with gel-filtration chromatography, steady-state enzyme assay and native gel electrophoresis. Native gel electrophoresis and gel-filtration chromatography confirmed the interaction between the proteins, respectively. A single glutamine → histidine amino acid change near the active site has led to more than 40% inhibiton of dUTPase enzymatic activity by Stl. Our results revealed that the 93rd position of the *E. coli* dUTPase is an important connection point which plays an important role in the inhibition of dUTPases by Stl.

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ME7

EFFECTS OF COMBINED HHP AND HEAT TREATMENT ON VISCOSITY AND MICROBIOLOGICAL SAFETY OF LIQUID EGG YOLK

Adrienn TÓTH* (toth.adrienn@etk.szie.hu)[1] – Csaba NÉMETH (nemeth.csaba@capriovus.hu)[2] – Emna AYARI (ayari.mna@gmail.com)[1] – Khabat NOORI (khagri86@yahoo.com)[1] – Ildikó ZEKE (zeke.ildiko.csilla@etk.szie.hu)[1] – László FRIEDRICH (friedrich.laszlo.ferenc@etk.szie.hu)[1]

[1]Szent István University, Department of Refrigeration and Livestock Product's Technologies, Ménesi út 43 – 45.1118 Budapest, Hungary
[2]Capriovus Ltd. Dunasor 073/72 hrsz. 2317 Szigetcsép, Hungary

Minimal processing technologies, like High Hydrostatic Pressure (HHP), heat treatments on law temperatures, ultra-sonication have an increasing tendency in food industry. Eggs are considered as functional foods, but for high retention of biological active compounds adequate minimal processing technologies are needed during preservation procedure. In our study liquid egg yolk was examined to meet consumer's expectations.
Several combinations of law temperature pasteurization (57 – 63 °C, 5 – 7 min) and High Hydrostatic Pressure (350 – 400 MPa, 5 min) were used to provide microbiological stability of liquid egg yolk. After treatments samples were examined for mesophyll aerobe and Enterobacteriaceae cell counts and viscosity attributes.
Our results show that microbiological stability is significant influenced by the different parameters of heat treatments and HHP have a strong effect.
Viscosity attributes (measured with Anton Paar MCR 92) analysed by Hershel-Bulkley models point out that higher pressure of HHP have a stronger influence on viscosity than the temperature of pasteurization. Our results show a great opportunity for industrial use of minimal processing technologies for liquid egg yolk. Microbiological safety is strongly influenced by the order of treatments, but viscosity is independent from order of HHP and heat treatment.

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ME8

SEARCHING FOR POTENTIALLY MYCOTOXIN DEGRADING BACTERIA IN *AGARICUS BISPORUS* MUSHROOM COMPOST PRODUCTION AND BEGINNING THE EXPLORATION OF THE COMPOST MICROBIAL COMMUNITY

Fanni TÓTH (fannytoth@ttk.elte.hu)[1] – Balázs VAJNA [1] – Mátyás CSERHÁTI[2] – Anita RISA[2] – Károly MÁRIALIGETI[1]

[1]*Department of Microbiology, Faculty of Science, ELTE Eötvös Loránd University Pázmány Péter sétány 1/C,, 1117 Budapest, Hungary*
[2]*Department of Environmental Safety and Ecotoxicology, Szent István University, Páter Károly str 1. 2100 Gödöllő, Hungary*

White button mushroom (*Agaricus bisporus*) is produced on a composted substrate in which process microorganisms have a substantial role. An important risk factor is that raw materials of the substrate are potentially contaminated with mycotoxins. Currently it is not known what kind of effects mycotoxins or their degradation products have on button mushroom and whether microorganisms in the mushroom compost are able to degrade or neutralize mycotoxins. The aims of this work were to isolate bacterial strains from different stages of the mushroom compost production and optimize DNA isolation for microbial community analysis. From the raw materials of the mushroom compost (wheat, rape straw, sunflower husk) bacterial strains were isolated using an extended spectrum, peptone containing medium (Corynebacterium Agar), and a more specific, starch casein medium. Based on 16S rRNA gene sequence analysis, mainly members of phylum Firmicutes (*Bacillus* spp., *Paenibacillus* spp. and *Siccibacter* spp.) were detected on the Corynebacterium Agar, while phyla Proteobacteria, Actinobacteria (*Massilia* spp., *Rhodococcus* spp. and *Streptomyces* spp.) and *Paenibacillus* spp. were isolated using starch casein medium. Thereafter samples were collected systematically from raw materials until phase III mushroom compost. Bacterial strains were isolated using Corynebacterium Agar and BYE enrichment media containing toxin analogues. Based on the research results obtained so far, there were some bacterial strains detected with degradation ability of aflatoxin B1, zearalenone, deoxynivalenol and T2 toxin from wheat straw. For optimizing community DNA isolation a few samples were homogenized with grinding in liquid nitrogen, then community DNA was isolated by a commercial kit using different time interval bead mill homogenization. The quantity and quality of the obtained DNA was analyzed with spectrophotometer. Community composition was compared using fingerprinting techniques: Terminal Restriction Fragment Length Polymorphism (T-RFLP) based on 16S rRNA gene for Bacteria, whereas Automated Ribosomal Intergenic Spacer Analysis (ARISA) based on nrDNA ITS-1 region for Fungi. The optimized DNA isolation technique was applied for the systematically collected samples. They will be subjected to bacterial and fungal amplicon sequencing (partial 16S rRNA gene and nrDNA ITS-2 region) and microbial community changes will be compared with the mycotoxin content of the samples.

This research was supported by the National Competitiveness and Excellence Programme (NVKP_16-1-2016-0035).

ME9

A *PENICILLIUM CHRYSOGENUM*-BASED EXPRESSION SYSTEM FOR BULK PRODUCTION OF *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEIN 2 (NFAP2)

Liliána TÓTH* (toth.liliana88@gmail.com)[1,2] – Györgyi VÁRADI (varadi.gyorgyi@med.u-szeged.hu)[3] – Attila BORICS (borics.attila@brc.mta.hu)[4] – Gyula BATTA (batta@unideb.hu)[5] – Zoltán KELE (kelezoltan@gmail.com)[3] – Roberta TÓTH (robertabellydance@hotmail.com)[2] – Hargita FICZE (ficze.hargita6@gmail.com)[2] – Csaba VÁGVÖLGYI (mucor1959@gmail.com)[2] – Florentine MARX (florentine.marx@i-med.ac.at)[6] – László GALGÓCZY (galgoczi@gmail.com)[1,2]

[1]*Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences; Szeged Temesvári krt. 62., 6726 Hungary*
[2]*Department of Microbiology, Faculty of Science and Informatics, University of Szeged; Hungary*
[3]*Department of Medical Chemistry, Faculty of Medicine, University of Szeged; Hungary*
[4]*Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences; Hungary*
[5]*Department of Organic Chemistry, Faculty of Science and Technology, University of Debrecen; Hungary*
[6]*Division of Molecular Biology, Biocenter, Medical University of Innsbruck; Austria*

In the last decades, systemic fungal infections caused by different *Candida* spp. have become one of the most frequent healthcare-associated infections worldwide. The treatment is problematic due to emerging number of resistant strains to the currently applied first-line therapeutic drugs. Based on our previous studies the *Neosartorya fischeri* antifungal protein 2 (NFAP2) is a promising candidate for novel antifungal drug development because it exerts high antifungal activity on clinically relevant *Candida* spp. One of the limiting factors of its further investigations and potential application is the low-yield expression by the native producer *N. fischeri* NRRL 181. To solve this problem a *Penicillium chrysogenum*-based heterologous expression system was developed for the bulk production. The applied *P. chrysogenum* Q176 strain is recognized as GRAS organism by the US Food and Drug Administration. The generated NFAP2-producer *P. chrysogenum* strain secreted recombinant NFAP2 (rNFAP2) in a defined minimal medium which allowed an easy, single-step chromatographic purification of the sufficient protein amount for functional and structural investigations. The average yield of rNFAP2 was 40-times higher than in the native producer. rNFAP2 showed the same high anti-yeast activity against different *Candida* species as the native NFAP2. Structural analyses revealed that rNFAP2 was correctly processed and folded. The described easy-production of high-yield functional rNFAP2 could open new perspectives for its practical application.

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ME10

CULTIVATION OF ALGAL-BACTERIAL BIOMASS FOR RENEWABLE ENERGY PRODUCTION

***Roland WIRTH* (roland.w@freemail.hu)[1] – *Tamás BÖJTI* (tamas.bojti@gmail.com)[1] – *Gergely MARÓTI* (maroti.gergely@brc.mta.hu)[2] – *Zoltán BAGI* (bagiz@brc.hu)[1] – *Gábor RÁKHELY* (rakhely@brc.hu)[1,3] – *Kornél L. KOVÁCS* (kovacs.kornel@brc.mta.hu)[1,4]**

[1]*Department of Biotechnology, University of Szeged, Közép fasor 52, 6726 Szeged, Hungary*

[2]*Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Temesvári krt. 62, 6726 Szeged, Hungary*

[3]*Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Temesvári krt. 62, 6726 Szeged, Hungary*

[4]*Department of Oral Biology and Experimental Dental Research, University of Szeged, Tisza L. krt. 64, 6720 Szeged, Hungary*

Alternative bioenergy production is one of the hot topics in both basic and applied research. Microalga based bioenergy production is a promising field with regard to the wide variety of algal species. Extensive previous studies employed microalgae for biohydrogen and particularly for biodiesel production, but relatively little attention has been paid to their utilization as biogas substrate. The algal biomass, grown under less stringent conditions and therefore containing symbiotic bacterial biomass as well, can be utilized in the biogas reactor and the total biomass is degraded and converted by the complex biogas producing microbial community. The critical elements of energetic use of microalgal biomass is the high cultivation cost and the cultivation efficiency. In this study simple and inexpensive cultivation methods were tested using waste streams of high organic content. *Chlorella vulgaris*, a green microalga was used in these experiments because this is a widespread strain in natural waters and is therefore easily accessible. During the cultivation process the pH, carbon, nitrogen, phosphorus, potassium, and organic acids were measured. The cultivation effectiveness depended on light limitation, pH and the organic acid content. The product, containing bacterial and *C. vulgaris* biomass, was investigated for its biogas potential and for the anaerobic process stability. The results showed that the algal-bacterial biomass had a biogas potential comparable to the pure and lyophilized *C. vulgaris* biomass. A potential difficulty in the sustained anaerobic digestion of microalgal biomass may be due to the low C/N ratio as increasing ammonium concentration in the fermentation medium has negative effect on methanogens. Therefore, alternative co-substrates were tested to improve the process stability and efficacy.

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NE1

GLPV – A 30-YEAR-OLD NEW VIRUS

***Emese DEMIÁN* (demian.emese@abc.naik.hu)[1] – Levente KONTRA (kontra.levente@abc.naik.hu)[1] – Nikoletta CZOTTER (czotter.nikoletta@abc.naik.hu)[1] – János LÁZÁR (lazar.janos@szbki.naik.hu)[2] – Éva VÁRALLYAY (varallyay.eva@abc.naik.hu)[1]**

[1]Diagnostic Group, Agricultural Biotechnology Research Institute, National Agricultural Research and Innovation Center, 2100 Gödöllő, Hungary
[2]Research Institute for Viticulture and Enology, National Agricultural Research and Innovation Center, Katona Zs. street 5. 6000 Kecskemét, Hungary

In 1987, a new virus-like disease was described by János Lehoczky in Hungary, called grapevine line pattern, the symptoms were various patterns of bright yellow lines or large rings on the leaves. The line pattern could be transmitted by grafting into three different, susceptible grapevine cultivars. In symptomatic leaves polymorphic virus-like particles were observed by EM. Based on its characteristics the virus was classified into the Ilarvirus genus, but no sequence information was gained. In the pathogenic garden at Kecskemét, established by Professor Lehoczky, the GLPV infected grapevine still exists.

The development of sequencing techniques allowed the spread of new metagenomic methods, which can be used to obtain sequence information from all pathogen present in the examined plant. Our group use the specific type, NGS of virus specific small RNAs, of these methods for virus diagnostic purposes. This technique provided an opportunity to gain information about the genome of the GLPV and its actual existence. Leaf, flower, tendril and shoot tip samples were collected from sprouted canes of the original, infected grapevine. After the RNA purification small RNA library was prepared from RNA pools of the organs using Illumina TruSeq small RNA library preparation kit. The library was sequenced and was analyzed by bioinformatics methods.

Small RNA sequences and the contigs from overlapping small RNAs were searched with BLAST on the NCBI reference genome database. The closest similarity was obtained with the Amazon lily mild mottle virus (ALiMMV), which is a member of the *Anulavirus* genus within the Bromoviridae family, such as ilarviruses. Primers for all of its 3 RNAs were designed and the presence of the putative GLPV was confirmed by RT-PCR. Traditional Sanger sequencing of the PCR products allowed the phylogenetic analysis of the virus genome and suggests a suspected new Anulavirus. The presence of both 3 RNAs of the tripartite genome was verified by Northern blot hybridization. However, to confirm the connection between this putatively new anulavirus in the anciently GLPV infected grapevine and the originally described symptoms of GLPV further investigation are needed.

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NE2

HISTONE ACETYLATION AND PROMOTER-BINDING BZIP TRANSCRIPTION FACTORS ENSURE TISSUE-SPECIFICITY OF HMW GS GENES IN WHEAT

Csaba ÉVA (eva.csaba@agrar.mta.hu)[1] – Szabolcs MAKAI (makai.szabolcs@agrar.mta.hu)[1] – Kitti SZŐKÉNÉ PÁZSI (kitti.pazsi@agrar.mta.hu)[1] – Blanka MONCSEK (moncsek.blanka@agrar.mta.hu)[1] – László SÁGI (sagi.laszlo@agrar.mta.hu)[1] – László TAMÁS (laszlo.tamas@ttk.elte.hu)[2] – Angéla JUHÁSZ (juhasz.angela@agrar.mta.hu)[1,3]

[1]Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik str. 2, 2462 Martonvásár, Hungary
[2]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary
[3]Present address: State Agricultural Biotechnology Centre, School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA 6150, Australia

High molecular weight glutenin subunits (HMW GS) are important storage proteins of wheat endosperm and their contribution is also remarkable to bread-baking. Their encoding genes are regulated in an endosperm-specific manner. Our intention was to get a clearer picture of this regulation. The possible role of histone acetylation has been studied by applying histone deacetylase-inhibitor treatment thereby causing histone hyperacetylation. The treatment resulted in HMW GS promoter activation in non-target vascular tissues, confirming here previous reports on the regulatory role of histone acetylation. Transcriptional regulation of the promoters has been studied both *in silico* and in wet lab. Six cis regulatory modules have been previously identified within the HMW GS promoters. Of them, the second one (CRM2) exerted tissue-specific control. Its deletion has strengthened the promoter in the leaf, a non-target tissue, as it was found during transient expression assays. Results with a mutant version of CRM2 indicated, that presumably a basic-leucine zipper (bZIP) transcription factor (TF) binds to this element and thereby blocks the HMW GS transcription outside endosperm. Efforts were also undertaken to identify the suppressor bZIP TF gene. Phylogenetic groups of bZIP TFs were silenced by transforming wheat leaves with antisense constructs and the effect was measured using transactivation assay. The results so far indeed showed that the suppression of many phylogenetic groups (C, D, F, G) boosts the activity of different HMW GS promoters. Suppressors may be present in endosperm as well, providing potential negative targets for breeding to improve bread-baking quality of wheat.

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NE3

PLANTSIZE: AN AFFORDABLE, NON-DESTRUCTIVE METHOD TO MEASURE PLANT SIZE AND COLOR *IN VITRO*

***Dóra FARAGÓ* (farago.dora@brc.mta.hu) – László SASS (sass.laszlo@brc.mta.hu) – Ildikó VALKAI (valkai.ildiko@brc.mta.hu) – Norbert ANDRÁSI (andrasi.norbert@brc.mta.hu) – László SZABADOS (szabados.laszlo@brc.mta.hu)**

Laboratory of Arabidopsis Molecular Genetics; Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Temesvári krt. 62., 6726 Szeged, Hungary

Plant phenotype is determined by the genetic background and environmental conditions. Interaction of the genotype and environmental factors influences plant growth and development, physiological and molecular traits. Characterization of phenotypes is crucial to understand the regulation of stress in responses to climate changes. Non-destructive analysis of plants through color imaging is an increasingly popular method to define growth parameters, characterize plant development in time.

We have developed a non-invasive method, which simultaneously measures basic morphological and physiological parameters of *in vitro* cultured plants such as *Arabidopsis thaliana*. Changes of plant size, shape and color is monitored by repeated photography with a commercial digital camera. Images are analyzed with the Matlab-based computer application PlantSize, which simultaneously calculates several parameters including projected rosette area (pixel area, fresh weight, convex area and ratio), and color (chlorophyll and anthocyanin contents). Numerical data are exported in MS Excel format. Subsequent data processing provides information on growth rates, chlorophyll and anthocyanin contents. Utility of the system is demonstrated by revealing small but significant differences between wild type and transgenic Arabidopsis plants overexpressing the HSFA4A transcription factor or the *hsfa4a* knockout mutant, subjected to different stress conditions. While HSFA4A overexpression was associated with better growth, higher chlorophyll and lower anthocyanin content in saline conditions, the mutant showed hypersensitivity to various stresses. Morphological differences were revealed by comparing rosette size, shape and color of wild type plants with phytochrome B (*phyB-9*) mutant.
The developed technology offers a simple, affordable and fast way to measure several morphological and physiological parameters of Arabidopsis plants. The methods are based on non-destructive imaging allowing repeated measurements and monitoring changes of various growth parameters in time. PlantSize is publicly available (http://www.brc.hu/pub/psize).

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NE4

A NEWLY IDENTIFIED SYMBIOTIC GENE IS REQUIRED FOR THE RHIZOBIAL INFECTION OF *MEDICAGO TRUNCATULA*

***Szilárd KOVÁCS* (kovacssz@brc.hu)[1] – *Sándor JENEI* (Jenei.sandor@brc.mta.hu)[1] – *Ernő KISS* (kiss.erno@brc.mta.hu)[2] – *Attila KERESZT* (kereszta@gmail.com)[1] – *Gabriella ENDRE* (endre.gabriella@brc.mta.hu) [1]**

[1]*Institute of Plant Biology, BRC-HAS, Temesvari krt. 62.. 6726. Szeged,Hungary*
[2]*Institute of Genetics, BRC-HAS, Temesvari krt. 6.2, 6726. Szeged, Hungary*

Legumes (Fabaceae) are unique among flowering plants in their ability to establish symbiotic associations with nitrogen-fixing bacteria colletctively known as rhizobia. This host specific relationship is taking place in a novel plant organ developing on the plant root termed nodule. The microsymbiont bacteria can enter into the nodule, convert into a bacteroid form and fix atmospheric nitrogen in a form that can be used by the host plant. The symbiotic process is triggered by the NOD-factors produced by the bacteria via inducing symbiotic gene expression in the host plant. Genetic studies during the last two decades have led to the identification and characterization of several plant genes involved in the early steps of legume-rhizobia symbiosis and it was found out that all of these genes are part of the so-called NOD-factor signal transduction pathway. On the other hand few genes are known that are involved in the later steps, like bacterial invasion of the nodule or formation of the symbiosomes. Here, we report the identification and characterization of a novel gene necessary for the bacterial invasion of the forming nodule.

By screening the European *Tnt1* insertional mutant collection a symbiotic mutant line has been isolated in wich the bacteria failed to invade the forming nodule. Detailed phenotypic analyses revealed that the earliest responses to the bacteria, like root hair curling and forming nodule primordia are intact. However, the formation of the tube-like structure called infection thread is impaired in the mutant, indicating that the mutated gene is required for the formation and progression of the infection thread. We uncovered the mutated gene and the symbiotic function of the gene was confirmed by complementation assays. By monitoring the promoter activity of this gene during the symbiotic interaction we proved that it is active only in the epidermis within a very short time window. Moreover, we provided an evidence that the induction of the gene is regulated by the NOD-factors. We have also checked the activity of this promoter in already known symbiotic mutants upon NOD-factor induction and so we could put this new symbiotic gene in relation to the already known compontents of the NOD-factor signal transduction pathway. Furthermore, when spontaneous nodule formation was induced in our mutant, this special organs appeared on the roots indicating that the nodule organogenesis itself was intact. Taken these data together, we discovered a novel symbiotic gene required for the Legume-Rhizobia nitrogen-fixing symbiosis and we termed it **N**OD-factor induced **E**pidermal **F**actor **1**.

NE5

PRODUCTION OF WHEAT/BARLEY TRANSLOCATION LINES

Andrea LENYKÓ-THEGZE (lenyko-thegze.andrea@agrar.mta.hu) – Eszter GAÁL (gaal.eszter@agrar.mta.hu) – Márta MOLNÁR-LÁNG (langne.molnar.marta@gmail.com) – Gabriella LINC (linc.gabriella@agrar.mta.hu)

Department of Plant Genetic Resources, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik Street 2, 2462 Martonvásár, Hungary

The cultivated and wild relatives of common wheat (*Triticum aestivum* L.) possess a wide range of genetic diversity. The aims of gene transfer from alien species are to produce intergenomic translocations that carry the chromosome sections of the alien species responsible for one of the important favourable characteristics. The final goal is to produce pre-breeding lines with favourable agronomical traits from barley (favourable chemical composition, high content of dietary fiber/β-glucan, salt tolerance etc.). Intergeneric rearrangements can be detected using genomic *in situ* hybridization (GISH) and molecular markers.

The plant material used in this study was produced earlier in Martonvásár. Wheat/barley disomic addition lines (2H, 3H, 4H, 6H and 7H) were developed between a Japanese facultative wheat cultivar ‘Asakaze' and a Ukrainian winter barley cultivar ‘Manas'. Our main goal was to produce genetically stable translocations, which only carry DNA segments responsible for a useful agronomical trait.

Two different genetic systems were used: pairing homoeologous (*Ph*) system and the gametocidal system. The *Ph1* gene located on the long arm of chromosome 5B. *Ph1* prevents recombination between homoeologous chromosomes, when the *Ph1* gene absent from the wheat genome, homoeologous chromosomes are also able to recombine. The 'Asakaze'/'Manas' 7H disomic addition line was crossed with the CS *ph* mutant line in order to induce chromosome breakage and rearrangements. Out of 60 plants analysed, 9 carried wheat/barley monosomic centric fusions. In the F_3 generation, out of 120 analysed plants, 21 plants carried the centric fusion in stable, disomic form.

In nature, there are certain species carrying gametocidal (Gc) chromosomes, which after being incorporated into another genome will cause genetic rearrangements, such as translocations. Wheat/barley translocations were induced with the gametocidal system using the 2C gametocidal chromosome originated from *Aegilops cylindrica* L. (2n = 4x = 28; CCDD). The Asakaze/Manas 7H addition × *Ae. cylindrica* 2C line was backcrossed with the 7H wheat/barley addition line. Out of 176 BC_1F_2 seeds, 6 wheat-barley (7H) translocations were detected and translocations were transmitted to the next generation. Seven plants carried a centric fusion with the short arm of the 7HS chromosome. In the F_3 generation, 3 plants carried the centric fusion in disomic form. We multiply these lines in order to analyse qualitative parameters and determine the phenotypic description of the plants.

This research was supported by the WHEALBI EU FP7 (613556) project.

NE6

ESTABLISHMENT OF FULLY PHOTOTROPHIC, EFFICIENT AND SUSTAINABLE H_2 PRODUCTION BY THE GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

***Valéria NAGY* (nagy.valeria@brc.mta.hu)[1] – Anna PODMANICZKI (podmaniczki.anna@brc.mta.hu)[1] – André VIDAL-MEIRELES (meireles.andre@brc.mta.hu)[1] – Roland TENGÖLICS (tengolics.roland@brc.mta.hu)[2] – Gábor RÁKHELY (rakhely.gabor@brc.mta.hu)[3] – László KOVÁCS (kovacs.laszlo@brc.mta.hu)[1] – Alberto SCOMA (alberto.scoma@bios.au.dk)[4] – Szilvia Z. TÓTH (toth.szilviazita@brc.mta.hu)[1]**

[1]*Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Temesvári krt. 62., 6726 Szeged, Hungary*
[2]*Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Temesvári krt. 62., 6726 Szeged, Hungary*
[3]*Department of Biotechnology, University of Szeged, Közép fasor 52., 6726 Szeged, Hungary*
[4]*Center for Geomicrobiology, Aarhus University, Ny Munkegade 116., 8000 Aarhus, Denmark*

Photobiological H_2 produced by the green alga *Chlamydomonas reinhardtii* is a potential CO_2-neutral energy source. The [Fe-Fe]-type hydrogenases of green algae, located at the acceptor side of photosystem I, are the most active molecular catalysts known for H_2 production. In nature, they serve as a safety valve upon the induction of photosynthesis following hypoxia and once the Calvin-Benson cycle is activated, the O_2 evolved by photosystem II inhibits the hydrogenases. To date, the most efficient method to sustain H_2 production in *Chlamydomonas* is based on nutrient deprivation, which results in the degradation of photosynthetic complexes and reduced O_2 evolution enabling hydrogenase expression. However, hydrogenase expression can be more rapidly induced by anaerobiosis in the dark. We show that by preventing the activation of the Calvin-Benson cycle via substrate limitation (by omitting CO_2 or acetate), the photosynthetic electron transport chain remains reduced in the light, resulting in low O_2 evolution and sustained H_2 production. Our method was further improved by the application of an iron-salt based O_2 absorber, which attenuated the inactivation of hydrogenases. As a result, we obtained yields that are about three times as high as observed for the standard sulphur deprivation procedure in acetate-containing medium and the cultures remained photosynthetically active even after several days of H_2 production. Thus, by eliminating the requirements for nutrient deprivation and acetate and preserving photosynthetic activity, we have simplified and significantly decreased the costs of algal H_2 production, enabling its industrial application in the more near future.

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NE7

BINDING OF MANGANESE TO CHLOROPLAST GLUTAMINE SYNTHETASE AND ITS EFFECT ON ENZYME ACTIVITY IN WHEAT

***Edit NÉMETH* (nemeth.edit@agrar.mta.hu)[1] – Lilla BÓNUS (bonuslilla8@gmail.com)[2] – Attila PÉCSVÁRADI (pecsvaradi@bio.u-szeged.hu)[2]**

[1]Department of Plant Physiology, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik str. 2., 2462 Martonvásár, Hungary
[2]Department of Plant Biology, University of Szeged, Közép fasor 52., 6723 Szeged, Hungary

Manganese toxicity is common in acidic soil. As the Mn^{2+} form increases in the soil solution the manganese stress reduces plant growth and development, and negatively affects yield production. Glutamine synthetase (GS, EC 6.3.1.2) is a key enzyme of nitrogen metabolism in every organism studied so far and plays a main role in wheat plants during grain filling. There are well known prokaryotic glutamine synthetase structures and several eukaryotic models were also born. These studies showed, that GS is a multi-subunit enzyme, which has two divalent cation binding sites at each active site. The two binding sites differ in their metallic ion affinity. In the case of prokaryotes manganese is required for the activation of the enzyme, and the binding of manganese alters the response of the enzyme to various feedback inhibitors. Our knowledge about eukaryotic GS is not as extensive. The binding of metallic ions and their effect on the enzyme differs among eukaryotic organisms, but the presence of Mg^{2+} is essential for enzyme activity.

In C3 photosynthetic plants GS is the most important enzyme in nitrogen assimilation, since GS is the first and only one in the concatenation of the enzymes of the nitrogen assimilatory pathway, which binds an inorganic nitrogen form to an organic compound. In wheat leaves there is a cytosol (GS1) and a chloroplast (GS2) localized isoform. The GS1:GS2 ratio is highly affected under stress conditions, which causes the early senescence of wheat plants and has an effect on grain filling process.

GS shows negative allosteric cooperativity to its substrate, glutamate, which is consumed in a concentration dependent way. We studied the effect of manganese *in vitro*, the binding of Mn^{2+} and its effect on enzyme activity, and allostery. We suggested that the Mn^{2+} displace the Mg^{2+} ions from the binding sites which are crucial for the catalytic activity. We separated the GS2 protein from Mn^{2+} and Mg^{2+} treated samples and measured the ion content by ICP-MS. The results show that the Mn^{2+} treatment caused the loss of Mg^{2+} content. Besides the enzyme activity was inhibited with the increasing amount of Mn^{2+} present in the system, but did not disturbed the concentration dependent glutamate consumption.

We suggest that Mn^{2+} binds to half of the available Mg^{2+} binding sites, and inhibits the activity of GS2 in wheat plants. The affinity of Mn^{2+} to the GS protein possibly plays a role during manganese toxicity.

NE8

THE ROLES OF A BRASSICACEAE-SPECIFIC miRNA IN HEAT STRESS RESPONSE

Henrik Mihály SZAKER* (szaker.henrik.mihaly@abc.naik.hu) – *Tibor CSORBA (csorba.tibor@abc.naik.hu)

Plant Biotechnology Section, Agricultural Biotechnology Institute, NAIK; Szent-Györgyi Albert str. 4., 2100 Gödöllő, Hungary

Considering the rise of global mean temperature, the exploration of plant heat stress response is increasingly important especially for agricultural reasons. Plants, as sessile organisms, are exposed to repeated diurnal temperature variations. By employing an experimental setup that mimics natural diurnal temperature changes, we searched for transcriptional alterations of long non-protein coding RNAs in *Arabidopsis thaliana* Col-0 seedlings, that may implicate relevancy during heat stress adaptation.

We have found the Brassicaceae-specific *miR824* precursor to strongly accumulate in response to heat. Previously it was shown that miR824 has roles in stomata complexity regulation and the timing of flowering transition as it post-transcriptionally inhibits its unique target AGAMOUS-like 16 (AGL16), a MADS-box containing transcription factor.

We have shown that both the *miR824* precursor and the mature microRNA accumulate in response to heat treatment. Using transgenic reporter lines, we have proved that miR824 accumulation is due to transcriptional activation. We have found one heat shock element (HSE) and two HSE-like sequences in the promoter of *miR824*. (HSEs are specific cis-elements directing heat shock factor (HSFs) binding. HSF protein family members are central transcription factors of the heat shock response.) Using *miR824-promoter::GUS* reporter lines, we have shown that HSE motifs within the miR824 gene promoter are *bona fide* functional *cis* elements and that members of the HSFA1 group and the HSFA2 are essential *trans* factors for the transcriptional activation of *miR824*. Furthermore, activation of *miR824* requires direct binding of HSFA1a. Beside these, we have found a drought responsive *cis* element (DRE), and although drought stress alone does not induce *miR824* expression, the combined stress (heat and drought) synergistically elevates miR824 transcription. Parallel to miR824 activation, a simultaneous decrease of AGL16 mRNA was observed in treated plants. By employing miR824 mutant and mimicry lines we have proved that miR824 has a role primarily in sustaining the low level of the AGL16 mRNA levels following heat stress.

Brassica napus (rapeseed) is an agronomically important crop plant, a close relative of Arabidopsis. To investigate whether miR824 ortholog in rapeseed has a similar regulation, we tested miR824 induction and AGL16 downregulation in reponse to acclimation: we have found elevated amounts of miR824 and decreased levels of AGL16 mRNA level in treated samples.

Our results suggest a conserved role for miR824/AGL16 module in heat acclimation in Brassicaceae.

NE9

DIFFERENCES IN THE MODE OF ACTION OF VARIOUS POLYAMINE PRE-TREATMENTS DURING CADMIUM STRESS IN WHEAT

***Judit TAJTI* (tajti.judit@agrar.mta.hu) – Tibor JANDA (janda.tibor@agrar.mta.hu) – Imre MAJLÁTH (majlath.imre@agrar.mta.hu) – Gabriella SZALAI (szalai.gabriella@agrar.mta.hu) – Magda PÁL (pal.magda@agrar.mta.hu)**

Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, POB 192462 Martonvásár, Hungary.

Polyamine are low-molecular-weight, organic cations which are found in all living organisms, and have role in plant stress responses and signalling. However, the individual polyamines have different roles and effects. Not surprisingly, their mode of action may also vary depending on the type of treatment. Different changes were induced by 0.5 mM putrescine or spermidine pre-treatments, when applied as seed soaking or added hydroponically, during 50 µM cadmium stress in young wheat plants. Putrescine pre-treatment provided protection both as seed soaking and applied hydroponically, while spermidine was only beneficial in the case of seed soaking, and enhanced the Cd-induced oxidative stress when it was added hydroponically. The polyamine pool is dynamic, changing over time, and polyamines also undergo rapid interconvertion in the polyamine cycle. According to these, the exogenous polyamines absorbed can be rapidly converted into each other already in the roots, or translocated into the shoot and then further metabolised. Thus, the differences observed between the polyamine pre-treatments under these conditions were related to the polyamine metabolism. The excess of polyamines induced several different mechanisms to control endogenous polyamine levels, especially putrescine. The accumulation of endogenous putrescine beyond a certain amount may be in relation with the negative effect of hydroponic spermidine pre-treatment during Cd stress. The polyamine metabolism is also related to the synthesis of plant hormones, such as salicylic acid. Salicylic acid is involved in general stress responses, in a complex relationship with other plant hormones, leading to the regulation of gene expression. The highest salicylic acid accumulation was found when hydroponic spermidine pre-treatment was followed by cadmium stress. In addition, the gene expression level of salicylic acid-induced protein kinase in the roots, which is a member of the mitogen-activated protein kinase family, also showed similar pattern to the salicylic acid content in the roots of wheat plants. In conclusion, in certain cases polyamines provide direct protection against heavy metals, but it should also be taken into consideration that polyamines are involved in a complex signalling system, and the fine balance in the polyamine pool may serve as a shuttle between the beneficial and deleterious effects of polyamines.

Poszter előadások összefoglalói

ÁPE1

THE EFFECTS OF VARIOUS SOLUBLE NON-DIGESTIBLE CARBOHYDRATES ON GUT HEALTH IN BROILER CHICKENS

***Andor MOLNÁR* (andor.molnar@georgikon.hu)[1] – Fanni DUBLECZ (dubleczfanni@gmail.com)[1,2] – László PÁL (pal-l@georgikon.hu)[1] – László WÁGNER (wagner@georgikon.hu)[1] – Claudia HESS (claudia.hess@vetmeduni.ac.at)[3] – Michael HESS (michael.hess@vetmeduni.ac.at)[3] – Ferenc HUSVÉTH (husvéth@georgikon.hu)[1] – Károly DUBLECZ (dublecz@georgikon.hu)[1]**

[1]Department of Animal Science, Georgikon Faculty, University of Pannonia, Deák Ferenc street 16., Keszthely, Hungary
[2]UBM Feed Kft., Kisvasút street 1., Pilisvörösvár, Hungary
[3]Clinic for Poultry and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Veterinaerplatz 1., Vienna, Austria

Nutritional strategies to promote gut health and safe broiler meat production have come into prominence due to the emerging challenges related to the ban of antibiotic growth promoters. The effects of diets, rich in various soluble non-digestible carbohydrates (sNDCs) were evaluated on different intestinal characteristics (histological, physico-chemical and microbiological) of chickens and compared with a maize based diet as a control.

A total of 160 Ross 308 male chickens were kept in deep litter pens (n=40) and fed their appropriate diets from day 1 to day 35 of life. Four isocaloric and isonitrogenous diets, differing in their sNDC content, were composed as follows: control (containing maize as the only cereal), maize-wheat based (M+W), and maize based supplemented with either 20 g kg^{-1} inulin (M+I) or 30 g kg^{-1} lactose (M+L). Samples were taken for investigating gut histology measures (morphology, goblet cell and intraepithelial lymphocyte numbers), ileal viscosity, cecal short-chain fatty acid concentration, pH, coliform and *Lactobacillus* counts, respectively.

All of the diets tested decreased ileal crypt depth, muscle layer thickness and increased cecal coliform counts relative to the control group. Villus-crypt ratio increased only in the M+L group. Ileal digesta of chickens fed the M+W diet had the highest ileal viscosity and the highest cecal butyrate, valerate and total short-chain fatty acid concentrations while the lowest pH was observed in cecal contents of chickens fed the M+I diet. The diet had no effect on ileal or cecal goblet cell and intraepithelial lymphocyte numbers. *Lactobacillus* counts in the cecal content remained unchanged.

According to the present study various sNDC sources may have beneficial gut health effect. However, some of the intestinal variables are dependent on the type of sNDCs.

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ÁPE2

IN VITRO PORCINE EMBRYOS SEX DETERMINATON BY SINGLE-CELL PCR

***Tímea PINTÉR* (timea.pinter90@gmail.com)[1] – Andrea MAGYAR (magyar.andrea@athk.naik.hu)[1,2,3] – Renáta FÁBIÁN (fabian.renata.@abc.naik.hu)[1,2] – Szilárd BODÓ (bodo.szilard@abc.naik.hu)[1,2,3]**

[1]*Szent István University; Páter Károly str. 11., 2100 Gödöllő, Hungary*
[2]*NAIK-MBK; Szent-Györgyi Albert str. 4., 2100 Gödöllő, Hungary*
[3]*NAIK-ÁTHK; Gesztenyés str. 1., 2053 Herceghalom, Hungary*

In order to improve productivity and competitiveness, in addition to traditional breeding procedures, biotechnological processes have been included. Preimplantation genetic diagnosis (PGD) is an useful technique for genetic profiling of the embryo. *In vitro* embryo production and embryo genotype identification can be used to obtain the production of a specific sex-proportionate breeding stock. However, during the process a limited amount of DNA (7-8 pg) is available for testing, so sensitive diagnostic procedure is required.

Our goal was develop to set single-cell PCR, which could be used to determine the sexes of *in vitro* embryos with high precision.

In the first step, we isolated DNA from known sex porcine tissues, (ovaries, uterus, epididymis).

We used a sex specific primers during the duplex PCR. The chromosome X was detected by pig mitochondrial 12S rRNA sequence (SUS12S), while the Y-chromosome was detected by the boar-specific repeating sequence (SUSYa / SUSYb). Thereafter was used gradient PCR to determine the optimum annealing temperature of the designed primers, then the reaction was adjusted on female and male dilution lines (15, 10, 5, 1 and 0.5 μg μl^{-1}). Then we collected oocytes from slaughterhouse ovaries to adjust the a cell-specific DNA contentrequired for the embryo sex determination.

Finally, the oocytes were matured for two days (solutions NCSU37-IVM 1, NCSU37-IVM2) then in vitro fertilized with frozen/thawed epididymis spermatozoa and the zygotes were cultured sequentially to 8 cells (solutions NCSU37-Glu-d2, NCSU37-PyrLac-d3).

Following removal of embryo zona pellucida, blastomeres were placed in individually numbered PCR tubes containing 2 μl distilled water and were tested for precision of the single-cell PCR. The PCR products were separated on a 2% agarose gel. In the gel could be detected a 235 bp long DNA fragment in female, while were 235 bp and 75 bp long fragments for males. Blastomers from one embryo received the same sex.

We developed successfully a PCR method, which could be used to determine the sex of pig embryos at a single-cell level. This method could be useful for determine the sex of biopsied embryos.

ÁPE3

SLOW COOLING AND VITRIFICATION OF EUROPIAN *CATFISH (SILURUS GLANIS)* TESTICULAR TISSUE

***Dóra SZERÉNYI* (dori.szerenyi94@gmail.com) – Jelena LUJIĆ (lujicjelena@gmail.com) – Ákos HORVÁTH (Horvath.Akos@mkk.szie.hu) – Zoran MARINOVIĆ (zor.marinovic@gmail.com)**

Szent István University; Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Páter Károly str. 1., 2100 Gödöllő, Hungary

Numerous fish species are about to extinct. Consequently, the importance of gene preservation is increasing year by year. Although sperm cryopreservation methods are well improved in case of fish species, even so number of studies conducted over the past three decades, cryopreservation of fish oocytes and embryos is not manageable with slow freezing, nor with vitrification. The cryopreservation of gonadal stem cells can be a substitute solution. In this study we established a metodology to preserve Europian catfish *(Silurus glanis)* spermatogonia with the comparison of different cryopreservation and vitrification methods for the first time.

For comparison of different slow cooling methods 18 test groups were formed. The following variables had been applied: **3 cryoprotectants (CPs):** methanol (MeOH), dimethyl sulfoxide (Me_2SO), ethylene glycol (EG); **3 concentrations of individual CPs:** 1M, 2M, 3M. Cryopreservation of the samples was carried out in 2ml cryotubes in CoolCell boxes with cooling rate of 1 ºC/min down to -80 ºC. After reaching -80 ºC, samples were transfered to liquid nitrogen. For thawing cryotubes were placed into 24 ºC water bath. For counting membrane-intact cells, trypane-blue staining was used.

For comparison of different vitrification methods 12 groups were formed. The following variables had been applied: **Equilibration media (E):** 15 min incubation time, **(E1)** 1,5M MeOH + 2,5M Me_2SO; **(E2)** 1,5M ethylene glycol + 1.5M Me_2SO; **Vitrification media (V):** Incubation times were 1 min, 1,5 min, 2 min (different test groups). **(V1)** 1,5M MeOH + 5,5M Me_2SO; **(V2)** 3M propylene glycol + 3M Me_2SO. Vitrification was performed by needle immersed method. For thawing each sample was warmed separately by plunging the needle into three warming solutions (**W1:** L15 + 10% FBS + 0,3 M trehalose; **W2:** L15 + 10% FBS + 0,1 M trehalose; **W3:** L15 + 10% FBS) at 23 ºC. Samples were serially transferred into W1 for 1 min, then into W2 for 3 min, and finally into W3 for 5 min.

According to our results, slow cooling was more effective for cryopreservation of catfish testicular tissue compared to vitrification. Following slow cooling, the highest number of viable cells (above 65%) was observed in the test groups 3 M Me_2SO and 3M ethylene glycol. This indicates that 3M is an optional cryoprotectant concentration in case of methanol and dimethyl sulfoxide for slow-freezing of catfish testicular tissue. Contrary, average viability of vitrified-thawed cells was below 20%.

Based on these results we can conclude that slow-rate freezing is more feasible for cryopreservation of catfish testicular tissue than vitrification, and the most effective protocol is as follows: Yoshizaki extender with 3M Me_2SO cryopreserved in CoolCell box.

BPE1

NOVEL COMPUTATIONAL METHODS TO EXPLORE THE INTERACTION NETWORK OF LINEAR MOTIF BINDING PROTEINS

Gábor ERDŐS (gerdos@caesar.elte.hu) – Tamás SZANISZLÓ (szanya82@gmail.com) – Mátyás PAJKOS (matyolade@gmail.com) – Borbála HAJDU-SOLTÉSZ (soltesz.borbala@gmail.com) – Zsuzsanna DOSZTÁNYI (dosztanyi@caesar.elte.hu)

ELTE Eötvös Loránd University; Department of Biochemistry, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary

A large number of protein-protein interactions are formed between a globular domain in one protein binding to a short protein segment in another protein. The short segment involves a stretch of approximately 3–10 residues that often shows a particular sequence pattern which captures the key residues that are specifically recognized by the binding partner. Such short linear motifs (SLiMs) are involved in a wide range of biological functions and can regulate the formation of transient protein complexes, orchestrate subcellular localization, modulate post-translational modification state, and determine the fate of proteins. SLiMs generally reside within intrinsically disordered regions (IDRs) and the interface created upon their binding is much smaller compared to the interface between globular proteins. Due to these specific properties the identification of SLiMs is challenging, both experimentally and computationally.

In this work we present several novel approaches that can be used to identify additional interaction partners for a given linear motif binding protein domain. These include an efficient procedure to collect known binding partners from multiple protein-protein interaction databases; a bioinformatic pipeline that combines various sequence features to enable the optimal filtering of the most likely binding peptides; a structure based method to identify novel binding motifs by estimating their binding affinities; and a new sequence alignment approach specifically developed to recognizes the island-like conservation of linear motifs.

The novel computational approaches are applied to expand the interaction network of dynein light chains, in particular, the LC8 protein. LC8 is a highly conserved eukariotyc hub protein. Although it was originally suggested to function as a cargo adaptor for the dynein motor complex, the more than 50 known interaction partners indicate a more general role independent of dynein. LC8 is now believed to function as a dimerisation or oligomerization engine for various proteins, and can have many yet undiscovered partners. Using the novel methods we identified new motif classes, new binding peptides within known partners and completely new binding partner proteins for LC8. Selected binding motifs were experimentally verified using surface plasmon resonance and fluorescence polarization techniques.

These studies help not only to further explore the interaction network of LC8 and gain better understanding of the functional and evolutionary properties of these interactions, but also pave the way to explore additional motif mediated interaction networks.

BPE2

PHYLOGENETIC ANALYSIS OF GRAPE TYPES USING WHOLE GENOME SEQUENCES

Schiller MÁTYÁS (matyasschiller@gmail.com) – Marincs FERENC (marincs.ferenc@abc.naik.hu) – Barta ENDRE (barta.endre@unideb.hu)

Mezőgazdasági Genetika és Bioinformatikai csoport, NAIK-MBK, Szent-Györgyi Albert u. 4. 2100 Gödöllő, Hungary

12 grape genomes have been sequenced in the NAIK MBK. The raw sequence data has been processed. We have determined and annotated the variations for each genome. Each genome yielded approximately 5 million single nucleotide polymorphisms (SNP) and 300–400 thousand short variations (INDEL) compared to the reference genome. This is a huge number considering that the propagation of the grape is done vegetatively.

Our next goal was to establish the relations between the genomes of the various grape types. Traditionally one would choose one or a select few genes which would undergo phylogenetic analysis after multiple fitting. We had the 12 whole genomes at our disposal and we supplemented our data from the NCBI SRA database. The phylogenetic analysis will be completed using all the valid SNP variation available. With all the metadata available to use we had 34+12 whole genome sequences to analyze. The previously used bioinformatic pipeline was heavily modified to fit for our purposes. This allowed us the automated download of the required raw sequences from the NCBI SRA database. The '.sra' files were converted to '.fastq', so all the files were the same format. From this point on, the downloaded and the previously owned data was treated in the same matter. The reads were compared to the newest (Vitis_vinifera.IGGP_12x.) grape reference genome. The variant calling took place using the 'samtools/mpileup' pipeline. The 'annovar' program was used to annotate the SNPs and INDELs and the 'SNPhylo' for the phylogenetic analysis. Experience has shown that even though the pipeline ran automatically, the differences between the sequences made it necessary to fine tune the process by hand. Four of the SRA genomes were discarded because of their quality. Finally 42 (12 own and 30 SRA) genomes made up the phylogenetic tree. The results show that the Israeli and Italian types make a different branch just as the Hungarian Hárslevelű, Ezerjó, Furmint and Portugieser samples.

In summary a new and functional pipeline has been developed. It can be used to fit the sequences to the genome, to find the variants and the annotate them. The pipeline is capable of comparing future sequences to the ones that are already processed, integrating them to the phylogenetic tree. The 'SNPhylo' program has been deemed useful when using 40 genomes for a phylogenetic tree. Our process was found to be successful and ready to adapt to the analysis of other species.

GPE1

SELECTIVE IMMOBILIZATION OF ACID PHOSPHATASES AND THEIR APPLICATION IN TRANSPHOSPHORILATION REACTIONS

***Flóra NAGY* (nagy.flora@mail.bme.hu)[1], Gábor TASNÁDI (gabor.tasnadi@uni-graz.at)[2,3], Diána BALOGH (dweiser@mail.bme.hu)[1,4], Kurt FABER (kurt.faber@uni-graz.at) [3], László POPPE (poppe@mail.bme.hu)[1,4]**

[1]*Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rkp. 3, 1111 Budapest Hungary*
[2]*Austrian Centre of Industrial Biotechnology, c/o*
[3]*Department of Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz Austria*
[4]*SynBiocat Ltd., Lövőház u 19/1, 1024 Budapest, Hungary*

An easy to use method combining the selectivity of metal chelate affinity chromatography with strong covalent linking was developed for immobilization of non-specific acid phosphatases bearing a His-tag from crude cell lysate. Silica nanoparticles were grafted with aminopropyl functions which were partially transformed further with EDTA dianhydride to chelators. The heterofunctionalized nanoparticles charged with Ni^{2+} as the most appropriate metal ion were applied as support. First, the His-tagged phosphatases were selectively bound to the metal-chelate functions of the support. Then, the enzyme-charged silica nanoparticles were further stabilized by forming a covalent linkage between nucleophilic moieties at the enzyme surface and free amino groups of the support using neopentylglycol diglycidylether as the most effective bifunctional linking agent. As model enzymes, nonspecific acid phosphatases PhoN-Sf from *Shigella flexneri* and PhoN-Se from *Salmonella typhimurium* LT2 were employed bearing histidine affinity tag. The phosphatase biocatalysts obtained by this method exhibited better phosphate transfer activity with a range of alcohols and PP_i as phosphate donor in aqueous medium applying batch and continuous-flow modes than the ones immobilized on conventional supports.

[1]Tasnádi *et al.* 2016, *Eur. J. Org. Chem*. 45-50.

GPE2

NEXT GENERATION SEQUENCING OF THE B CELL REPERTOIRE

***Bence SZIKORA* (szykora17@gmail.com)[1] – Anita MARX (marxanita94@gmail.com)[1] – Orsolya PIPEK (porsika@gmail.com)[2] – István CSABAI (csabai@complex.elte.hu)[2] – Imre KACSKOVICS (imre.kacskovics@ttk.elte.hu)[1]**

[1]*Department of Immunology, ELTE Eötvös Loránd University, Pázmány Péter stny 1/C., 1117 Budapest, Hungary*
[2]*Department of Physics of Complex Systems, ELTE Eötvös Loránd University, Pázmány Péter stny 1/A., 1117 Budapest, Hungary*

The B cells and the antibodies they produce are the most important and key elements of the humoral immune response. At the end of the B cell development and the selection events, every naïve B cell will produce a unique immunoglobulin, which are the basic elements of the immune repertoire. The diversity of these immunoglobulins (Ig) comes mainly from the various gene segments (V(D)J) and randomly built-in nucleotides. The healthy repertoire's composition consists many different Ig sequences with a relatively even distribution. But this composition can change because of infections or other disorders such as autoimmunity. These can be discovered and followed by analysing the Ig repertoire with the help of next-generation sequencing (NGS) and bioinformatical evaluation techniques, as NGS produces millions of reads in parallel at the same time, covering the Ig repertoire in a reasonable quality and relatively short time.

The rheumatoid arthritis (RA) is a common autoimmune disease, manifesting in synovial inflammation. Before the onset of RA, typical disease markers can be detected from blood: autoantibodies of given specificity, typically anti-citrullinated protein antibodies (ACPA) and/or IgM rheumatoid factors (IgM-RF), but these markers are not entirely reliable.

Our aim is to develop a highly sensitive and accurate diagnostic marker in connection with RA. We try to initialize a system which can reliably detect highly expanded clones from blood of RA patients to predict disease progression and follow treatment's efficiency as well. In addition, we also would like to use this repertoire analysis in other diseases, such as myeloma multiplex and other B cell malignancies, to monitor the disease progressions, and minimal residual disease (MRD).

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MPE1

STEVIA PLANT PROCESSING AND ENZYMATIC BIOCONVERSION OF STEVIOL GLYCOSIDES

Réka CZINKÓCZKY* (czreka1@gmail.com) – *Áron NÉMETH (naron@f-labor.mkt.bme.hu)

Department of Applied Biotechnology and Food Science, B*udapest University of Technology and Economics, Szent Gellért tér 4., 1111 Budapest, Hungary*

Stevia (*Stevia rebaudiana* Bertoni) is a perennial shrub, stem from South America mostly Paraguay and Brazil. This herb has been used to sweeten tea of the Guarani Indians in South America. These plants sweeteners were discovered in 1931. Stevia plant and its extracts have been used for a long time in China, Japan, Asia, South America and different countries in Europe. Stevia leaves and highly refined extracts are used for low-calorie sweetener in Korea, Japan and Brazil. Stevia extracts have been approved by the US FDA as GRAS food additives (i.e. generally recognized as safe).
Stevia produces steviol glycosides which are 200–300 times sweeter, than sucrose solution. The main component in the leaves is stevioside, but it has a bitter aftertaste[1]. Stevioside bioconversion to rebaudioside A (reb A) is required to reduce this unpleasant quality of taste. Reb A has a sweet aftertaste, and also exists in the plant leaves, but in lower amount than stevioside. UDP-glucosyltransferase UGT76G1 enzyme does the catalysis in the stevioside to rebA conversion in the plant. Researchers isolated the mRNA sequence of the enzyme, and cloned into *E. coli*[2] or *S. cerevisiae*[3] to produce recombinant enzyme. Researchers used other non-plant originated enzymes for this bioconversion as well, like β-1,3-glucanase[4].
The purpose of our previous studies was the extraction of steviol glycosides from the plant at different circumstances. We optimized hot water extraction with a central composite design (under publication). Then we also examined stevia extract decolorization to eliminate the brown colour from the crude product. Our aim was to perform the enzymatic bioconversion and gain high reb A : stevioside ratio after the reaction with different enzymes. Another target was the decolorization of the stevia extract with different methods (precipitation, enzymatic degradation, filtrations etc).

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MPE2

EFFECT OF SALT TREATMENT ON BIOTECHNOLOGICALLY IMPORTANT MICROALGAL STRAINS

Sai Divya KANNA (divya.kanna@brc.mta.hu)[1] – Izabella LEITNER (leitner.izabella@brc.mta.hu)[1] – Tímea Ottilia KOBORI (Ottilia.Kobori@bayzoltan.hu)[2] – Ágnes DERGEZ (agnes.dergez@bayzoltan.hu)[2] – Ottó ZSÍROS (zsiros.otto@brc.mta.hu) [1] – Renáta ÜNNEP (runnep@gmail.com)[3] – László SZILÁK (laszlo.szilak@gmail.com)[4] – Győző GARAB (garab.gyozo@brc.mta.hu)[1] – Bettina UGHY (ughy.bettina@brc.mta.hu)[1]

[1]Institute of Plantbiology, Biological Research Centre of HAS, Temesvari krt 62, 6726 Szeged, Hungary
[2]Division for Biotechnology, Bay Zoltán Nonprofit Ltd., Derkovits fasor 2, 6726 Szeged, Hungary
[3]Wigner Research Centre for Physics of HAS, Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary
[4]Department of Biology, Savaria University Centre, ELTE Eötvös Loránd University, Karolyi G. ter 4, 9700 Szombathely, Hungary

Microalgae, i.e. unicellular algae and cyanobacteria, are good model organisms to study photosynthetic processes and they have eminent biotechnological importance. Growth and productivity of photosynthetic organisms are significantly affected by stress factors. Photosynthesis is very sensitive to changes in the environmental conditions because it balances the absorbed light energy with the energy consumed by metabolic processes of the organisms. In general, photosynthetic functions are controlled by multilevel regulatory mechanisms and depend largely on the molecular composition and (macro-) organization of the thylakoid membranes, which contain virtually all protein complexes that are involved in the light reactions of photosynthesis. The stress signal is first perceived at the membrane level. Adjustment of the characteristics of membrane structure enables the membrane components to maintain and perform their physiological functions upon changes in the environmental conditions. The understanding of adaptation to higher salt concentration of biotechnologically important microalgal strains is highly important since every continent is affected by salinized soil and water, and because of the possible use water sources with high salt concentrations. We have investigated the adaptation of different strains to salt treatments using CD (circular dichroism) spectroscopy and SANS (small-angle neutron scattering) measurements, to provide information about the organization of pigment-protein complexes and to characterize the changes in the periodic organization of the thylakoid membrane system. These measurements revealed different susceptibilities of the (macro-) organization of thylakoid membranes in different microalgae to salt stress. The applied biophysical techniques can thus be used to monitor the adaptation capability of various strains and contribute to the better understanding of the underlying physical mechanisms.

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MPE3

MONITORING THE COMBINED EFFECT OF BIOCHAR AND PLANT GROWTH PROMOTING RHIZOBACTERIA SOIL AMENDMENTS ON ACIDIC SANDY SOIL

<u>András KARI</u> (andraskari@yahoo.com)[1] –
Zsuzsanna NAGYMÁTÉ (zsuzsanna.nagymate@gmail.com)[1] –
Csaba ROMSICS (rcsaba@elte.hu)[2] – József KUTASI (biokutasi@gmail.com)[2] –
Éva KÁRPÁTI (karpatieva1@gmail.com)[3] – Nikolett UZINGER (uzinger@rissac.hu)[4] – Károly MÁRIALIGETI (mariak@caesar.hu)[1]

[1]Department of Microbiology, ELTE Eötvös Loránd University, Pázmány P. sétány 1/C, 1117 Budapest, Hungary
[2]BioFil Ltd., Váci út 87. 1139 Budapest, Hungary
[3]Saniplant Ltd., Raktár str. 19, 1035 Budapest, Hungary
[4]Institute for Soil Sciences and Agricultural Chemistry CAR HAS, Herman Ottó út 15., 1022, Budapest, Hungary

Biochar as being distinguished from charcoal by its use as a soil amendment, is produced by the thermal "degradation" of organic materials in the absence of oxygen (pyrolisis). It has shown a great role to mitigate climate change by carbon sequestration and reducing non-CO_2 greenhouse gases emissions. As a soil amendment biochar has the potential of improving crop productivity with increased soil nutrient retention and structure as well as moderate soil acidity. PGPR's have the evidenced potential to enhance plant productivity and yields by production of phytohormones, favouring nutrient uptake, induction of systematic resistance (ISR) of host plants and decreasing inhibitory effects of pathogens. The synergistic use of biochar and PGPR microorganisms offers a long term method for soil quality improvement and yield reliability.

Five different PGPR bacterium strains were applied alongside biochar produced from grain husk and paper fibre sludge, as an amendment of acidic sandy soil. The aim of the study was to detect the effect of biochar on soil biota and inoculated bacterium strains. To evaluate the bacterial community structure, the genotypic terminal restriction fragment length polymorphism (T-RFLP) with clone libraries and the phenotypic phospholipid fatty acid analysis (PLFAs) fingerprint methods were applied. Based on the applied strains unique terminal restriction fragments (T-RFs) the detection and monitoring of the strains relative abundances were determined with T-RFLP method. Two different restriction endonucleases had been *in silico* selected for specific detection of the inoculated stains.

The treatment of biochar and PGPR inoculant combination affected positively the relative abundance of the two *Azospirillum brasilense* strains, and *Arthrobacter crystallopoietes*, while only the PGPR inoculation form affected positively the relative abundance of *Bacillus aryabhattai* strains. Treatments containing high doses of biochar (0.5; 1 ww^{-1} %) showed a great influence, by T-RFLP analysis, on the autochthonous microbial community structure by increased abundance of *Alphaproteabacteria* and decreased abundance of *Acidobacteria* class compared to the untreated fields. PLFA analysis has not shown correlation between the treatments and microbial community dynamics.

MPE4

DEVELOPMENT OF LACTIC ACID FERMENTED, FUNCTIONAL, PROBIOTIC SOUR CHERRY JUICE

***Judit PERJÉSSY* (perjessy.judit@eki.naik.hu) – Ferenc HEGYI (hegyi.ferenc@eki.naik.hu) – Magdolna NAGY-GASZTONYI (gasztonyi.magdolna@eki.naik.hu) – Zsolt ZALÁN (zalan.zsolt@eki.naik.hu)**

National Agricultural Research and Innovation Centre, Food Science Research Institute; Herman Ottó út 15, 1022 Budapest, Hungary

Introduction: Nowadays, demand for products which beyond the overall nutritional value, have a feature that protects the consumers health, have increased. Fruits could be ideal substrates for a fermented product, as they have an important role in human nutrition and contain many beneficial ingredients (minerals, vitamins, dietary fibers). Fruit juice has been suggested as a novel and appropriate medium for fortification with probiotic cultures, since they are rich in nutrients, high amounts of sugars which could enhance the survival of the incorporated probiotic bacteria and often have oxygen scavenging agents, which can protect probiotic bacteria that can be sensitive to oxygen. However the fruit juice is less suitable for the probiotic viability than the dairy products, because it is considered as being too acidic to enable good stability of probiotics during storage. Nevertheless several studies have proved that fruit juices can become suitable carrier or medium for probiotic organisms. Therefore the aim of our study was to investigate the possibility of the probiotication of sour cherry juice by fermentation with probiotic starter culture.
Materials and methods: For the fermentation 9 - among them 6 probiotic - *Lactobacillus* strains were used and two types of sour cherry as raw material. The Újfehértói fürtös and Petri species were provided by NARIC - Fruitculture Research Institute. During the research, the properties of the strain - such as reproduction and metabolism (organic acid production) - and its effect on the raw material (carbohydrate and titratable acid content) were studied by microbiological and analytical methods, and the lactic acid fermented sour cherry juice's polyphenol and antioxidant capacity were also measured.
Results: From preliminary experiments it was observed that sour cherry juice in its natural form does not provide an adequate environment for the growth of *Lactobacillus*. Although the original pH adjustment to a neutral value resulted in a one-tenth increase in order of magnitude (10^8 CFU ml^{-1}), to reach the recommended probiotic cell count we investigated addition of nutrients - such as protein, carbohydrate. The effect of dilution of sour cherry juice was also investigated. Based on the experiments carried out, the added yeast extract as well as the pH adjusting and dilution resulted in the highest (10^9 CFU ml^{-1}) cell number and the pH decreasing to the optimal value after 24 hours. Therefore, central composite design (CCD) was used to determine the optimal values for these parameters. Despite the fact that all investigated strains reached the desired 10^9 CFU ml^{-1} cell density, a significant difference was observed between the numbers of live cells of some *Lactobacillus* strains. Furthermore, our results show that the type of sour cherry also affects the fermentation, so it is important to select the starter culture for the raw material.

MPE5

STRESS SENSITIVITY OF AN *ATFA* DELETION MUTANT OF *FUSARIUM VERTICILLIOIDES*

***Zsuzsa SZABÓ* (szabo.zsuzsa@science.unideb.hu) – *Éva LEITER* (leiter.eva@science.unideb.hu) – *István PÓCSI* (pocsi.istvan@science.unideb.hu)**

Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Egyetem square 1., 4032 Debrecen, Hungary

Adaptation to changes in intra- or extracellular conditions is a universal requirement to all organisms. The cell homeostasis is achieved through a highly coordinated mechanism of transcription regulation involving transcription factors. Dimeric basic leucine zipper (bZIP) proteins are transcriptional enhancer factors occuring in all eukaryotes. These transcriptional factors play a critical role in ontogenesis. In S*accharomyces cerevisiae* a distinct subset of proteins within the bZIP family, the yeast activator protein (Yap) transcriptional factors, is well characterized. The Yap family comprises eight members, all containing a conserved sequence in their basic region similar to the conventional Gcn4 factor. The Gcn4 factor plays a critical role in the activation of the General Amino Acid Control and the regulation of genes involved in both purine and amino acid synthesis. Despite their similarity, the DNA-binding properties of Yap differs from the Gcn4 factor. The DNA binding site of the Yap family was characterized as TTAC/GTAA. Yap proteins often mediate stress responses and are often associated with resistance to reactive oxygen species (ROS), as well as tolerance to osmotic and heavy metal stress. Yap1, the first member of the family plays a critical role in response to oxidative stress; it regulates several genes involved in the detoxification of ROS. Yap1 orthologues have been characterized in a number of fungi, including *Aspergillus nidulans*, *Fusarium oxysporum* and *Fusarium graminearum*. In *A. nidulans*, deletion of the *atfA* gene caused increased sensitivity to menadione sodium bisulfite, H_2O_2, *t*-butyl hydroperoxide and diamide but not to NaCl. The *ΔFgap1* deletion mutant of *F. graminearum* was sensitive to $CdCl_2$, sorbitol, NaCl and H_2O_2 compared to the wild-type strain. Although the pathogenecity was not affected, the deletion mutant showed increased trichothecene B production. Loss of the *Foatf1* gene in *F. oxysporum* caused higher sensitivity to H_2O_2 and loss of pathogenicity on Cavendish banana.

The main aim of our study was generating *FvatfA* deletion mutant strains in the phytopathogenic fungus *Fusarium verticillioides* using the double joint PCR method. To evaluate the stress sensitivity of the mutant, freshly grown conidia were point-inoculated on Czapek-Dox agar plates, supplemented with one of the following stress generating agents: sorbitol, NaCl, KCl, $CdCl_2$, menadione, diamide, *tert*-butyl hydroperoxide, hydrogen peroxide, and Congo Red, in various concentrations. Deletion of *FvatfA* resulted in increased stress sensitivity to menadione, H_2O_2 and Congo Red.

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MPE6

NITROGEN REMOVAL FROM NH4-N RICH SIDE-STREAM WASTEWATER BY ANAMMOX SYSTEM DEMON®

***Anna SZENTGYÖRGYI* (Anna.Szentgyorgyi@vizmuvek.hu) – *Bernadett LEMAIRE* (Bernadett.Lemaire@vizmuvek.hu)**

Institution Budapest Waterworks, Budapest Central Wastewater Treatment Plant H-Nagy-Duna sor 2., 1211 Budapest, Hungary

The Anammox (Anaerobic ammonium oxidation) is an innovative and energy efficient way to treat nitrogen-rich wastewater. At the Budapest Central Wastewater Treatment Plant (BCWWTP) the installed DEMON® is performing the one-way reaction nitrification-anammox process. This technology combines the success of biotechnology and engineering design. Specially developed anammox system, DEMON®, is used for the treatment of NH_4-N rich side-stream wastewater. Anammox technology has a higher nitrogen removal rate (NRR) lower operational cost and smaller space demand than the traditional nitrification-denitrification nitrogen-removal system. The main objective is to reduce the total nitrogen (TN) load of the plant by approx. 10 to 15% compared to the inlet load. According to literature the reduction of stoichiometric oxygen demand is up to 40% compared to the conventional method. The sludge is granulated with a special technology, thereby it is possible to maintain its concentration in the reactors at 2-2.3 g l^{-1} in average. Maintenance of appropriate sludge medium, the necessary separation and recirculation is carried out by hydro cyclones. The air supply is provided with 4 in-duty plus 1 standby blowers with a maximum capacity of 45kW. The NH_4-N content of the incoming wastewater and technology spill water reduces in annual average by 5-7%. According to operational experiences DEMON® currently removes approx. 1-1.2 tons of NH_4-N and the average removal rate of the reactor is 80-85%. This saves up to 1 tons NH_4-N loads in the activated sludge system. The energy demand of the main-stream nitrogen removal is much more significant, approx. 3.6 kWh kg^{-1} of NH_4-N compared to the DEMON® reactor which is 1,4 kWh kg_{-1} for NH_4-N removal. So this new technology saves up to approx. 25 million HUF for the BCWWTP. DEMON® technology is implemented in SBR-type (Sequencing Batch Reactor) reactors with specific culture of activated sludge for ammonia removal. The concept of SBR reactors is to operate a single biological reactor basin using repetitive cycles. At the BCWWTP aeration, mixing, settling, decanting and sludge extraction processes alternate with strictly defined cycle times. In the conventional N removal method: at first ammonium is converted into nitrite by a special bacterial group *Nitrosomonas*, thereafter *Nitrobacter* bacteria oxidize nitrite to nitrate. Finally, nitrate is converted into nitrogen gas by anaerobic denitrifying microorganisms. The DEMON® uses an alternative way to nitrification-denitrification pathway, as the second oxidation step leading from nitrite to nitrate is suppressed; instead the produced nitrite and the ammonium ions are converted directly into nitrogen gas. The bacteria that perform this anammox process belong to the bacterial phylum *Planctomycetes*. By stopping ammonia oxidation at the nitrite level and by performing denitritation, 25% of air blowing and 25% of the carbon requirement can be saved so the overall energy demand can be reduced by 40%.

MPE7

INVESTIGATION OF THE MICROBIAL ELECTROHYDROGENESIS CELLS

Piroska TAKÁCS (tpiroska94@gmail.com) –
László KOÓK (kook@almos.uni-pannon.hu)

Research Institute on Bioengineering, Membrane Technology and Energetics, University of Pannonia; Egyetem str. 10., 8200 Veszprém, Hungary

Several researches are currently in progress to cope with the energy crisis, to treat wastewater produced during household and industrial processes, to modernize their processing or solve numerous other problems related to environment protection. The role of bio electrochemical systems in achieving these goals is increasing; their various types can be applied for producing electric energy, hydrogen or methane, as well as for soil remediation, and they can also be integrated into wastewater treatment technologies.

During my research, I have been dealing with a special type of microbial fuel cells, namely the microbial electrohydrogenesis cells. In this system, the electrons originating from the substrates broken down by exoelectrogenic microorganisms can be used for biohydrogen production, decreasing the chemical oxygen need of the wastewater used.

The subject of the current study is the presentation of the result and challenges related to the construction and operation of the microbial electrohydrogenesis cell and the development of the monitoring of electrochemical processes.

Keywords: bio electrochemistry, hydrogen production, electrogenic microorganisms

NPE1

SCREENING OF ENDOPHYTIC FUNGI AND METABOLITES OF PLANT MATERIALS USED IN MUSHROOM COMPOST

Péter János BEREK-NAGY (nagyberek92@gmail.com) – Imre BOLDIZSÁR (boldizsarimi@gmail.com) – Dániel G. KNAPP (knappdani@gmail.com) – Gábor M. KOVÁCS (gmkovacs@caesar.elte.hu)

Department of Plant Anatomy, Institute of Biology, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C., 1117 Budapest, Hungary

Raw plant materials – used in several agricultural and industrial activities – contain secondary metabolites produced by the plants and endophytic microbes as well. Both the metabolites and endophytic fungi (fungi colonizing plants internally and asymptomatically) might have influence on the subsequent use of plant materials. Such an example is the compost production for mushroom cultivation, where the compost is produced mainly from plant materials. Our aim was to determine the endophytic fungal community and metabolic composition of plant materials, used in compost production of white button mushroom cultivation at the Bio-Fungi Ltd. (Áporka, Hungary).
The raw plant materials of the compost, barley-, rapeseed-, wheat straw and sunflower seed hulk, as well as the compost samples from different stages of the process were screened. To isolate endophytic fungi, plant materials were surface-sterilized with different methods. The isolated fungi were identified based on the sequence of their nrDNA ITS region, the DNA barcode region of fungi. Different staining techniques were carried out to visualize fungi, colonizing the raw plant materials. To determine the metabolic composition of the samples, methanolic extracts were made, and their components were separated by RP HPLC using gradient elution, and detected by DAD UV/Vis detector.
We present the results of the identification of fungal isolates, majority isolated from the raw plant materials, especially from barley and wheat. The isolates, identified till now represent common endophytic fungi of these plants. By using different staining techniques on the raw plant materials, we detected extensive fungal colonisation. We could reproducibly detect three metabolites in the extracts of barley and wheat, which, based on their UV spectra, might be flavonoids. The amount of these metabolites decreased notably during the first steps of composting.
Further experiments are in progress to test, whether these endophytes and metabolites can influence the efficiency of compost production and mushroom cultivation.

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NPE2

PRODUCTION OF DOUBLED HAPLOID CUCUMBER (*CUCUMIS SATIVUS* L.) LINES BASED ON COMPARATIVE EXPERIMENTS OF *IN VITRO* OVARY CULTURE

***Zsófia FEKETE* (fekete.zsofia@abc.naik.hu) – Judit MITYKÓ (mityko.judit@abc.naik.hu)**

NARIC Agricultural Biotechnology Institute; Szent-Györgyi A. u. 4., 2100 Gödöllő, Hungary

Nowadays, *in vitro* haploidy became one of the most intensely researched fields in plant biotechnology. The spread of practical application of the scientific results mainly predominates in cereal and vegetable farming.

Cucumber is a widely cultivated vegetable plant in Hungary. Breeders are facing dual expectations: they have to improve complex disease resistant hybrids while keeping all morphological (shape) and nutritional (taste) properties fancied by the consumers. Due to growing market competition, the high variability of viruses and bacteria requires up-to-date tracking as well as continuous and prompt improvement of new resistant breeding lines.

Developing genetically stable homozygous lines of cucumber with improved traits takes 6–8 years by using classical breeding methods. On the other hand, it requires only 1 year by using *in vitro* ovary culture.

In our laboratory, comparative investigations were carried out on a Japanese [P1] (Diao *et al*. 2009), a Hungarian [P2] (Gémes-Juhász *et al*. 2002) and a self-developed [P3] ovary culture protocol. Three different cucumber hybrid types were used as plant material. All they were parthenocarpic hybrids, characterized by indeterminate growth and purely female flowering (American pickling-, Beit alpha- and Salad type cucumber).

In spite of using different genotypes, our results have followed closely those of published, even have given a bit higher ovary response (published results are listed in brackets): [P1] - 67,9% (62,0%) and [P2] 15,9% (13,2%). In our self-developed [P3] protocol 42,8 % of the ovaries produced embryos, showing intermediate response. Highest embryo induction frequencies were as follows: [P1] 100,0% (72,7%), [P2] 29,5% (18,4%) and 49,1 % in our [P3] protocol. All experiments were also evaluated for plant regeneration efficiency, data obtained as follows: [P1] 1,3% (1,6%), [P2] 4,2% (7,1%) and [P3] 0,4%, respectively.

In summary, total of 33 rooted cucumber plants could be obtained and potted in greenhouse from 2 different genotpes. Flow cytometry analysis showed the following ploidy level distributions: 16 haploid (n), 16 doubled haploid (2n) and 1 tetraploid (4n) plants. Considering our investigated genotypes, Beit alpha proved to be the most responsive type with total of 23 rooted and acclimatized plants. Salad type cucumber was followed it up with 10 plants. Although highest number of embryos could be regenerated from the American pickling type, no plants could be obtained from this genotype.

In conclusion, further investigations are necessary to obtain higher doubled haploid (DH) yield to meet the conditions of safe and wide range practical application.

NPE3

MYB GENE BASED PHYLOGENY OF *VITIS* SPECIES

***Adrienn KEREKES* (adrienn.kerekes@gmail.com)[1] – Antal SZŐKE (Szoke.Antal@mkk.szie.hu)[2] – A. Kitti TÓTH-LENCSÉS (lencses.kitti@mkk.szie.hu)[2] – Gabriella BEDZSÓ (bedzso.gabriella@gmail.com)[2] – Erzsébet KISS (kiss.erzsebet@mkk.szie.hu)[2]**

[1]*Department of Genetics and Plant Breeding, Szent István University, Budapest, Hungary*
[2]*Institute of Genetics, Microbiology and Biotechnology, Szent István University, Gödöllő, Hungary*

The grape family of *Vitaceae* consists of about 14 genera and 900 species which distributed in the north temperate region. The main growing areas are East Asia and North America with 25–30 and approximately 30–40 taxa, respectively. In Europe *Vitis vinifera* (*V. vinifera* subsp. *sylvestris*, *V. vinifera* subsp. *vinifera*) is the only species which distributed over the Mediterranean Refuge is. Nowadays huge interest in the wild grapes is due to the high disease resistance. *Vitis* genus is a phylogenetically ambiguous group. Several works have attempted to clarify the relationship within *Vitis* genus with various DNA markers without unrelieved conclusions.

Based on our preliminary studies with 20D18CB9 CAPS marker – which linked to *VvMybA1* transcription factor gene regulating the anthocyanin biosynthesis – polymorphism between the *Vitis vinifera* and wild species was detected. According to this we assumed that it can be suitable for further examinations and we can set up a new phylogenetic tree within the Vitaceae family.

Based on the sequenced PCR fragments, generated with the 20D18CB9 primer pair, the North American species have a 34-bp deletion, so they form a separate group on the dendrogram. The Asian species, which do not contain this deletion form a distinct group, but some North American ones also lacked this 34 bp DNA deletion.

In this work a new phylogenetic relationship of *Vitis* species was constructed using the 20D18CB9 marker linked to *VvMybA1* transcription factor gene. Our study is the first attempt to use the 20D18CB9 non-coding sequences as a phylogeny marker.

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NPE4

DE NOVO SEQUENCING AND CHARACTERIZATION OF MALE AND FEMALE FLORAL TRANSCRIPTOME OF THE MONOCEIUS WEED, *AMBROSIA ARTEMISIIFOLIA L.*

***Eszter VIRÁG* (virag@georgikon.hu)[1] – Kinga MÁTYÁS (petrovicsnemkk@gmail.com) [1] – Géza HEGEDŰS[2] (hegedus@georgikon.hu)[1] – Eszter FARKAS (peterne.farkas.eszter@gmail.com)[1] – Kincső DECSI (decsikincso@gmail.com)[1] – János TALLER (taller@georgikon.hu)[1]**

[1]*Department of Plant Science and Biotechnology, Georgikon Faculty, University of Pannonia, Festetics György út 7. 8360 Keszthely, Hungary*
[2]*Department of Economic Methodology, Georgikon Faculty, University of Pannonia, Festetics György út 7. 8360 Keszthely, Hungary*

Ambrosia artemisiifolia is a highly allergenic, rapidly spreading weed. Flowering repression may be an opportunity to ragweed control, however there is limited knowledge concerning the underlying molecular genetics.

In order to perform expression pattern during floral progression we sequenced pistillate flower tissues in early and late developmental stages collected from *in vitro* cultivated plants using the Illumina platform (NGS). For expression diversity analysis of gender specific floral transcriptomes we used an earlier published NGS dataset of wild growing male, female flowers and leaves (NCBI TSA GEZL00000000).

De novo Trinity assembly of combined read set of male (M), female (F) and leaf (L) of wild growing plants was performed to get an *A. artemisiifolia* reference transcriptome representing both vegetative and generative organ specific transcripts in different developmental stages. Sequence analysis were performed by using Trinotate software. The transcript datasets were extracted from Trinotate heat map and post processed using SQL Database Manager. The queries for unique organ specific sequences resulted in 5659 (M), 1691 (F) and 4267 (L) founds. Separately two sets of reads representing two classes of female (F) development such as early (1F) and late (2F) stages were generated from sterile *in vitro* plants. 1F and 2F unique transcripts were clustered aligning to the F contig sets. The number of unique transcripts were: 987 (1F) and 1321 (2F). Functional annotation were performed using Blast2GO. The three-specific RNA pools (M, F, L) resulted in 81.56% translated contigs in total. Based on GO annotation the cell part, organic cyclic compound binding and organic substance metabolic process were the most abundant GO slims in the categories cellular component, molecular function and biological process, respectively. The high physiological activity of *Ambrosia* reproductive tissues was suspected from the high representation of primary metabolic process and cellular metabolic process groups within the biological process category.

Focusing on the photoperiodic and vernalisation pathway expression profiles, we defined a theory according that the floral gender determination is tightly regulated by day-length in this species.

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Poszterek összefoglalói

ÁP1

SEMAPHORE – A REPORTER SYSTEM FOR DSBR ACCURACY IN ZEBRAFISH

***Tamás ANNUS* (tamasannus@gmail.com)[1] – Dalma MÜLLER (dalma.muller2@gmail.com)[1] – Mihály KOVÁCS (kovacsm@elte.hu)[2] – Máté VARGA (mvarga@ttk.elte.hu)[1, 3]**

[1]*Department of Genetics, ELTE Eötvös Loránd University Pázmány Péter prom 1/C, 1117 Budapest, Hungary*
[2]*Department of Biochemistry, ELTE Eötvös Loránd University Pázmány Péter prom 1/C, 1117 Budapest, Hungary*
[3]*MTA-SE Nephrogenetic Laboratory, 1st Department of Paediatrics, Semmelweis University, Bókay János str 53-54., 1083 Budapest, Hungary*

Genome integrity maintenance is one the most fundamental cellular processes. Lacking the means to repair DNA damage may lead to severe consequences such as oncogenesis, premature aging, or metabolic insufficiencies. Although valuable information can be derived from *in vitro* and *in silico* studies owing to their flexibility and high throughput capabilities, *in vivo* approaches hold the most promise when it comes to gaining information about phenomena occurring in living organisms in their natural context. Our aim with the development of the Semaphore system is to create a method through which DNA double stranded break repair (DSBR) accuracy can be analyzed. The core components of the construct are an *mStrawberry* and an *eGFP* cassette, the latter of which is flanked by homology arms. Through Cas9 induced cleavage and the use of two fluorophores, the ratio between homology directed repair (HDR) and non-homologous end joining (NHEJ) pathways can be visualized and possibly quantified *in vivo* – eGFP fluorescence marking HDR events, while *mStrawberry* expression NHEJ events. Because our reporter system targets the zebrafish *golden* locus, in its present state it is only applicable in zebrafish. However it could be relatively simply modified for any other model organism given a suitable gRNA target sequence that is sufficiently susceptible to Cas9 cleavage. We propose that such a reporter system could prove to be useful in both basic research and applied sciences.

ÁP2

INVESTIGATION OF THE GENETIC BACKGROUND OF A COMMON CATTLE DISEASE WITH HRM ASSAY

***Bálint BIRÓ* (biro.balint@abc.naik.hu)[1] – Zoltán GÁL (zoltan.gal89@gmail.com)[1,2] – Olivér SZABÓ (szabo.oliver7@gmail.com)[3] – László HIRIPI (hiripi@yahoo.com)[1], András HORVÁTH (horvath.andras@aotk.szie.hu)[4] – Orsolya Ivett HOFFMANN (hoffmann.orsolya.ivett@abc.naik.hu)[1]**

[1]*Department of Animal Biotechnology, Agricultural Biotechnology Institute; NARIC, Gödöllő, Hungary*
[2]*Faculty of Agricultural and Enviromental Science, Szent István University, Gödöllő, Hungary*
[3]*Faculty of Food Science, Szent István University, Budapest, Hungary*
[4]*Large Animal Clinic; University of Veterinary Medicine, Üllő, Hungary*

Left-sided displacement of the abomasum (LDA) is one of the most common disorders of the digestive system in many dairy cattles. The LDA is associated with high yielding, intensively fed dairy cows in late or early gestation lactation. In the LDA affected animals the gastrointestinal track has reduced movement and abomasum starts bloating due to the accumulating gases. It has to be surgery treated to fix the abomasum in its correct position. Even if the treatment succeeded without any incidents, a significantly reduced milk production and increased culling risk for affected cows can be observed. LDA is a multifactorial disease, in which environmental effects play a role, however heritability rates of LDA are higher than in other dairy cattle diseases. Although the prevalence of LDA is usually 1-7% in German Holstein-Friesian populations. It is known that the genetic influence is important in the development of the disease, the genetic background of the disease is unclear.
The two main reasons for the developing of the disease are the abomasal hypomotility and the disfunction of the intrinsic nervous system. The motilin gene (MLN) located on BTA23 is an appropriate candidate for the development of LDA in bovine. Motilin is a gastrointestinal hormone which regulates the peristalsis. A single nucleotide polymorphism within this gene showed significant association with the disease thus could be used as a marker to predict the occurence of LDA.
The intention of the present work is to develop a rapid procedure to observe this SNP in the bovine motilin gene. We used high-resolution melting analysis (HRM) for detection of the SNP. HRM is an innovative technique which is easier and more cost-effective than probe-based genotyping assays and less time consuming than conventional sequencing.

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ÁP3

DEVELOPMENT OF GENETIC MONITORING METHODS FOR HUNGARIAN LARGE CARNIVORES: *CANIDAE*

Péter FEHÉR* (feher.peter@abc.naik.hu)[1] – Kinga SZEPESI (szepesi.kinga@abc.naik.hu)[1,2] – Krisztián FRANK (frank.krisztian@abc.naik.hu)[1] – Botond HELTAI (heltai.botond@abc.naik.hu)[1] – Bendegúz MIHALIK (mihalik.bendeguz@abc.naik.hu)[1] – Dóra ÚJVÁRY (ujvarydori@gmail.com)[3] – István SZILÁGYI (szilagyi@feherkeresztliga.hu)[4] – Péter GOMBKÖTŐ (gombkoto.peter@gmail.com)[5] – László SZEMETHY (laszlo.szemethy@gmail.com)[6] – Viktor STÉGER (steger.viktor@abc.naik.hu)[1]

[1]*Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Szent-Györgyi Albert str. 4, 2100 Gödöllő, Hungary*
[2]*Department of Animal Science and Animal Breeding, Georgikon Faculty, University of Pannonia, Festetics str. 7, 8360 Keszthely, Hungary*
[3]*Foundation for the Preservation of European Wildlife, Brassó str. 42, 1112 Budapest, Hungary*
[4]*Bear Farm, Patak str. 39, 2112 Veresegyház, Hungary*
[5]*Bükk National Park Directorate, Sánc str. 6, 3304 Eger, Hungary*
[6]*Institute for Wildlife Conservation, Faculty of Agricultural and Environmental Sciences, Szent István University, Páter Károly str. 1, 2100 Gödöllő, Hungary*

Large carnivores like grey wolf (*Canis lupus*) count as the most controversial animal groups in our modern and crowded world. Nowadays, the population of several large carnivores have an increasing tendency in Europe which is due to conservation programs and legal protection. Non-invasive monitoring methods are suitable for investigate these species. Genetic testing is required for species and individual identification and population characterisation. Non-invasive samples like urine, scat and hair are feasible genetic analysis. Thus, we adopted genetic methods for monitoring of these species. Individual recognition was performed using microsatellite genotyping from non-invasive samples. Hair and scat samples were provided by the Bükk National Park Directorate. Additional reference samples were obtained from the Hungarian Natural History Museum, Foundation for the Preservation of European Wildlife and the Bear Farm of Veresegyház. Twelve microsatellites were optimized for multiplex PCR and used for genotyping. Initial species identification based on morphological characters was done by the sample collectors. Genetic clustering was carried out with STRUCTURE and PAST softwares. The amelogenin gene was used for sexing the samples. Grey wolf are genetically distinct from dogs and zoo's wolf samples, so markers are suitable for separating the species. Additionally, our results show that there is a substantial genetic difference between the closed and field wolf samples. Thus, the method is feasible for genetic monitoring purposes. Because of low sample numbers detailed population genetic analyses were not carried out, but a long-term goal is to study the genetic diversity of these protected predators from non-invasive samples. The larger effort in sampling could result in better population assignment, furthermore the acquired data could be used also for forensic investigations.

ÁP4

DEVELOPMENT OF MICROSATELLITE MARKERS RELATED TO THE HYGIENIC BEHAVIOUR OF HONEY BEES

Botond HELTAI* (heltai.botond@abc.naik.hu)[1,2] – Kinga SZEPESI (szepesi.kinga@abc.naik.hu)[1,3] – Krisztián FRANK (frank.krisztian@abc.naik.hu)[1] – Péter FEHÉR (feher.peter@abc.naik.hu)[1] – Alexandra TOKÁR (tokar.alexandra@abc.naik.hu)[2] – Bendegúz MIHALIK (mihalik.bendeguz@abc.naik.hu)[1] – Gréta BOGNÁR (greta.bognar@gmail.com)[3] – Éva KOLICS-HORVÁTH (horvathevica@gmail.com)[3] – Endre BARTA (barta.endre@abc.naik.hu)[1] – Szilárd BODÓ (bodo.szilard@abc.naik.hu)[1,2] – Balázs KOLICS (bkolics@gmail.com)[3] – Viktor STÉGER (steger.viktor@abc.naik.hu)[1]

[1]*Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Szent-Györgyi Albert str. 4, 2100 Gödöllő, Hungary*
[2]*Institute of Animal Science and Animal Husbandry, Szent István University, Páter Károly str. 1, 2100 Gödöllő, Hungary*
[3]*Department of Plant Science and Biotechnology, Georgikon Faculty, University of Pannonia, Festetics str. 7, 8360 Keszthely, Hungary*

The honey bee's most damaging ectoparasite is the *Varroa destructor* mite. Some bees can effectively defend themselves against this parasite showing hygienic and Varroa Sensitive Hygiene (VSH) behaviour. Hygienic bees uncap and remove dead pupae and larvae from the nest, while VSH bees remove infected brood too, thus preventing further infection. Hygienic and VSH behaviour is a heritable genetic trait. Genomic studies make the development of DNA markers possible, which could help to create nucleus populations of animals, selected for some economic characteristics. Our goal was to support the domestic beekeepers with genetic data to improve the breeding of bee families and disease resistant bee populations.

Sequence data were generated for Pannonian honey bee specimens. We identified tandem repeat motifs in the available bee genome with the Perl based QDD package, and designed primers for the eligible ones with Primer3. Then we selected microsatellite markers for the QTL region which is linked to the hygienic and the *Varroa* mite sensitive behaviour.

We used two type of field assays to screen colonies for hygienic behaviour: bee brood freezing and the pierced brood assays. Bee families in the experiment showed a wide range of removal rates. Bee hives were grouped as hygienic, intermediate or non-hygienic according to the amount of the removed brood within 24 hours. These families provide the possibility to validate the markers developed. The linked microsatellite markers may help the marker-assisted selection of the hygienic trait, reducing the field assays.

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ÁP5

VITRIFICATION OF ZEBRAFISH (*DANIO RERIO*) GERM CELLS FOR GENE CONSERVATION PURPOSES

Eszter KÁSA (Kasa.Eszter@mkk.szie.hu) – Tímea KOLLÁR (timi.kollar@gmail.com) – Jelena LUJIĆ (lujicjelena@gmail.com) – Zoran MARINOVIĆ (zor.marinovic@gmail.com) – Béla URBÁNYI (Urbanyi.Bela@mkk.szie.hu) – Ákos HORVÁTH (Horvath.Akos@mkk.szie.hu)

Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Szent István University; Páter Károly str. 1., 2100 Gödöllő, Hungary

The aim of this study was to develop vitrification methods for testicular tissue, isolated spermatogonial cells and sperm of the animal model zebrafish *(Danio rerio).* In case of fish species, the methods of gene conservation are limited due to the fact that fish embryos and eggs cannot be frozen (because of their high yolk content). In addition, in small-bodied model fish species sperm cryopreservation is problematic, due to low individual sperm quantity (1 µl per fish). With cryopreservation of gonadal stem cells the genome of both sexes can be stored. Vitrification protocols had been applied for gonadal stem cells in different fish species.

For vitrification ultra-rapid cooling is applied (10^6-10^{10}°C sec^{-1}), due to this ultra-fast cooling, the creation of crystalline structure of water molecules is inhibited. During vitrification, the ice crystals do not damage cells, altough the required cryoprotectant concentration (30–50%) can be harmful for the cells.

Spermatogonia vitrification was carried out by two methods: whole testis vitrification (fixed on acupuncture needles), and digested tissue vitrification. For counting membrane-intact spermatogonia, trypane-blue staining was used. Numerous intracellular cryoprotectants (methanol, propylene-glycol, dimethyl-sulfoxide) had been tested in different combinations, besides trehalose and FBS (Foetal Bovine Serum) supplementation had been used as extracellular cryoprotectant. Sperm and digested tissue was vitrified on Cryotop, while whole testes were vitrified on acupuncture needles.

Each tested cryopreservation method (sperm-, testes- and digested tissue vitrification) had been applied succesfully for the gene preservation of zebrafish. Following vitrification of the sperm (protocol: 15% methanol + 15% propylene-glycol, 1:4 dilution ratio, HBSS extender) the progressive motility was 10.8±5.2% (fresh control: 84.5±8%). Fertilization test with vitrified sperm resulted in 0.7±0.3% hatching (control: 59.8±3%). After vitrification of digested tissue the viabiltity of spermatogonia was 18.5±0.1% (protocol: 1.5M propylene-glycol + 1.5M DMSO, 0.5M trehalose, 0.25M Hepes, 10% FBS, 1:1 dilution ratio (v/v) in L-15). Vitrification of whole testes resulted in 47.43±1.7% of viable spermatogonia following thawing (protocol: equilibration media: 1.5M methanol + 1.5M propylene-glycol, vitrifying media: 3M propylene-glycol + 3M DMSO, both solutions were supplemented with 0.5M trehalose, 0.25M Hepes and 10% FBS).

According to our results, the genetic material of zebrafish can be preserved both with sperm- and spermatogonia vitrification. With the protocols described above, it is possible to generate an *in vitro* gene bank for special zebrafish lines.

ÁP6

INVESTIGATION OF EMBRYOGENESIS FOLLOWING FERTILIZATION WITH SPERM EXPOSED BY HEAVY METALS, IN ZEBRAFISH (*DANIO RERIO*)

***Tímea KOLLÁR* (timi.kollar@gmail.com) – Eszter KÁSA (Kasa.Eszter@mkk.szie.hu) – Zsolt CSENKI-BAKOS (Csenki.Zsolt@mkk.szie.hu) – Ákos HORVÁTH (Horvath.Akos@mkk.szie.hu)**

Department of Aquaculture, Institute of Aquaculture and Environmental Safety, Szent István University; Páter Károly str 1., 2100 Gödöllő Hungary

During the experiments, the effects of 5 heavy metals (Cr, Zn, Ni, Cd, Cu) were investigated on fertilization and embryogenesis of zebrafish following the exposure of sperm (30 or 120 minutes). Due to the physiological features of fish (aquatic living, external fertilization), fish sperm can be contaminated during the breeding with toxicants which are found in the water. Their effect do not manifest on the motility parameters of sperm in some cases (e.g. oxidative DNA damage), however, toxic exposure of sperm can lead to several embryonic deformities and reduction in fertilization. These impairments can be examined on sperm directly only with expensive methods and equipments (e.g. flow-cytometer). Consequently, carrying out of fertilization experiments with exposed sperm are needed to investigate the effects of heavy metals on fertilization and embryogenesis.

The similar pattern was observed in cases of Cr, Ni and Cu, where the exposure duration of sperm affect neither to the fertilization rate nor to the 48^{th}-hour of survival of embryos, however, the effect of concentration was significant in case of both examined endpoints. Following exposure to Cr, the fertilization rate and the 48^{th} hour of survival decreased at 100, 150 and 200 mg L^{-1} after 120 minutes of exposure significantly. In case of Ni, already 600 mg L^{-1} decreased the fertilization rate and the 48^{th} hour of survival significantly after 30 minutes of exposure. Following exposure to Cu, the fertilization rate and the 48^{th} hour of survival decreased significantly at 25 mg L^{-1} after 120 minutes of exposure and at 50 mg L^{-1} after 30 as well as 120 minutes of exposure. In cases of Cd and Zn, neither the exposure duration of sperm, nor the concentration of the tested metals affected to the fertilization rate and the 48^{th} hour of survival significantly. The rate of embryonic deformities (yolc oedeme, pericardial oedeme, tail malformation) did not differ from the control in case of any of the tested heavy metals significantly.

It can be concluded that the fertilization rate of eggs and the 48-hour survival of embryos are less sensitive endpoints than the motility parameters of sperm (e.g. progressive motility, velocity) to the toxic exposure of sperm in the most cases (exc. Ni, Cr). Beside of that the examination of motility parameters is not dependent on the availability and the quality of eggs, it can give more reliable results. In addition, the motility parameters are able to indicate the toxic effects real-time, while the fertilization rate can be determined only 20–24 hours after the fertilization. Nevertheless, the fertilization rate and the 48 hours of survival can sign the toxic exposure of sperm, so their investigation can be reasonable in the absence of CASA system. *This work was supported by the ÚNKP-17-3 New National Excellence Program of the Ministry of Human Capacities.*

ÁP7

SHORT TERM EFFECTS OF STERIGMATOCYSTIN ON THE REGULATION OF THE ANTIOXIDANT SYSTEM IN COMMON CARP

Benjámin KÖVESI* (benjamin.kovesi@gmail.com)[1] – Csilla PELYHE (pelyhe.csilla@gmail.com)[2] – Erika ZÁNDOKI (Zandoki.Erika@mkk.szie.hu)[2] – Krisztián BALOGH (Balogh.Krisztian@mkk.szie.hu)[1,2] – Miklós MÉZES (Mezes.Miklos@mkk.szie.hu)[1,2]

[1]Department of Nutrition, Faculty of Agricultural and Environmental Sciences, Szent István University, , , Páter Károly str. 1., 2100 Gödöllő, Hungary
[2]Mycotoxins in the Food Chain Research Group, Hungarian Academy of Sciences-Kaposvár University, Guba Sándor str. 40., 7400 Kaposvár, Hungary

Sterigmatocystin (STC) is a secondary metabolite of *Aspergillus* moulds, which is structurally similar to aflatoxins. The main target organs of STC toxicity are the liver and kidneys. It has mutagenic, carcinogenic and cytotoxic effects and also has immunomodulatory activity. It is classified as a 2B carcinogen (possibly carcinogenic to human) by the International Agency for Research on Cancer (IARC). STC is metabolized by the cytochrome P450 3A4 to a reactive epoxide, which reacts with nucleic acids to form guanyl adducts. It is known that the formation of guanyl adducts increases the production of reactive oxygen species which may lead to oxidative stress. Due to the different level of oxidative stress different redox-sensitive pathways are activated and different biological responses can be observed as it was described by the hierarchical oxidative stress model. During mild oxidative stress conditions the transcriptional activation of phase II antioxidant enzymes and the expression of antioxidant genes, including glutathione peroxidases, occurs via the Nrf2-Keap1/ARE pathway.

The aim of this study was to evaluate the short term effects of sterigmatocystin on the glutathione redox system along with Nrf2-Keap1/ARE pathway in common carp liver (n=96). After a week of adaptation period experimentally contaminated (1, 2 and 4 mg STC kg^{-1} feed and 400 µg AFB_1 kg^{-1} feed) or control complete feed was given by gavage directly into the gut. Liver samples were taken from 6 carps of each group at every 8th hour during a 24-hour long experimental period. Expressions of glutathione peroxidase genes (*gpx4a* and *gpx4b*), and also *nrf2* and *keap1* genes were analysed.

Sterigmatocystin caused similar changes in the expression of both *gpx4a* and *gpx4b* genes. At 8^{th} hour sampling the gene expression was at the control level in all experimental groups, but at 16^{th} and 24^{th} hour induction was observed. This dual response was also observed in case of *nrf2* and *keap1* genes, however *keap1* gene showed earlier response as its level was significantly higher even at 8^{th} hour in case of lower doses.

According to the results it can be concluded that the short term sterigmatocystin exposure probably emerged ROS formation therefore activated the glutathione redox system and its encoding genes.

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ÁP8

REGIONALLY DISTINCT ALTERATIONS IN THE COMPOSITION OF THE LUMEN- AND THE MUCOSA-ASSOCIATED MICROBIOTA IN CHRONIC ETHANOL-TREATED RATS

***Diána MEZEI* (mezeidiana810@gmail.com)[1] – *Nikolett BÓDI* (bodi.nikolett@bio.u-szeged.hu)[1] – *Roland WIRTH* (wirth@bio.u-szeged.hu)[2] – *Gergely MARÓTI* (maroti.gergely@brc.mta.hu)[3] – *Mária BAGYÁNSZKI* (bmarcsi@bio.u-szeged.hu)[1] – *Lalitha CHANDRAKUMAR* (lalitha.chandrakumar@bio.u-szeged.hu)[1] – *Zita SZALAI* (zszalai@bio.u-szeged.hu)[1] – *Bettina SZUCSÁN* (szucsan.bettina@gmail.com)[1] – *Kornél L. KOVÁCS* (kovacs.kornel@brc.mta.hu)[2,4]**

[1]*Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Közép fasor 52, 6726 Szeged , Hungary*
[2]*Department of Biotechnology, University of Szeged, Közép fasor 52, 6726 Szeged, Hungary*
[3]*Biological Research Centre, Hungarian Academy of Sciences, Temesvári krt. 62., 6726 Szeged, Hungary*
[4]*Facultry of Dentistry, University of Szeged, Tisza L. krt. 64-66, 6720 Szeged, Hungary*

Chronic alcohol administration did not result significant neuronal loss in the rat myenteric plexus, but primarily impaired the nitrergic pathways in gut region-specific way. These results imply the importance of the neuronal microenvironment along the gastrointestinal tract in the pathogenesis of nitrergic neuropathy in alcoholism. Our aim was to map the composition of the lumen-associated (LAM) and the mucosa-associated microbiota (MAM) in different intestinal segments in a chronic ethanol-treated rat model. Rats were randomly divided into a chronic alcohol-treated and a control group receiving tap water. Chronic alcohol-treated rats were given increasing concentrations of ethanol in tap water (v/v%) starting with 10% in the first, 15% in the second and 20% in the 3rd to 8th weeks.

Lumen material of the duodenum, ileum and colon were used to characterize LAM, and tissue samples were used to demonstrate the MAM by using next-generation DNA sequencing. Characteristic rearrangements in the microbiome composition and diversity were detected in both LAM and MAM after chronic ethanol consumption.

LAM: In the duodenum the ratio of class Bacteroidia was almost 3-times higher in the ethanol treated group, while the abundance of classes Bacilli and Clostridia decreased compare to the control rats. In the ethanol treated ileum the composition of LAM changed moderately. In the colon the ratio of class Bacteroidia was higher after alcohol treatment.

MAM: In the duodenum significant rearrangements were detected in the abundance of classes Bacteroidia, Betaproteobacteria, Bacilli and Clostridia. In the ileum the ratio of class Chlamydia decreased, while the class Clostridia was increased in the ethanol-treated group. In the colon the ethanol-related alterations in the composition of MAM were moderated.

In conclusion, chronic ethanol treatment affected the composition of both LAM and MAM in gut region-dependent manner.

ÁP9

EFFECTS OF ENDOGENOUS AND EXOGENOUS PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE ON EARLY MOUSE EMBRYO DEVELOPMENT

Bálint PETRÁNYI (balint0705@gmail.com)[1] – Dóra TÖRÖK[1] – Dóra REGLŐDI[2] – Andrea TAMÁS[2] – Bence SOMOSKŐI (somoskoi.bence@univet.hu)[1] – Sándor CSEH (cseh.sandor@univet.hu)[1]

[1]*University of Veterinary Medicine, István str. 2., 1078 Budapest, Hungary*
[2]*University of Pécs, Vasvári Pál str.4., 7622 Pécs,Hungary*

Due to the antiapoptotic effect of pituitary adenylate cyclase activating polypeptide (PACAP) and its widespread presence in the organ system, PACAP is considered as a general cytoprotective peptide. The peptide was found in the gonads in high levels, that is what drew attention to the peptide might play a central role in reproduction.
The objective of our research is to investigate the effect of endogenous (Exp.1) and exogenous PACAP (Exp.2) on early mouse embryo development in vitro.
PACAP-producing (wild type) and KO CD1 mice were examined through the Exp.1. Zygotes produced by natural mating were obtained from donor superovulated females 5-6 hours after copulation. Embryos were cultured for 96 hours in vitro and developmental state, cell number and micronuclei rate were assessed. In the Exp.2, embryos were obtained from BDF1 donor females. Zygotes were treated with 0.95 mM PACAP1-38 or 0.95 mM PACAP6-38 (antagonist). 5% PBS was used as diluent control.
In Exp.1, higher blastomer number was found in PACAP KO embryos than in wild ones ($p=0.0022$). However, the advanced PACAP KO embryos have significantly higher micronucleus rate compared to advanced wild type embryos ($p = 0.01$). In the Exp.2, all of the PACAP treatments decreased the blastocyst rate. However, embryos treated with PACAP6-38 reached the blastocyst stage in a lower manner (27.1%) than in the PACAP1-38 treatment (41.6%). 5% PBS did not cause any reduction of blastocysts from the control group. Our data show that PACAP has a remarkable effect on embryo development. Due to our controversial data on endogenous and exogenous PACAP, further experiments will be carried out.

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ÁP10

DETECTING ASIAN ORIGIN OF HONEY USING GENETIC MARKERS

Kinga SZEPESI* (szepesi.kinga@abc.naik.hu)[1,2,3] – Botond HELTAI (heltai.botond@abc.naik.hu)[2] – Krisztián FRANK (frank.krisztian@abc.naik.hu)[2] – Péter FEHÉR (feher.peter@abc.naik.hu)[2] – Endre BARTA (barta.endre@abc.naik.hu)[2] – Viktor STÉGER (steger.viktor@abc.naik.hu)[2] – Balázs KOLICS (bkolics@gmail.com)[3]

[1]*Festetics Doctoral School, University of Pannonia, Deák Ferenc str. 16, 8360 Keszthely, Hungary*
[2]*Department of Genomics, Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Szent-Györgyi Albert str. 4, 2100 Gödöllő, Hungary*
[3]*Department of Plant Science and Biotechnology, Georgikon Faculty, University of Pannonia, Festetics str. 7, 8360 Keszthely, Hungary*

Honey bees are one of the most important insects, having a huge agricultural value. First of all they are essential for the pollination of the main crops. Furthermore the products they provide are used in a large range by several industries; for example honey, propolis, beeswax, pollen and the very valuable royal jelly are remarkable products in themselves, but also count as raw material for many products in the food, pharmaceutical and also the beauty industry.

Despite the fact that Hungary is a small country, in the European Union we occupy the sixth place in point of number of bee hives, and second place if we look at the bee density. Due to this, Hungary is among the major honey producer countries in Europe; more than 1 million bee colonies are registered, producing 30000 tons of honey annually.

Being a popular food product, we chose to characterize different honey samples. There are numerous ways to describe it. The main analyses monitor the chemical composition (sugars, nitrogenous compounds, phenolic contents, acids, sugar decomposition products), nutritional value (carbohydrates, vitamins, minerals, trace elements), medicinal and antimicrobial value. Our main objective was the genetic detection of the Asian honey bee (*Apis cerana)* components in honey, this way we can make a difference between the honey samples depending on the genetic background of the bee that collected the nectar.

Beekeepers in Europe use western honey bees (*Apis mellifera)* in their apiaries, this way we determined to detect sequence differences specific to *Apis mellifera* and *Apis cerana.* In total 51 complete honey bee genomes were used to find these discrepancies using bioinformatics softwares. There were tens of thousands of genomic differences identified between the western honey bee and the Asian honey bee. We prefered the mitochondrial region and searched for insertions and deletions located on this part of the DNA to develop a real-time PCR DNA diagnosis essay to detect Asian and European components of the honey.

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BP1

DNA METHYLATION PATTERNS AND HUMAN AGING

István CSABAI (csabai@complex.elte.hu)[1] – Judit BÖRCSÖK (judit.borcsok27@gmail.com)[2]

[1]*Department of Physics os Complex Systems, ELTE Eötvös Loránd University, Pf. 32, 1518 Budapest, Hungary*
[2]*Faculty of Sciences, ELTE Eötvös Loránd University*

In mammals, epigenetic mechanisms are essential for normal development and maintenance of tissue-specific gene expression levels. It has been observed that epigenetic patterns change over the lifetime, suggesting that these changes may play an important part in the aging process. The epigenetic mark that has been mostly studied is DNA methylation, the presence of methyl groups at CpG dinucleotides. These dinucleotides are often located close to gene promoters and linked to gene regulation. It has been shown by numerous studies that certain CpG sites are highly associated with age enabling to accurately estimate the chronological age of tissues and cell types. In my poster, I would like to present a comparison of several regression models of aging and demonstrate their applicability to predict age. For building the models, I used methylation data sets of different human tissues measured by Illumina methylation platforms from TCGA and GEO databases. Selecting the best fit model and CpG sites highly associated with age may provide a better insight into the aging process and age-related diseases.

BP2

IDENTIFICATION OF FLORAL ORGAN IDENTITY GENES IN THE COMMON RAGWEED *(AMBROSIA ARTEMISIIFOLIA L.)*

Kinga Klára MÁTYÁS* (mkinga@georgikon.hu)[1] – János TALLER (taller@georgikon)[1] – Géza HEGEDŰS (hegedüs@georgikon.com)[2] – Balázs KOLICS (bkolics@gmail.com)[1] – Eszter FARKAS (peterne.farkas.eszter@gmail.com)[1] – Tamás PARRAG (parrag.tamas@georgikon.hu)[1] – Zoltán FARKAS – (farkas.zoltan@georgikon.hu)[1] – Eszter VIRÁG (virag@georgikon)[1]

[1]Department of Plant Science and Biotechnology, Georgikon Faculty, University of Pannonia, Festetics str. 7, 8360 Keszthely, Hungary
[2]Department of Economic Methodology, Georgikon Faculty, University of Pannonia, Festetics str. 7, 8360 Keszthely, Hungary

The floral architecture development depends on the regulation of floral homeotic genes based on the –so called– ABC combinatorial model. The highly allergenic weed, *Ambrosia artemisiifolia* is a monoecious plant with separate male and female flowers on the same individual which flowering regulation is still a revealing field. The objective of this study was to annotate ABC homologous and their expression pattern in reproductive tissues of *A. artemisiifolia* male and female inflorescences.

RNA extraction and sequencing were performed from early and late developmental stages of *A. artemisiifolia* female flowers. cDNA synthesis and library construction were prepared for Illumina NextSeq paired-end sequencing. The complete CDS of each genes were predicted using our previously reported transcript dataset (NCBI) and using UNIPROT databases. Normalized expression level values (RPKM) were calculated applying our self-developed application GenoUtils.

ABC homologous such as AP1, AP2, AP3 1, AP3 2, PI, AG, SEP1, SEP2 1, SEP2 2, SEP3 1, SEP3 2, SEP3 3 and SEP4 revealed open reading frame for putative proteins of 248, 488, 223, 229, 235, 196, 247, 250, 252, 254, 245, 263 and 248 amino acids, respectively. According to our results the following specification of floral organ identity may be concluded: (i) AP2, AP3 2 and PI homologous were not expressed in pistillate tissues indicating that these genes are involved for staminate flowers during floral morphogenesis. High expression levels of four SEP genes were detected in early developmental stages of female flower. These genes SEP1, SEP2 1, SEP3 1 ad SEP4 probably participate in petals and carpel primordia development. Genes SEP3 2 and SEP 2 2, which have higher level in late stage, may specify carpel prolongation.

The described unique expression patterns of these genes typically involved in reproductive development have implications on our understanding of sex-specific flower induction in *A. artemisiifolia.*

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BP3

TMCRYS: PREDICT PROPENSITY OF SUCCESS FOR TRANSMEMBRANE PROTEIN CRYSTALLIZATION

Julia K. VARGA (varga.julia@ttk.mta.hu) – Gábor E. TUSNÁDY (tusnady.gabor@ttk.mta.hu)

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok körútja 2, 1117 Budapest, Hungary

Transmembrane proteins play vital roles in the life of cells, anchoring cells to the extracellular matrix, joining cells and acting as gatekeepers and receptors in the membranes. They make up to 25–30% of the human proteome and are targeted by around 50% of marketed drugs. However, only 2% of the structures deposited in structure databases belong to transmembrane proteins.

One of the most widely used methods for solving protein structures is X-ray crystallography. Transmembrane proteins, due to their special physical-chemical properties, are hard to be solubilized and crystallized, thus making the determination of their structures difficult. The process is time and money consuming, thus prediction for the success or failure of the crystallization may help the crystallization projects.

Our aim was to develop a prediction method for the crystallization process of transmembrane proteins. So far, no method is available that would be specific for transmembrane proteins. We used the TargetTrack and PDBTM databases to create training and test sets for several steps of the crystallization process. XGBoost Decision Tree algorithms were used to train and optimize models for all steps.

GP1

HIGH DIMENSIONAL CHARACTERISATION OF CELLULAR FEATURES BY SINGLE CELL MASS CYTOMETRY

***József Ágoston BALOG* (balog.jozsef@brc.mta.hu) – László G. PUSKÁS (puskas.laszlo@brc.mta.hu) – Gábor János SZEBENI (szebeni.gabor@brc.mta.hu)**

Laboratory of Functional Genomics, Biological Research Centre, Hungarian Academy of Sciences; Temesvári krt. 62, 6726 Szeged, Hungary

High resolution measurement characterizing the large number of cellular features is in the focus of recent cell biological research. To achieve these goals single cell mass cytometry combines advantages of the single cell resolution of traditional fluorescence-based flow cytometry with the multiplexicity of inductively coupled plasma-mass spectrometry. Instead of fluorophores detection for mass cytometry is based on stable heavy-metal isotope labeled antibodies. Thus, the autofluorescence and spectral overlapping are eliminated. The current state-of-the-art mass cytometer is capable of measuring up to 135 different stable isotopes of rare earth metals, although the current availability of these tags in high purity limits the usage to around 45 different rare earth metal tags. This unique feature enables researchers to multiplex up to 45 different antibodies in one single tube.

Extensive mapping of signaling networks in single cells, cell surface receptor quantification has been also achieved by single cell mass cytometry. Sample multiplexing is also possible by barcoding prior the antibody labeling which enables the combination of 20 different samples in one single tube. Cell types, cellular populations of interest can be visualized on dot plots and the protein expression levels are demonstrated by histograms. Furthermore, gating hierarchy above 10–12 level is also manageable. Mass cytometry deeply reveals cellular heterogeneity on the basis of highly multiplex phenotypical and functional characterization. There are several novel algorithmic approaches to process large datasets such as: SPADE, viSNE, Citrus.

The monitoring of the complex immunophenotype is highly relevant in several human diseases which have been previously restricted to limited number of markers with flow cytometry compared to single cell mass cytometry. Human systemic autoimmune diseases (rheumatoid arthritis, systemic lupus erythemathosus and systemic sclerosis) are under investigation. The cellular complexity and functional heterogeneity of solid tumors, inflammatory diseases and animal models (tumor, bone marrow, spleen, lymph nodes) will be also analyzed in our laboratory by single cell mass spectrometry.

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GP2

OPENING A NEW DOOR TO COST-EFFECTIVE LARGESCALE VACCINE EFFECTIVENESS STUDIES

***Katalin BÖRÖCZ* (borocz.katalin@pte.hu)[1] – *Ákos MARKOVICS* (markovics424@gmail.com)[2] – *Timea BERKI* (berki.timea@pte.hu)[1] – *Péter NÉMETH* (nemeth.peter@pte.hu)[1]**

[1]*Department of Immunology and Biotechnology, Clinical Centre, University of Pécs; Szigeti Street 12, 7624 Pécs, Hungary*
[2]*Department of General and Physical Chemistry, Faculty of Natural Sciences, University of Pécs, Ifjúság Street 6, 7624 Pécs, Hungary*

Motivated by nowadays epidemiological threats, our aim was to establish a straightforward and standardized anti-measles IgG indirect ELISA assay protocol, which is smooth to introduce in the basic laboratory practice, hence opening a new door to cost-effective largescale long term vaccine effectiveness studies. Opposite to the common development practice of positivity-centred assays, our goal was the designation of a test destined for negativity detection.

For the development process, the most prominent criterion was the maximal elimination of all the potential false-positivity inducing factors. The sample multitude was categorized based on alterations in immunization schedules and/or in vaccine components since the introduction of the measles vaccine in Hungary (Ethical License number 2015/5726).Samples were analysed using our self-developed test in comparison with multiple commercial assays. For the confirmation of the results anti-measles nucleocapsid-based (anti-N) sandwich ELISA and indirect immunofluorescence (IIF) were used.

We established the operational protocol of a standardized, feasible and cost-saving assay which overlaps well with commercial kits and shows strong correspondence with anti-N based sandwich ELISA and IIF results (in both cases correspondence analysis p-value< 0.0001 with alpha=0,05). Based on the measurement of more than 2000 sera; the highest ratio of low and questionable antibody tittered samples is evident in the cluster “1978–1987” (≈25.4%), followed by the cluster “1969–1977” (≈15.4%).

Since both clusters that we found particularly susceptible encompass individuals who have been immunized during the initial times of vaccination, when the age and the way of vaccination were poorly specified and consequent vaccine failure of this era later on became a matter of a fact, we can pronounce that the actual measurement data are in accordance with real life trends; suggesting the reliability of the self-developed assay. We consider the here described series of experiments a pilot for a complete MMR vaccine effectiveness study aided by an immunological binding-based quick-test.

GP3

ANTIMICROBIAL PEPTIDE BIOSYNTHESIS FOR THE DEVELOPMENT OF A NANOFIBER-BASED WOUND DRESSINGS

Álmos DÓSA* (dosa.almos@vimspectrum.ro)[1] – Pál SALAMON (salamonpal@uni.sapientia.ro)[2,3] – Dr. Csongor ORBÁN (orbancsongor@uni.sapientia.ro)[3] – Ferenc BODA (boda.francisc@umftgm.ro)[4] – Zoltán SZABÓ (szabo.zoltan@umftgm.ro)[4] – Dr. Edit SZÉKELY (edit.székely@umftg.ro)[4]

[1]Vim Spectrum Pharmaceuticals, Corunca no. 409, 547367 Mureş County, Romania.
[2]University of Pécs, Faculty of Sciences, Institute of Chemistry, Ifjúság útja 6, 7624 Pécs, Hungary
[3]Department of Bioengineering, Faculty of Economics, Socio-Human Sciences and Engineering, Sapientia Hungarian University of Transylvania, Piaţa Libertăţii nr. 1, Harghita County, 530104 Miercurea Ciuc, Romania.
[4]Departament of Fundamental Pharmaceutical Sciences, University of Medicine and Pharmacy of Tîrgu Mureş, Gheorghe Marinescu Street 38, 540139, Tîrgu Mureş, Romania.

Since technology has had a dramatic impact on healthcare, biomedical engineering techniques gained more and more popularity within biotechnology. The developments in medicine and pharmaceutics and a better understating of protein-protein interactions pave the way for highly-specific biologics obtained through biosynthesis.

Currently, there are more than 60 peptide-based therapeutic agents. Produced with the help of solid-phase chemical synthesis (SPPS), the annual commercial trade of those seven peptides, which are produced in the biggest quantity reaches 5,2 billion dollars. Apart from being very expensive, the chemical manufacturing process of these peptides is quite slow and with increasing peptide length procedure effectiveness and turnaround decreses drastically.

If a suitable fusion partner and vector construction are employed, peptide synthesis, realized through an expression system, is a lot cheaper than chemical synthesis. The aim of the present study was to achieve the high-yield production of the antimicrobial peptide Buforin II in *Escherichia coli* host, using a lab-scale bioreactor. The purified peptide would be afterwards loaded into a nanofibrous topical delivery system for the treatment of suprainfected topical wounds.

Production of Buforin II was achieved in *Escherichia coli* BL21 DE3 Rosetta host, using a Biostat A+ bioreactor. The first goal was to achieve high cell count and optimize the conditions of the expression. Peptide purification was performed on a FPLC system, using a Ni-histidine tag affinity chromatography, followed by gel filtration.

Based on our results, the selected host and optimized expression conditions are suitable for the high-yield biosynthesis of Buforin II, yielding adequate quantities for further studies.

GP4

HOW TO PRODUCE *CIS* 4-SUBSTITUATED CYCLOHEXYLAMINES USING ω-TRANSAMINASE AND PYRUVATE DECARBOXYLASE?

***Emese FARKAS* (farkas.emese@mail.bme.hu)[1] – *Dániel János INCZE* (inczedani96@gmail.com)[1] – *László NAGY-GYŐR* (nagy-gyor.laszlo@mail.bme.hu)[1] – *Péter SÁTORHELYI* (peter.satorhelyi@fermentia.hu)[3] – *Zoltán SZAKÁCS* (Z.Szakacs@richter.hu) – *János ÉLES* (J.Eles@richter.hu)[2] – *László POPPE* (poppe@mail.bme.hu)[1,4*] –*Gábor HORNYÁSZNKY* (hornyanszky@mail.bme.hu)[1]**

[1]*Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, , Szent Gellért tér 4., 1111 Budapest, Hungary*
[2]*Gedeon Richter Plc., PO Box 27, 1475, Budapest, Hungary*
[3]*Fermentia Ltd, Berlini u. 47-49., 1045, Budapest, Hungary*
[4]*SynBiocat Ltd, Lövőház u. 19, 1024, Budapest, Hungary*

In recent years, stereoselective synthesis of amines has received considerable attention due to their important role in the preparation of various active substances in pharmaceutical, chemical and agrochemical industry. In principle, *ω*-transaminase (*ω*-TA) as a PLP-dependent (pyridoxal-5-phosphate) enzyme can be used in an asymmetric synthesis of prostereogenic ketones to the corresponding amines[1]. The problem with the asymmetric synthesis is that the equilibrium of the reaction lies strongly on the side of substrate ketone.

The aim of our present study is to investigate the diastereomer selectivity of the ω-transaminase catalyzed transamination reaction of 4-substituted cyclohexanones to the corresponding amines. If instead of (*S*)-methylbenzylamine L-alanine is used as an amine donor then the equilibrium shift can be achieved by removing the co-product pyruvate with the enzyme pyruvate decarboxylase (PDC). The Trp60Cys mutant of the *ω*-TA from *Chromobacterium violaceum*[2,3] and PDC from *Lodderomyces elongisporus* in immobilized form could perform transamination of the 4-substituted ketones with excellent *cis*-diastereomeric selectivity (up to 95% *de*).

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GP5

qPCR METHOD FOR IDENTIFYING COPY NUMBER VARIATIONS IN CYP2C8 GENE

Evelyn GABRI (gabri.evelyn@ttk.mta.hu)[1] – Katalin MANGÓ (mango.katalin@ttk.mta.hu)[1] – Katalin TÓTH (toth.katalin@ttk.mta.hu)[1] – Máté DÉRI (deri.mate@ttk.mta.hu)[1] – Ádám KISS (kiss.adam.ferenc@ttk.mta.hu)[1] – Réka MEZEI (reka.mezei95@gmail.com)[1] – Judit MOLDVAY (drmoldvay@hotmail.com)[2] – Katalin MONOSTORY (monostory.katalin@ttk.mta.hu)[1]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok körútja 2, 1117 Budapest, Hungary
[2]National Korányi Institute for TB and Pulmonology, Pihenő út 1, 1122 Budapest, Hungary

Cytochrome P450 (CYP) enzymes are the key factors in drug-metabolism responsible for the biotransformation of >70% of the drugs in clinical use. CYP enzymes are highly polymorphic that contributes to the substantial inter-individual differences of drug-metabolism.
Clinically relevant variations in CYP genes containing single nucleotide polymorphisms generally produce non-functional CYP enzymes or enzymes with decreased activity. In tumorous cells, displaying genome instability, copy number variations (CNV) of drug metabolizing CYP genes may occur resulting in altered drug metabolism and therapy resistance.
Paclitaxel is a mitotic inhibitor used to treat a number of types of cancer, including breast, ovarian and non-small cell lung cancer. Paclitaxel is primarily metabolized by CYP2C8 and to a lesser extent by CYP3A4. Our aim was to develop a PCR method to identify the *CYP2C8* polymorphic alleles most frequently occur in Caucasian populations (*CYP2C8*3* and *CYP2C8*4*). The further aim was to develop a quantitative method for the identification of CNVs of *CYP2C8* using quantitative real-time PCR (qPCR). *CYP2C8*3* and *CYP2C8*4* loss-of-function alleles as well as CNVs of *CYP2C8* gene were determined in lung adenocarcinoma tissues.

GP6

IN VIVO AXONAL REGENERATION IS ACCELERATED BY NON-MUSCLE MYOSIN 2 ALLOSTERIC INHIBITION

***Ádám I. HORVÁTH* (horvath1@caesar.elte.hu)[1] – Demeter TÚRÓS (rockdeme@gmail.com)[1] – Áron ZSIGMOND (aron.zsigmond@yahoo.com)[2] – Máté VARGA (mvarga@ttk.elte.hu)[2] – András MÁLNÁSI-CSIZMADIA (malna@elte.hu)[1]**

[1]*Department of Biochemistry, Motor Pharmacology Research Group, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C., 1117 Budapest, Hungary*
[2]*Department of Genetics, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C., 1117 Budapest, Hungary*

Non-muscle myosin 2 (NM2) motor proteins play central roles in mechanical processes of the cell such as cytokinesis, migration and neuronal development. The NM2 inhibitor blebbistatin can induce neurite outgrowth *in vitro*. It has been a pivotal question whether *in vivo* axonal regeneration can be accelerated by NM2 inhibition. However, blebbistatin cannot be used *in vivo* because of its low solubility and non-specific cytotoxic effect. To overcome these drawbacks, we developed a non-cytotoxic and highly soluble blebbistatin derivative, para-aminoblebbistatin (neurelaxin), which maintained blebbistatin's inhibitory properties while lacking its adverse effects. By using neurelaxin, laser ablated Mauthner axon regeneration in zebrafish embryo was several times faster than with the vehicle control. These results demonstrate that novel NM2 inhibitors may serve as tool compounds in drug development.

GP7

PHYSIOLOGICAL ROLE OF MITOCHONDRIAL PERMEABILITY TRANSITION IN INFLAMMATORY REPROGRAMMING

Nikoletta KÁLMÁN* (knikoletta@gmail.com)[1] – Csenge ANTUS (csenge.antus@aok.pte.hu)[1] – Fruzsina FÓNAI (fruzsina.fonai@aok.pte.hu)[1] – Péter B. JAKUS (peter.jakus@aok.pte.hu)[1] – Éva BOROS (boros.eva88@gmail.com)[3] – Zoltán HEGEDŰS (hegedus@brc.hu)[4] – Krisztián ERŐS (krisztian.eros@aok.pte.hu)[1] – István NAGY (nagyi@seqomics.hu)[3,5] – László TRETTER (tretter@eok.sote.hu)[6] – Ferenc GALLYAS Jr (ferenc.gallyas@aok.pte.hu)[1,2,7] – Balázs SÜMEGI (balazs.sumegi@aok.pte.hu)[1,2,7] – Balázs VERES (balazs.veres@aok.pte.hu)[1]

[1]Department of Biochemistry and Medical Chemistry, Medical School, University of Pécs, Pécs, Hungary
[2]MTA-PTE Nuclear-Mitochondrial Interactions Research Group, Pécs, Hungary.
[3]Institute of Biochemistry Biological Research Centre of the Hungarian Academy of Sciences, 6726 Szeged, Hungary
[4] Institutes of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, 6726 Szeged, Hungary
[5]SeqOmics Biotechnology Ltd., 6782 Mórahalom, Hungary
[6]Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary.
[7]Szentágothai János Research Center, University of Pécs

Cyclophylin D (CypD)-regulated mitochondrial permeability transition (mPT) in its physiological low-conductance mode regulates mitochondrial membrane potential, matrix pH and Ca^{2+}, thereby adjusting energy production to match cell metabolic demand; in its high-conductance state, it is actively involved in the cell death process in various pathophysiological conditions. Here, we demonstrate for the first time that CypD-regulated mPT plays a critical role in bacterial lipopolysaccharide-induced gene expression reprogramming and tissue damage in endotoxemia using CypD-deficient mice. CypD-deficient mice had a 75% survival rate vs. 0% for the wild type. Additionally, the expression levels of 966 genes were markedly decreased in CypD-deficient mice, including several transcription factors associated with oxidative stress, inflammation and apoptosis, which were significantly altered in septic shock. We verified our key mRNA findings at the protein and/or functional levels. These results suggest a novel role for CypD-dependent mPT, namely, global modulation of inflammatory gene expression. This role may not be restricted to the inflammatory response, but may apply to other oxidative-stress-related disorders, such as cardiovascular, cerebrovascular and neurodegenerative diseases, which provides a novel target for therapeutic intervention.

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GP8

ANTI-TUMOR ACTIVITY OF METHOTREXATE FUNCTIONALIZED SILICA-GELATIN AEROGEL MICROPARTICLES

***Gábor KIRÁLY* (kiraly.gabor@science.unideb.hu)[1] – *József KALMÁR* (kalmar.jozsef@science.unideb.hu)[2] – *Péter VERES* (veres.peter@science.unideb.hu)[2]– *Adrienn FEHÉR* (adrienn112@gmail.com)[1] – *Patrícia GUBÓ* (plujzus@gmail.com)[1] – *Gábor SZEMÁN-NAGY* (szeman-nagy.gabor@science.unideb.hu)[1] – *István JUHÁSZ* (ijuhi@yahoo.com)[3]**

[1]*Department of Microbial Biotechnology and Cell Biology, University of Debrecen; Egyetem Square 1, 4032 Debrecen, Hungary*
[2]*Department of Inorganic and Analytical Chemistry, University of Debrecen; Egyetem Square 1, 4032 Debrecen, Hungary*
[3]*Deparment of Dermatology, University of Debrecen; Nagyerdei körút 98., 4032 Debrecen, Hungary*

Nowadays the different potential drug delivery systems are in the focus of medical biotechnology research. Aerogels are excellent candidates for drug delivery due to their properties, such as their huge specific surface area, open mesoporous structure and easily tunable surface characteristics. The pharmaceutical applications of aerogels were shown in numerous studies[1]. Applying these porous matrices both immediate and sustained drug release is feasible. In this study, we focused on a silica-gelatin hybrid aerogel, which is an excellent drug delivery system[2,3]. For biocompatibility investigations, a potential method is the *in vitro* time-lapse video-microscopy, which enables long-term dynamical observations. The well-characterized murine SCC cell line (SCC VII) was used for the in vitro studies. This squamous carcinoma cell line was isolated from the abdominal wall of the C3H mouse and has significant metastasis properties. The collagen containing silica-gelatin aerogel particles had no harmful effect on the SCC line, furthermore, the surface of the aerogel particle is suitable for the adhesion of the cells. In addition, we found that in the presence of aerogel particles the cells had a directed and faster movement towards the aerogel particle, probably due to the collagen content. In other experiments, we have investigated the anti-tumour effect of antineoplastic agent containing silica-collagen particles on the SCC VII cells. Methotrexate (MTX), which is a dihydrofolate-reductase inhibitor cytostatic drug[4], was bound covalently to the collagen of the silica-gelatin particles. The collagenase activity of the cancer cells liberated the MTX from the silica-collagen particles. The results of the viability tests showed that the MTX containing particles have the same effect on the SCC cancer cells as a free MTX in drug equivalent amounts. Based on our results these silica-gelatin particles can be potential drug-delivery systems in further *in vitro* and *in vivo* cancer experiments.

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GP9

CYP2C19 AND *CYP2D6* GENOTYPE-PHENOTYPE MISMATCH

Ádám Ferenc KISS* (kiss.adam.ferenc@ttk.mta.hu) – Dorottya VASKÓ (vasko.dorottya@gmail.com) – Cintia JUHÁSZ (cintijuhasz@gmail.com) – Máté Tamás DÉRI (deri.mate@ttk.mta.hu) – Katalin TÓTH (toth.katalin@ttk.mta.hu) – Katalin MONOSTORY (monostory.katalin@ttk.mta.hu)

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok 2, 1117 Budapest, Hungary

Genetic polymorphism of cytochrome P450s results in clinically significant modifications in patients' drug metabolizing capacities. CYP2C19 and CYP2D6 have crucial role in the elimination of several clinically important drugs (*e.g.* beta adrenergic blockers, psychopharmacons, proton pump inhibitors, antiplatelet drugs); however, the prediction of the phenotypic appearance of CYP2C19 and CYP2D6 is a challenge. Single nucleotide polymorphisms frequently occur in both *CYP2C19* and *CYP2D6* genes and, moreover, gene copy number variations (gene deletion and multiplication) also occur in *CYP2D6* gene; thus, enzyme activities particularly depend on the genetic factors.

Hepatic CYP2C19 and CYP2D6 activities (mephenytoin 4'-hydroxylation and dextromethorphan O-demethylation, respectively) as well as *CYP2C19* and *CYP2D6* genotypes for the most frequent allelic variants (*CYP2C19*2, *3, *4, *17* and *CYP2D6*3, *4, *5, *6, *10, *41*, duplication , respectively) were determined in human liver samples, then genotype-phenotype associations were investigated. Finally, clinical data of the donors were considered in the genotype-based phenotype prediction.

Substantial inter-individual variations were observed in both CYP2C19 and CYP2D6 metabolic activities. The underestimation of CYP2D6 phenotypes was assumed to be originated from the overlapping ranges of CYP2D6 activity among similar diplotypes or from the presence of *-1584C>G* in the promoter region evoking increased transcription of the wild-type *CYP2D6* allele. Underestimation of CYP2C19 phenotype could be explained by medication which could be accountable for the activation of nuclear receptors (CAR/PXR or GR). In appreciable number of donors, the genotype-based phenotype prediction was overestimated in the case of both CYP2C19 (47% of the donors) and CYP2D6 (20.3%) because of rare allelic variants which were not included or some external factors that could alter CYP2C19 and CYP2D6 activities (medication with substrate/inhibitor) and hepatic function (amoxicillin-clavulanic acid therapy, chronic alcohol consumption, disease).

CYP2C19 and *CYP2D6* genotypes only partially determine the phenotypic appearance; co-medication, diseases with inflammation processes and aspecific factors, such as chronic alcohol consumption and amoxicillin-clavulanic acid therapy seem to be potential phenotype-modifying factors.

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GP10

SOL-GEL IMMOBILIZATION OF A MUTANT ω-TRANSAMINASE FROM *CHROMOBACTERIUM VIOLACEUM*

Zsófia MOLNÁR* (molnar.zsofia@mail.bme.hu)[1,2] – Szabolcs KOHÁRI (szabolcs.kohari@fermentia.hu)[2] – Péter SÁTORHELYI (peter.satorhelyi@fermentia.hu)[2] – Viktória BÓDAI (viktoria.bodai@fermentia.hu)[2] – Diána BALOGH (dweiser@mail.bme.hu)[1] – László POPPE (poppe@mail.bme.hu)[1]

[1]*Department of Organic Chemistry and Technology, Budapest University of Technology and Economics, Műegyetem rkp. 3., 1111 Budapest, Hungary*
[2]*Fermentia Microbiological Ltd.; Berlini str. 47-49., 1045 Budapest, Hungary*

Transaminases are typical pyridoxal 5'-phosphate (PLP)-dependent enzymes which are ubiquitous in nature being responsible for transferring amino groups. Most transaminases exclusively transfer the amino group of α-amino acids. However, a special subgroup of these enzymes, the ω-transaminases can transfer amino groups from carbon atoms that do not bear a carboxyl group. This capability can be used to produce a wide variety of optically pure amines, which are highly valuable for the pharmaceutical industry (e.g. sitagliptin, valinol, unnatural amino acids, 3-arylGABA derivatives).

Although enzymes offer a green alternative against conventionally used catalyzators in organic chemistry, their low stability and the difficulties in their recovery hinder their widespread industrial application. Enzyme immobilization methods offer several potential improvements, such as easier handling, efficient enzyme recovery and recycling and higher productivities. The entrapment of biocatalyzators in silica sol-gel matrices is a suitable method for this purpose, since silica is biocompatible and has excellent mechanical properties. Furthermore, the sol-gel matrices are capable of preventing the diffusion of the enzyme and stabilizing the enzyme structure, thereby improving the stability of the catalyzator.

In our work, we immobilized an engineered ω-transaminase mutant Trp60Cys from *Chromobacterium violaceum* in silica sol-gel matrices. The effect of several additives to the sol-gel matrices and the stability of the preparations were examined. The activity of the immobilized enzymes was evaluated in kinetic resolution of several racemic amines.

GP11

EXPLORATION OF FUNCTIONAL MECHANISMS OF ELAFIN, A SERINE PROTEASE INHIBITOR, BY DIRECTED EVOLUTION

Bálint Zoltán NÉMETH ***(nemeth.b93@gmail.com) – Gábor PÁL (gabor.pal@ttk.elte.hu)***

Department of Biochemistry Faculty of Science, ELTE Eötvös Loránd University,; Pázmány Péter sétány 1/c, 1117 Budapest, Hungary

Elafin is one of the most intensively studied canonical protease inhibitors. This small protein is expressed by epithelial and immune cells and is secreted into the inter- and extracellular space. There, it controls inflammatory processes by inhibiting neutrophil serine proteases (leucocyte elastase and proteinase 3). Moreover other anti-inflammatory and antibacterial functions of elafin have also been described. Based on these properties elafin is a promising candidate to treat inflammatory diseases. In order to better understand the mechanisms of the interaction between the inhibitor and the serine proteases, we subjected elafin to directed evolution. By randomizing the amino acids of the canonical enzyme-binding loop, we created billions of elafin variants, and expressed them on the surface of phage particles. We selected the phage-library against two of the most thoroughly characterized serine protease model enzymes (bovine chymotrypsin A and porcine elastase). By comparing the sequence patterns of the two variant pools capable of binding to the individual enzymes we could assess the function of each randomized amino acid position. Positions, which have evolved to the same direction independent of the target enzyme, most likely play a role in stabilizing the canonical structure of the inhibitory loop. On the other hand, positions that evolved in an enzyme-dependent manner could be responsible for enzyme-selectivity. In all, these results outline the “functional anatomy” of the protease-binding surface of elafin.

GP12

RAPID NEURITE OUTGROWTH CAN BE TRIGGERED BY BIOLOGICALLY SAFE NON-MUSCLE MYOSIN 2 INHIBITORS IN HUMAN NEURONS.

Máté PÉNZES ***(matthew.penzes@gmail.com) – Máté GYIMESI (mate.gyimesi@ttk.elte.hu) – András MÁLNÁSI-CSIZMADIA (malnalab@yahoo.com)***

Department of Biochemistry, Motor Pharmacology Research Group, ELTE Eötvös Loránd University, Budapest, Hungary

Blebbistatin, a myosin 2 specific inhibitor, has been implicated *in vitro* in having effects on neurons by promoting dendritic and axonal cone growth as well as resulting in dynamic filopodia-like spine precursors. Due to low solubility, chemical instability, high fluorescence and cytotoxicity of blebbistatin, its prolonged effects on neurons cannot be studied. Recently, our laboratory developed para-nitroblebbistatin (pNBleb) and para-aminoblebbistatin (pAminoBleb or Neurelaxin) which have the same target specificity as blebbistatin but without any significant adverse effects. Hence our efforts were aimed to examine the effects of these NM2 inhibitors on living human neuronal cells. Our experiments were conducted on neural stem cells which were differentiated into neurons. The quantitative analysis of neurite outgrowth revealed that direct myosin 2 inhibition by the newly developed blebbistatin derivatives can cause significant dendritic and axonal architectural changes in human neurons.

MP1

XYLITOL FERMENTATION ON WHEAT BRAN HYDROLYSATE BY USING *OGATAEA ZSOLTII*

***Botond ANTAL* (botond_antal@msn.com) – Csaba *FEHÉR* (csaba_feher@mail.bme.hu)**

Biorefinery Research Group, Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics. Műegyetem rkp. 3. 1111 Budapest, Hungary

Biorefinery technologies can offer a sustainable way to produce a wide range of marketable bioproducts and bioenergy from the extensively available lignocellulosic residues. This study focuses on the production of xylitol - a valuable sugar alcohol – by fermentation from wheat bran hydrolysate. A two-step acidic fractionation of wheat bran was applied to obtain an arabinose-rich liquid fraction, a xylose-rich liquid fraction and a cellulose-rich solid fraction. The xylose-rich liquid fraction was further utilized for xylitol fermentation in benchtop fermenter by *Ogataea zsoltii*, a facultative methylotroph yeast. Xylitol fermentation from xylose is a one-step reduction, which generally requires microaerobic conditions. In order to achieve high xylitol yield, the effect of oxygen supply was first investigated using xylose-containing model medium with four different OTR settings in benchtop fermenter. Focusing on the attainable xylitol yield, the tested OTR values ranged from 2.6 mmol l^{-1} h^{-1} to 3.9 mmol l^{-1} h^{-1}. A maximum xylitol yield was achieved in the case of 3.5 mmol l^{-1} h^{-1} OTR. The most favorable OTR was applied during the fermentation on the wheat bran hydrolysate, resulting in a promising xylitol yield of 52.3% of theoretical. In conclusion, wheat bran was found to be a highly potential raw material for xylitol fermentation and could serve as a raw material for the production of other valuable products integrated within the same process.

MP2

INVESTIGATION OF A PUTATIVE COPPER AND CADMIUM ION TRANSPORTER IN *ASPERGILLUS NIDULANS*

Imre BOCZONÁDI* (boczonadi.imre@science.unideb.hu)[1] – Beatrix CSERNAI (csernaibeatrix1994@gmail.com)[1] – Klaudia GYURCSÓ (gyurcsoklaudiadoci@gmail.com)[1] – Gábor KÓNYA (konyabiom@gmail.com)[1] – Éva LEITER (leiter.eva@science.unideb.hu)[1] – Ágnes JAKAB (jakab.agnes@science.unideb.hu)[1] – Mi-Kyung LEE (mikyung.lee@wisc.edu)[2]– Jae-Hyuk YU (jyu1@wisc.edu)[2] – István PÓCSI (pocsiistvan@unideb.hu)[1]

[1]*Department of Biotechnology and Microbiology, Faculty of Science and Technology University of Debrecen, Egyetem tér 1., 4032 Debrecen, Hungary*
[2]*The University of Wisconsin, Madison, WI 53706, USA.*

Fungi have several protein families to maintain copper homeostasis, including copper-binding transcription factors and copper transporters. The latter transporters are required to survive in low or high copper ion environments. To the best of our knowledge, there is still no information available on how copper transporters participate in the acquisition and detoxification of copper in the model filamentous fungus *Aspergillus nidulans.*

In this study, we characterized the locus AN3117, which is predicted to encode a copper and cadmium ion transporter in *A. nidulans*. The prototrophic TNJ36 strain (genotype: *pyrG89 AfpyrG*$^{+}$ *pyroA4 veA*$^{+}$) was used as the control strain. Asexual spores of the mutant and control strains were collected from the cultures grown on *Aspergillus* minimal medium (AMM) containing 0.05% (v/v) pyridoxin at 37 °C for 6 days. Growth and stress sensitivity assays were also performed on solid AMM, which were supplemented with 0.1- 2.5 mM $CdCl_2$ and 0.025- 0.2 mM $CuCl_2$, and incubated at 37 ºC for 5 days. We performed experiments for each stress condition in three sets. Fungal growth was determined by colony diameters, and the stress sensitivities were quantified by percentage decreases in the colony growth in comparison to the control cultures. Somewhat surprisingly, colony growth was minimally influenced by the deletion of AN3177 under non-stressed conditions, where the ΔAN3117 mutant showed slightly decreased growth (~10% decrease in colony diameter) in comparison to the control strain. On the other hand, the ΔAN3117 mutant displayed significantly increased sensitivity to 0.1 mM cadmium (~30% growth inhibition). When grown on AMM with 0.75 mM $CdCl_2$, the control and mutant strains showed reduced colony growth approximately 80 and 90%, respectively. Conversely, the deletion of AN3117 resulted in a significantly increased tolerance to heavy metal stress initiated by 0.1 mM $CuCl_2$ (15% growth stimulation), whereas 0.15- 0.175 mM $CuCl_2$ blocked the sporulation of the mutant cultures. The mutant strain showed decreased growth at 0.175 mM $CuCl_2$ concentration (~50% less in colony diameter) in comparison to the control strain, and exposure to 0.2 mM Cu^{2+} inhibited completely the growth of the ΔAN3117 mutant. Our findings suggest that the AN3117-encoded transporter plays a distinct role in responding to cadmium and copper heavy metals.

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MP3

THERMOMYCES LANUGINOSUS IN THE BIODEGRADATION OF AGRO-INDUSTRIAL MATERIALS

***Csilla FARKAS* (Csilla.Farkas@etk.szie.hu) – Judit M. REZESSY (Szabo.Judit@etk.szie.hu) – Quang D. NGUYEN (Nguyen.Duc.Quang@etk.szie.hu)**

Department of Brewing and Distilling Faculty of Food Science; *Research Centre for Bioengineering and Process Engineering, Szent Istvan University,Ménesi street 45., 1118 Budapest Hungary*

Ten strains of the thermophilic fungus *Thermomyces lanuginosus* from different culture collections, namely ATCC 16455, ATCC 84400, ATCC 36350, IMI 140524, IMI 131010, IMI 110803, IMI 096218, DSM 5826 and CBS 288.54a, CBS 395.62b were investigated for their ability to degrade various lignocellulosic substrates, as wheat bran, wheat straw, wood chips and brewers' grains. The strains were cultured on these substrates for 2-week period to simulate pre-treatment process. Among investigated strains, *T. lanuginosus* ATCC 16455 exhibited the best results on wheat bran within 7 days of solid state pre-treatment. The maximum reducing sugar was 7.27 g/L. Wheat bran seemed to be the most easily biodegradable substrate and the best inducer for production of extracellular xylanase and other hydrolytic enzymes. Maximum xylanase activity was 326.04 U g^{-1}, while CMC-ase activity and β-glucosidase activity were 47.44 U g^{-1} and 57.12 U g^{-1}, respectively. The moisture level between 60% and 70%, pH around 6.5 and 65 °C were determined as optimum conditions for microbial degradation. Effects of supplement of different carbon sources such as glucose, fructose, maltose, sucrose, soluble starch as well as nitrogen sources as ammonium sulphate, ammonium nitrate, meat extract, peptone, yeast extract on released soluble sugars were investigated. Addition of ammonium sulphate and maltose resulted 10% and 5% increase in reducing sugar content, respectively, while addition of the others did not show any significant change in sugar content or production of the xylanolytic enzymes. Our results were preliminary but may provide good possibility to exploit the *T. lanuginosus* filamentous fungi in development of microbial pretreatment of lignocellulosic raw materials.

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MP4

IDENTIFICATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) BY MALDI-TOF MS METHOD

***Brigitta HORVÁTH* (horvath.brigitta920108@gmail.com)[1] – Ferenc PELES[1] – László BERECZKI[2] – Ágnes ERŐS[3] – Rita SIPOS[3] – Csaba LOVÁSZ[4] – Adrienn MICSINAI[4]**

[1]*Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Böszörményi str. 138. 4032 Debrecen, Hungary*
[2]*MikroMikoMed Ltd. Lajos str. 74-76. 1036 Budapest, Hungary*
[3]*Biomi Ltd. Szent-Györgyi Albert str. 4., 2100 Gödöllő, Hungary*
[4]*WESSLING Hungary Ltd. Anonymus str. 6. 1045 Budapest, Hungary*

Infections caused by MRSA in the hospital or other associated environments can often lead to severe or in extreme cases to lethal consequesnces. However, these microbes can also be isolated from community and livestock associated sources. In order to be able to identify these multiresistant microbes faster, cheaper and more reliably in a routine diagnostic laboratory we aimed to develop a method that accomplish this goal by using the MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) technique.

The MALDI TOF MS technique is based on coupling a laser ion source and a time-of-flight mass spectrometer. The energy of the laser emitted is absorbed by the matrix which in turn ionises the target compounds of the sample and thus they can enter the vacuum of the mass spectrometer and finally reach the detector. The obtained mass spectra provide information on the protein and macromolecule profiles of the sample. This fingerprint pattern serves as a base for the routine identification of the microbes, compared to a validated database. Also, this fingerprint pattern can potentially be used to distinguish between MRSA and MSSA (methicillin sensitive *Staphylococcus aureus*) isolates, by aiming to uncover markers or marker sets which can reliably differentiate such microorganisms from a wide variety of sources, including isolates from clinical, livestock, food, feed or environmental sources.

The method development includes an assessment of the effects of the culture media and age of cultures, sample preparation (direct mounting, ethanol/formic acid), and the use of different MALDI matrices (HCCA, SA, DHB) on the obtained mass spectra. The strains used are characterised biochemically and genetically by 16S rDNA sequence and MLST (multi locus sequence typing) techniques.

MP5

TYROSOL INFLUENCES THE PHYSIOLOGY AND VIRULENCE OF *CANDIDA PARAPSILOSIS*

Ágnes JAKAB* (jakab.agnes@science.unideb.hu)[1] – Fruzsina NAGY (nagyfru@freemail.hu)[2] – Zoltán TÓTH (tothi5@hotmail.com)[2] – Dániel NEMES (nemes.daniel@pharm.unideb.hu)[2] – László MAJOROS (major@med.unideb.hu)[2]– Ildikó BÁCSKAY (bacskay.ildiko@pharm.unideb.hu)[2] – István PÓCSI (pocsiistvan@unideb.hu)[1] – Renátó KOVÁCS (kovacs.renato@med.unideb.hu)[2]

[1]*Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen; Egyetem tér 1., 4032 Debrecen, Hungary*
[2]*University of Debrecen, Nagyerdei krt. 98., 4032 Debrecen, Hungary*

Tyrosol has a pivotal role in fungal morphogenesis because it stimulates germ tube formation in yeast cells and hyphae development in the early stage of biofilm at least for *Candida albicans*. Additionally, it may have inhibitory effect at supraphysiological concentration, which is well-documented in case of *C. albicans*. However, the effect exerted by tyrosol has remained unknown so far among non-albicans species (e.g.: *C. parapsilosis*) regarding physiological properties and virulence. Hence, the aim of our experiments was to investigate the effect of tyrosol against *C. parapsilosis* with regard to morphology, cytotoxicity on intestinal epithelial cells and pathogenicity.

In our experiments to determine the effect of tyrosol on growth, CLIB214 *C. parapsilosis* reference strain was grown overnight in YPD medium and diluted to an A_{640} of 0.2 at 37 ºC. The YPD cultures were supplemented 10-15 mM tyrosol at 4 h incubation time. Growth was monitoring either by counting cells or by measuring A_{640} while the morphological forms were recorded by phase-contrast microscopy. Growth experiments demonstrated that tyrosol at supraphysiological concentration (15 mM) significantly inhibits the growth of *C. parapsilosis* but had no effect on the morphological transition. Counting viable cells showed that growth is markedly inhibited within 2 hours of the adding of tyrosol. Based on our two cytotoxicity assays (MTT assay and Real Time Cell Electronic Sensing method), 15 mM tyrosol had no any cytotoxic effect on Caco-2 cells; therefore, the usage of tyrosol in alternative antifungal therapies to be considered, which was confirmed using invasive candidiasis mouse model.

Our findings may result new biofilm-related alternative treatment in the future.

MP6

MARJORAM ESSENTIAL OIL: CHANGES IN COMPOSITION AND EFFECTIVENESS AGAINST BACTERIAL BIOFILMS

***Erika Beáta KEREKES* (kerekeserika88@gmail.com)[1] – Csilla GÖMÖR[1] (gmri.csilla@gmail.com)[1] – Anita VIDÁCS (anitavidacs@gmail.com)[1,3] – Elvira NACSA-FARKAS (elvirafarkas@gmail.com)[1] – Andrea BÖSZÖRMÉNYI (aboszormenyi@gmail.com)[2] – Nadeen Moustafa ZOUABI (nadeenzouabi99@gmail.com)[1] – Csaba VÁGVÖLGYI (csaba@bio.u-szeged.hu)[1] – Judit KRISCH (krisch@mk.u-szeged.hu)[3]**

[1]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Közép fasor 52., 6726 Szeged, Hungary
[2]Department of Pharmacognosy, Semmelweis University, Üllői út 26. 1085 Budapest, Hungary
[3]Institute of Food Engineering, Faculty of Engineering, University of Szeged, Mars tér 7., 6724 Szeged, Hungary

Essential oils (EOs) are hydrophobic liquids originated from plant materials produced mainly by steam distillation. Their use as aroma compounds is frequent and due to their good antimicrobial effect they could also provide natural preservation of foods. The yield and quality of an EO depends on its composition which is influenced by edaphic and climatic conditions under which the plants grow. Due to these factors chemical composition of EOs can be highly variable, thus it is important to optimize both growing conditions and the genotype. Biofilm formation is common among bacteria; in this form they become more resistant to antimicrobials and sanitizing agents compared to planktonic cells. They can be present on almost any surface and can easily contaminate food products leading to spoilage. The aim of our study was to examine if changes in the composition of marjoram (*Origanum majorana*) EO influenced its antimicrobial and anti-biofilm forming efficiency. Biofilm formation of food spoilage bacteria and food borne pathogens were included in the study. Marjoram EO was used in two compositions representing two different years of harvest (2013 and 2014). The composition of the oils was determined by GC-MS, minimal inhibitory concentrations (MIC) by microdilution and biofilm formation by crystal violet staining. GC-MS measurements showed changes in the composition of the oil: in 2013 the major component was terpinene-4-ol (33.5%) and in 2014 this changed to p-cymene (21.2%). MIC concentrations were significantly higher for the oil from 2014 in all cases and leading to changes in inhibition of biofilm formation. Compared to the oil from 2013 the efficiency of the EO decreased. This can be a result of the accumulation of the less effective component p-cymene. Another important conclusion of our finding is that, as EO compositions cannot be standardized, MIC values and anti-biofilm forming effects need to be established for every new batch of EO.

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MP7

CHARACTERIZATION OF A GLYCEROL 3-PHOSPHATE DEHYDROGENASE GENE DELETION STRAIN OF THE FILAMENTOUS MODEL FUNGUS *ASPERGILLUS NIDULANS*

***Anita KIRÁLY* (kiraly.anita@science.unideb.hu) – Anna MOLNÁR (ann.molnar92@gmail.com) – Éva LEITER (leiter.eva@science.unideb.hu) – István PÓCSI (pocsiistvan@unideb.hu)**

Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Egyetem square 1., 4032 Debrecen, Hungary

Glycerol 3-phosphate dehydrogenase (G3PDH) is an essential enzyme in glycerol biosynthesis because it is responsible for the conversion of dihydroxyaceton phosphate to glycerol 3-phosphate, which is thereafter converted into glycerol. Two homologous G3PDH isoforms exist in the budding yeast *Saccharomyces cerevisiae*[1] In *Aspergillus nidulans*, there are also two isoforms of G3PDH (*gfdA, gfdB*), and this study is focused on the phenotypic characterization of the Δ*gfdB* gene deletion strain of *Aspergillus nidulans*. The *gfdB* (AN6792) gene was deleted by the Double-Joint PCR method[2].
The *ΔgfdB* strain showed reduced growth on minimal medium at 37°C, higher sensitivity to oxidative stress inducing agents diamide, *tert*-butyl hydroperoxide (*t*BOOH) and hydrogen-peroxide. As oxidative stress response has been connected to secondary metabolite production, we also quantified and compared the sterigmatocystin productions of the strains tested, and significant increases in the mycotoxin production have been found in the mutant strain. Significant differences were also observed in the reactive species productions of the strains; it was higher in the *ΔgfdB* strain as compared with the wild-type either with or without 0.6 mM *t*BOOH addition. Some antioxidant enzyme activities, including glutathione peroxidase (GPx), glutathione reductase (GR), catalase and superoxide dismutase (SOD) were measured to study the oxidative stress defense systems of the mutant and control strains. The deletion of the *gfdB* gene increased the specific SOD and GR activities, while the GPx and the catalase activities were lower than those found in the control strain. In addition, the specific SOD, catalase and GPx activities decreased in the mutant strain under *t*BOOH exposures.
Currently, we use NMR-based metabolomics to map the changes in the metabolite fluxes through the glycolytic and glycerin biosynthetic biochemical pathways caused by the elimination of *gfdB.*

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MP8

IMMOBILIZATION OF MORTIERELLA ECHINOSPHAERA LIPASE ENZYME ON ACCUREL MP1000 SUPPORT

Alexandra KOTOGÁN* (primula15@gmail.com) – *Tamás PAPP* – *Csaba VÁGVÖLGYI* – *Miklós TAKÓ

Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Közép-fasor 52., 6726 Szeged, Hungary

Immobilization of enzymes can improve their activity and stability and allow their reusability in multiple reactions. Therefore, enzyme-support complexes are applicable more economically in the industry than free enzyme preparations. Earlier studies revealed that Accurel MP1000 polypropylene matrix (particle size < 1500 μm) could be suitable as solid support for lipase immobilization due to the adsorption occur by hydrophobic interaction which makes the process very selective and efficient.

In this study, immobilization of the previously purified extracellular lipase enzyme from the oleaginous fungus *Mortierella echinosphaera* on Accurel MP1000 as support was carried out. The polypropylene matrix was stirred in 50% ethanol for 30 min to remove air from the support pores, then particles were filtered and washed with distilled water. Activated support was mixed with enzyme solution having 33.4 U hydrolyzing activity in 25 mM phosphate buffer (pH 6.8) and the mixture was incubated under gentle shaking (150 rpm) at 4 °C for 24 h. After incubation, the enzyme-carrier complex was filtrated and washed. The adsorption was monitored by measuring the residual hydrolytic activity in the supernatant, and immobilization efficiency (IE, %) and activity yield (AY, %) were calculated. Time course of the adsorption showed very fast initial loading of the lipase onto the support, within the first 8 hours of incubation. IE proved to be above 90% after the full 24 hours of incubation, indicating high affinity of the lipase to interact with the hydrophobic surface. The enzyme-support preparation showed AY of 29.9%, and 0.2 and 0.07 U/mg of support specific activities for *p*NPP hydrolysis and transesterification reactions, respectively. Investigating the reusability of the immobilized lipase complex indicated its high stability up to 5 cycles; resulting 83% and 59% residual hydrolytic and transesterification activities after the fifth reaction, respectively. Besides, improved storage stability of the enzyme-carrier complex was also displayed at 5 °C. Based on our preliminary tests, higher optimal temperature of the activity and enhanced thermal stability are assumed by the immobilized lipase compared to the free enzyme.

This is the first report describing the successful immobilization of a *Mortierella* lipase to a support, after which it remained stable and active in both aqueous and water-free environment. Our results confirmed the applicability of Accurel MP1000 as solid support for immobilization of the *M. echinosphaera* lipase, and the industrial potential of the complex.

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MP9

SECONDARY METABOLITE PRODUCTION BY ATOXINOGENIC AND TOXIGENIC ASPERGILLUS FLAVUS ON CORN KERNELS

***Szilvia KOVÁCS* (kovacs.szilvia@agr.unideb.hu) – Tünde PUSZTAHELYI (pusztahelyi@agr.unideb.hu)**

Central Laboratory of Agricultural and Food Products, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Böszörményi út 138. , 4032 Debrecen, Hungary

Aflatoxins are secondary metabolites produced by filamentous fungi. Many chemically modified compounds of aflatoxins are known, for example aflatoxin B1, B2, G1, G2 and aflatoxin M1 occurring in milk. Their strong physiological effects on humans and animals are known: carcinogenic, immunosuppressive, mutagenic, teratogenic compounds, and the most dangerous is aflatoxin B1. Mainly *Asperillus flavus* and *Aspergillus parasiticus* filamentous fungi produce it, but other *Aspergillus* and some *Rhizopus* strains are also well-known producers of aflatoxins. Principally aflatoxin contamination of corn causes the highest economic damage worldwide, but other seeds, spices, cottonseeds may also contain aflatoxin. One of the new possibilities to control aflatoxin contamination is the biocontrol, by the help of atoxigenic *Aspergillus flavus* that prevents the aflatoxin productions of toxigenic strains on agricultural fields. In our experiments we isolated *Aspergillus flavus* strains from different feedstuff and identified them by PCR. We selected aflatoxin B1 producing and non-producing *Aspergillus flavus* strains from our isolates. These were inoculated on autoclaved corn kernels, and we examined metabolites produced by the fungi using HPLC-MS detection. By our results we can conclude that the most significant difference between the compounds produced by the toxigenic and atoxigenic isolates was that the atoxinogenic *Aspergillus flavus* strains produced speradine F. Other fungal secondary metabolites were not detected.

The danger of the aflatoxins contamination is increasing in Hungary today, because of the rising average temperature and extreme weather conditions. We concluded that monitoring of atoxigenic *Aspergillus flavus* secondary metabolite production is necessary prior to use in biocontrol and important as a new information base of fungal secondary metabolite production.

MP10

STUDY OF GROWTH CURVES AND INHIBITION OF *E.COLI* APPLYING IMPEDIMETRIC METHOD

***Dóra KOZÁK* (kozakdori@gmail.com)[1] – *Áron NÉMETH, DR. (naron@f-labor.mkt.bme.hu)[1] – *Tamás NEMCSOK (tamas002@hotmail.com)[2]**

[1]Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Szt Gellért tér 4., 1111 Budapest Hungary
[2]Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Budafoki str. 8. F.III. building, 1111 Budapest Hungary

Escherichia coli is one of the most widely known and used microorganism in modern biotechnology. It has a very short generation time, which makes it ideal for quick, successive experiments in lab scale and even good productivity in larger scales.

During our experiments, we examined *E.coli* strain's reproduction with an innovative impedimetric method, using BacTrac monitor. This method has such advantages, like it allows us to measure up to 40 parallel samples in small volumes, which makes this method fast, effective and economical.

We examined different substances, whether they can be utilized and degraded by the microorganisms in minimal media or have any antimicrobiotic effects. Antibiotic effects of unknown samples are compared with standard and well-known antibiotic compound.

MP11

FERMENTATION OF PINEAPPLE JUICE BY SOME PROBIOTIC *LACTOBACILLUS* SP.

***Bao Toan NGUYEN* (toannguyen1905@gmail.com) – Erika BUJNA (Bujna.Erika@etk.szie.hu) – Anh M. TRAN (tranthimaianh_2807@yahoo.com) – Quang D. NGUYEN (Nguyen.Duc.Quang@etk.szie.hu)**

Department of Brewing and Distilling, Faculty of Food Science, Szent István University, Ménesi út 45, 1118 Budapest, Hungary

Fruit juices contain essential nutrients such as vitamins, minerals, antioxidants which have a health-promoting effect for human body. They are also proved to be good media for growth of probiotic bacteria, therefore they could serve as a great alternative to milk in some of traditional *probiotic* dairy *products, which* cannot be accepted by a *consumer suffered from lactose intolerance or allergic to milk protein*. In this study, lactic-fermentation of pineapple juice was focused. Three probiotic lactobacilli strains *Lb. acidophilus La*-5, *Lb. casei*-01 and *Lb. acidophilus La*-150 were selected to study their ability to grow in pineapple juice and survive during storage and through the model of gastro-intestinal tract. The *fermentation* period lasting *from 16 to 24 h* at 37 ºC to reach the pH 4.0. The growths of *Lb. acidophilus La*-5 and *Lb. casei*-01 were more quickly than *Lb. acidophilus La*-150 in pineapple juice. The fermentation was initiated with cell concentrations around 10^7 cfu ml^{-1} and reached the maximal concentration of 10^9 cfu/ml at the 16^{th} of fermentation, while the pH decreased from pH 6.3 to pH 4.0. The *Lb. acidophilus La*-5 strain exhibited the fastest utilization of sugar and reduction of pH in the pineapple juice comparing to the other strains. The ferric ion reducing antioxidant power (FRAP) and total phenolic content (TPC) of the fermented juice decreased by 32% and 7%, respectively, after 24 h of incubation with *Lb. acidophilus La*-150. Conversely, *Lb. acidophilus La*-5 and *Lb. casei*-01 showed no significant decrease in FRAP during 16^{th} hour of fermentation and remained above 2.7 mM $FeSO_4$/ml. After fermentation, the resistance of lactobacilli to simulated gastro-intestinal (GI) conditions were studied. *Lb. acidophilus La*-150 has the average 1.7 log cfu ml^{-1} given higher viability losses than the *Lb. acidophilus La*-5 (0.1 log cfu ml^{-1}) when exposed for 135 minutes in gastric fluid (pH 2.0) containing pepsin in concentration of 0.3%, followed 2.5 h incubation in presence of 0.6% bile salts. It can be concluded that all investigated *Lactobacillus* sp. strains are able to grow on pineapple juice without any supplementation of nutrients. These results provide good bases for development of technology for the production of probiotic drink with high antioxidant activity.

Keywords: probiotics, pineapple, lactic acid bacteria, fermentation, probiotic drinks

MP12

IMPEDIMETRIC STUDY OF PROBIOTIC BIFIDOBACTERIA

***Krisztina OLÁH* (olahkriszta95@gmail.com) – *Áron NÉMETH, dr* (naron@f-labor.mkt.bme.hu)**

Department of Applied Biotechnology and Food Science, Faculty of Chemical technology and Biotechnology, Budapest University of Technology and Economics, Budafoki str 6-8., 1111 Budapest, Hungary

Whey is a highly polluting by-product of cheese and casein powder-production. Our purpose was to produce probiotic on waste whey.

$190*10^6$ tons of whey is produced per year all over the world, and this number is still increasing. Whey was generally considered as environmentally damaging by-product, but in the last decades, many researchers tried to find a way to utilize it, rather than just dispose it. In the last 50 years, the number of biotechnological applications of whey increased, but high amount of this dairy side product still gets into the wastewater untreated as industrial waste. This is dangerous because of its high biochemical oxygen demand (40,000–60,000 mg l^{-1})[1].

In Hungary this is also a significant problem, of which solution may be the utilization of waste whey as nutrient for the fermentation of probiotic bacteria. Probiotics are microorganisms, which offer health benefits to the host by improving the original intestinal flora, so their production is important for health-protecting, as well. Growing and/or activity of probiotic bacteria can be enhanced by prebiotics[2]. This aspect will also be an important part of our work.

Our purpose was to test the fermentation of Hungarian waste whey by probiotic bacteria, and to find the limiting components of the fermentation. Another purpose was to find a fast and simple method for determining the prebiotic effect. Major part of the experiments were done in an innovative impedimetric equipment called BacTrac.

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[2]He *et al*., 2011 International Conference on Human Health and Biomedical Engineering August 19-22, 2011, Jilin, China

MP13

TECHNO-ECONOMIC EVALUATION OF A BIOREFINERY PRODUCING XILYTOL, ARABINOSE AND BIOGAS FROM WHEAT BRAN

Bettina PASZERBOVICS ***(paszerbovics.betti@gmail.com) – Csaba FEHÉR (csaba_feher@.mail.bme.hu)***

Departmentof Applied Biotechnology and Food Science. Biorefinery Research Group, Budapest University of Technology and Economics. Műegyetem rkp. 3. 1111 Budapest, Hungary

In the biorefinery concept biomass is used as an input to produce a wide variety of bio-based products and/or bio-based energy. In this study wheat bran was used as an input, since it is an abundant lignocellulosic by-product coming from the milling industry. It should be emphasized that it is not only available in high volume but also rich in carbohydrates which makes it a promising raw material for biorefining.

In this study techno-economic evaluation of a biorefinery producing arabinose, xylitol and biogas from wheat bran was performed by Aspen Tech. software. The models are based on literature data and laboratory experimental results of our Biorefinery Research Group. The simulated biorefinery plant consists of the following process steps: two-step acidic fractionation of wheat bran, yeast-mediated biopurification of the arabinose-rich hydrolysate, purification of arabinose solution, arabinose crystallization, purification of the xylose-rich hydrolysate, xylitol fermentation, purification of xylitol solution, xylitol crystallization and anaerobic digestion of organic residues. The two-step acidic fractionation results in an arabinose-rich supernatant, a xylose-rich supernatant and a cellulose-rich solid fraction. The arabinose-rich fraction is utilised to produce arabinose and the xylose-rich fraction is used to produce xylitol, while the cellulose-rich solid fraction and the residual organic streams are used to produce biogas. The produced biogas, or part of that, is burned to generate heating steam, covering the heat demand of the process steps. Heat integration of the process steps results in significant decrease in the amount of heating steam required. Thus heat integration within the process has a major role in terms of the technologic and economic feasibility of the process. The economic feasibility of the process also strongly influenced by the purity of crystalline arabinose and thus by the price of that.

Wheat bran was found to be an appropriate raw material to produce high-value bioproducts (arabinose and xylitol) and bioenergy (biogas) through a biorefinery process having a great potential to be economically viable.

MP14

EFFECTS OF COMMERCIAL *SACCHAROMYCES CEREVISIAE* STRAINS ON FERMENTATION OF PEAR JUICES AND PRODUCTION OF VOLATILE COMPOUNDS

Tuan M. PHAM (phamminhtuan1508@gmail.com) – Réka VARJÚ (Varjureka95@gmail.com) – Gergely AGÓCS (Zabothegyezek@vipmail.hu) – Erika BUJNA (Bujna.Erika@etk.szie.hu) – Ágoston HOSCHKE (Hoschke.Ágoston@szie.etk.hu) – Quang D. NGUYEN (Nguyen.Duc.Quang@etk.szie.hu)

Research Centre for Bioengineering and Process Engineering, Faculty of Food Science, Szent Istvan University, Ménesi út 45., 1118 Budapest, Hungary

Pálinka is a traditional spirit drink produced exclusively by the alcoholic fermentation and distillation of any fruit grown in Hungary. The character of individual pálinka definitely depends not only on primary aroma compounds came from fruit, it is strongly influenced by using yeast strain. In this study, achievements (fermentation of pear juice and production of volatile compounds) of nine commercial yeast strains namely Uvaferm SLO, Uvaferm PM, Uvaferm Danstil A, Fermiblanc Arom, Viniflora Melody, Vin-O-Ferm Roses, Fermicru AR2, Oenoferm x-treme F3 and Oenoferm x-thiol F3 were aimed. Samplers were analysed by both classical (reducing sugar, Brix, alcohol content and pH) and instrumental methods (HPLC: sugars, acids and alcohol, GC-FID: volatile compounds). The pH values ranged from pH 3.06 to pH 3.15 during fermentation period (8 days). The concentration of citric, malic and fumaric acids statistically significant increased from the beginning to the end of fermentation. Sugars (glucose, fructose and sucrose) decreased and were almost exhausted at the end of fermentation indicated good fermenting dynamics. While the ethanol level reached about 7.32 v/v % at the end of fermentation in all cases of fermented juice, whereas the ethanol production capacity was negligibly different. The ability of sugar consumption of strain Danstil A and AR2 was the faster than the others (only 3 days). The spectrum of volatile components was almost similar in all nine yeasts, but the concentrations of some volatiles varied with yeast strains. Major volatile compounds found in nine fermented pear juices were ethyl laurate, izo-pentyl acetate, 2-pentanol, 2-methyl-1-butanol as well as some miscellaneous compounds. These results provide the possibility to produce pear pálinka with different characteristics using different yeast strains.

Keywords: Pálinka, spirit, *Saccharomyces cerevisiae*, volatile compound, pear

MP15

STRESS SENSITIVITY OF A *MnSOD* DELETION MUTANT OF *FUSARIUM VERTICILLIOIDES*

Zsuzsa SZABÓ (szabo.zsuzsa@science.unideb.hu) – Klaudia PÁKOZDI (pakozdiklaudia94@gmail.com) – Bálint SZUNYOG (balintszunyog@gmail.com) – Éva LEITER (leiter.eva@science.unideb.hu) – István PÓCSI (pocsi.istvan@science.unideb.hu)

Department of Biotechnology and Microbiology, Faculty of Science and Technology, University of Debrecen, Egyetem square 1., H-4032 Debrecen, Hungary

Both prokaryotic and eukaryotic organisms sense and respond to various types of environmental challenges. One of these challenges is the generation of intracellular reactive oxygen species (ROS) under aerobic conditions. ROS production is mainly generated by environmental changes and as a side effect of mitochondrial respiration in the cell. In the case of pathogenic fungi, ROS can be produced by the host as a defense reaction during infection. Although molecular oxygen is thought to be relatively unreactive, it can undergo reduction to form hydrogen peroxide, superoxide anion and hydroxyl radicals; ROS can than interact with essential biological molecules causing damages, such as mutations in DNA, oxidation of proteins and lipid peroxidation. To cope with the damage caused by ROS all organisms have antioxidant defense mechanisms capable of eliminating ROS and maintaining a reduced redox environmental within the cell. Antioxidant defenses include a number of enzymes, such as catalases, superoxide dismutases, methionine sulfoxid reductase, thioredoxins, peroxiredoxins and also the gluthatione system. Superoxide dismutases convert the superoxide anions to hydrogen peroxide that can successively be eliminated by catalases or peroxidases. The mitochondrial manganase superoxid dismutase (MnSOD) is well characterised in several microorganisms, like in *Saccharomyces cerevisiae* and *Aspergillus nidulans*, model organisms of molecular microbiology. Deletion of the *mnSOD* gene in *S. cerevisiae* resulted in reduced growth ability under aerobic conditions. The *ΔmnSOD* mutant of *A. nidulans* showed high sensitivity to menadione and increased sensitivity to the apoptotic cell death caused by the antifungal protein PAF.

The goal of our study was to generate *ΔmnSOD* deletion mutant strain in the phytopathogenic fungus *Fusarium verticillioides* using the double joint PCR method. To screen the stress sensitivity of the mutant, freshly grown conidia were point-inoculated on Czapek-Dox agar plates, supplemented with one of the following stress generating agents: sorbitol, NaCl, KCl, $CdCl_2$, menadione, diamide, *tert*-butyl hydroperoxide, hydrogen peroxide, and Congo Red, used in various concentrations. The deletion of *FvmnSOD* resulted in increased sensitivity to menadione.

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MP16

QUANTITATIVE CHANGE IN SMALL RNA DUE TO ARBUSCULAR MYCORRHIZA INOCULATION IN TOMATO

***Viktor SZENTPÉTERI* (szviki213@gmail.com) – Katalin POSTA (posta.katalin@mkk.szie.hu)**

Institute of Genetics, Microbiology and Biotechnology, Szent István University Páter Károly str. 1. 2100 Gödöllő, Hungary

Around 90% of land plants are able to form symbiosis with arbuscular mycorrhizal fungi. This mutually beneficial interaction helps our host plant to survive in nutrient deficient environment moreover increased their tolerance to different stresses.
Plant microRNAs, are a class of small non-coding RNAs their function is to regulate gene expression at transcriptional and post-transcriptional levels in plants. Moreover, some research works show that they are involved in the regulation of AM symbiosis. The mir171 family frequently reported as key in symbiosis between plants and fungi.
The aim of this work is to compare the quantity of a special type of miRNA(miR171a) in mycorrhizal and non-mycorrhizal plants.
For mycorrhizal treatment, tomato (*Solanum lycopersicum var.* Moneymaker) seedlings were inoculated with *R. irregularis (*mixture of spores, hyphae and roots) while the control sample received the same amount of autoclaved inoculum. Plants were cultivated under 16h light at 24 °C and 8h dark at 20 °C in a growth chamber. 56 days after inoculation plants were harvested and fresh root and shoot measured. Total RNA from roots of both treatments extracted and used for cDNA synthetizes. Quantitative change in one miRNA estimated using PCR with specific primers for miR171a.
Our preliminary result shows difference in quantity of miRNA during mycorrhizal inoculation, but more research is required to make it sure and clear.

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MP17

COMPARISON OF ADHESION AND INFECTION DYNAMICS OF CANDIDA ALBICANS ISOLATES WITH DIGITAL IMAGE ANALYSIS

Laszlo TALAS (talas88laszlo@gmail.com) – Zsuzsa SZIGETI MATHENE (szigeti.zsuzsa@science.unideb.hu) – Imre BOCZONADI (imre.boczonadi@gmail.com) – Gaspar BANFALVI(bgaspar@unideb.hu) – Gabor NAGY (bigdegu@gmail.com)

Department of Biotechnology and Microbiology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary.

In the hospitals the infection of fungus has significantly increased in the last few years. Mortality rates of systematic *Candida albicans* infections result in 30–50%. Adhesion to abiotic medical devices and implants are the most common forms of infection. Effective adhesion can provide the establishment of aggressive biofilms that are difficult to handle. A more accurate knowledge of the adhesion dynamic of *Candida albicans* isolates can provide an opportunity to control invasive fungal diseases.
In this research, we combined a high-time resolution video microscopic technique with digital image analysis to create a faster and more accurate picture of adhesion and growth dynamic of *Candida albicans* isolates.
With this system we have not only described the bacterial and fungal infections of mammalians cells but in practice it was used to detect mycoplasma infections. Bacteria belonging to the Mycoplasma genus have no cell wall therefore insensitive to penicillin and other beta-lactam antibiotics. They cause serious damages in the cell cultures so their identification and early detection results in considerable financial savings.
The purpose of the study was to detect the growth and adhesion dynamic of *Candida albicans* isolates. We examined 3 important aspects which underlie the growth and adhesion dynamic: stopping of Brownian motion, appearance of the first hypha and further branching process.
Literature data support the hypothesis that there is a close connection between the period of adhesion, dynamic and the rate of growth of filamentous fungi that may affect their virulence. These three factors were 106nalysed for individual yeast cells. We wanted to examine if there is a difference between the adhesion and infection strategies of mutant strains and clinical isolates. Physiological conditions were maintained in a CO_2 incubator.

The real time moment of the adhesion and branching were determined by using digital image analysis. This allowed us to avoid variables that affect the mistakes of digital image analysis. Moreover, we are able to get a better knowledge of the mechanism of monitored drugs.
Time lapse video microscopy combined with image analysis can provideus with a convenient and reliable method to examine fungal cultures. Further benefits of the system is to observe individual fungal cells and specific hyphal regions for long time periods.

MP18

CHARACTERIZATION OF A PLANT HOMOLOGUE IZOFLAVONE REDUCTASE GENE DELETION MUTANT IN *ASPERGILLUS NIDULANS*

***Dániel TÓTH* (7danieltoth7@gmail.com) – István PÓCSI (pocsi.istvan@science.unideb.hu) – Éva LEITER (pocsi.istvan@science.unideb.hu)**

Department of Biotechnology and Microbiology, University of Debrecen, Egyetem tér 1., 4032 Debrecen, Hungary

Under long-term oxidative stress induced by menadione sodium bisulfite, a plant isoflavone reductase homologue gene (*ANID_08815*) was induced at both mRNA and protein levels in *Aspergillus nidulans*. Isoflavone reductases by producing isoflavonoids in plants play an important role not just in the protection against environmental stresses but also in lignin biosynthesis and, in *Fabaceae*, in the initiation and procession of symbiosis with nitrogen-fixing bacteria.

In this study, we constructed the gene deletion mutant of the plant isoflavone reductase homologue gene *ANID_08815* by double-joint PCR method and phenotypically characterized the mutant. The mutant showed increased oxidative stress sensitivity in the presence of menadione sodium bisulfite and hydrogen-peroxide in comparison to the control strain. We also tested the stress sensitivities of wild-type and mutant conidiospores. Although conidia were either incubated with 200 mM hydrogen peroxide or were subjected to heat stress at 50 ^{o}C, we did not find any differences in the viability of the asexual spores of the control and mutant strains. We also quantified fruiting body formation and conidiospore production of the mutant. The deletion of *ANID_08815* did not influence either cleistothecia number and maturation or conidiospore production. As oxidative stress response regulation can be connected to secondary metabolite production, we determined the sterigmatocystin production in carbon-starving submerged cultures of *ΔANID_08815*. In the gene deletion mutant we observed lower sterigmatocystin yield than in the control strain after 48 h incubation in carbon-free culture medium. To study the oxidative stress defence system of the mutant, catalase and superoxide dismutase enzyme activities were measured in carbon-starving cultures. The deletion of *ANID_08815* resulted in approximately 60% lower catalase activity than that found in the control strain but did not influence the SOD activity of the cultures.

Our results demonstrated that the *ANID_00815* isoflavone reductase in *A. nidulans* - similarly to plants - plays role in the stress defence of the fungus.

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MP19

OPTIMIZATION OF MEDIUM COMPONENTS FOR NARINGINASE PRODUCTION BY *LACTOBACILLUS FERMENTUM D13*

Anh M. TRAN (tranthimaianh_2807@yahoo.com)[1,2] – Erika BUJNA (bujna.erika@etk.szie.hu)[1] – Bao Toan NGUYEN (toannguyen1905@gmail.com)[1] – Mai S. DAM (damsaomai@yahoo.com)[2] – Quang D. NGUYEN (nguyen.duc.quang@etk.szie.hu)[1]

[1]Research Centre for Bioengineering and Process Engineering, Faculty of Food Science, Szent István University; Ménesi út 45, 1118 Budapest, Hungary
[2]Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, No. 12, Nguyen Van Bao, Ward 4, Go Vap District, Ho Chi Minh City, Vietnam

Naringin is main compound that causes bitter taste in citrus juice and can be eliminated by application of naringinase. Naturally, naringinase is an enzyme complex consisted of α-L-rhamnosidase (EC.3.2.1.40) and *β*-D-glucosidase (EC.3.2.1.21) activities. In debittering process, naringin can be firstly hydrolyzed by α-L-rhamnosidase producing prunin and rhamnose. Then *β*-D-glucosidase hydrolyses prunin into non-bitter naringenin and glucose. Products formed in the hydrolytic reactions have a great potential, especially in the food and pharmaceutical industries, because of their antioxidant, anti-inflammatory, anti-ulcer, and hypocholesterolemic effects. Furthermore, naringenin has also shown anti-mutagenic and neuroprotective activities, whereas prunin has antiviral activity. Recently, naringinase is commercially used in debittering and clearance of citrus fruit juices as well as enhancement of wine aromas in the food applications. In this study, fermentation medium was optimized for enhancing naringinase production by *L. fermentum D13*. First, effects of some physical parameters on production of naringinase were investigated using MRS medium. Carbohydrate (sucrose), inducer (naringin) and pH levels were observed to be important factors that significantly affect naringinase activity. Central composite design (CCD) was applied to find the optimal concentration of each variables. The second-order polynomial model was applied for evaluation of experimental data. The following model: $y = 3.973 – 0.341x_1 + 0.51x_2 – 0.492x_1^2 – 0.584x_3^2 – 0.223x_2^2 – 0.121x_1x_2 – 0.552x_2x_3$ was able to describe the changes of naringinase activity (y) with 3 independent factors pH (x_1), sucrose content (x_2) and naringin concentration (x_3). The optimum values of pH, sucrose and naringin were pH 5.8, 7.5 g L^{-1}, 0.08 (m/v) %, respectively. Application of optimal medium resulted 4.57 IU mL^{-1} enzyme titer. Our results can serve a good basic for study of naringinase by *Lactobacillus fermentum* D13 strain.

Keywords: Naringinase, optimization, naringin, debitter, probiotic bacteria

MP20

EFFECT OF GLUCOSE AND MALTOSE SUBSTRATES ON GROWTH AND RIBOFLAVIN PRODUCTION OF *SHEWANELLA XIAMENENSIS* IN MICROBIAL FUEL CELL

Hoang Duy TRUONG (hoangduy511@gmail.com)[1,2] – Edina NAGY (Nagy.Edina.Szandra@etk.szie.hu)[1] – DAM Sao Mai (damsaomai@gmail.com)[2] – Erika BUJNA(Bujna.Erika@etk.szie.hu)[1] – Quang D. NGUYEN (Nguyen.Duc.Quang@etk.szie.hu)[1]

[1]Research Centre for Bioengineering and Process Engineering, Faculty of Food Science, Szent Istvan University, , Ménesi út 45, 1118 Budapest, Hungary
[2]Industrial University of Ho Chi Minh city, Institute of Food Technology and Biotechnology, 12 Nguyen Van Bao Street, Go Vap District, HCMC, Vietnam

Microbial fuel cell is an electrochemical device that can directly convert the chemical energy in organic matter into electricity by living microorganisms. *Shewanella* species are reported to be able to produce extracellular electrons and transfer them onto electrode, thus these species can act as biocatalysts in MFC systems. It is well known that the *Shewanella oneisensis* MR-1 strain is able to secrete extracellular riboflavin to improve the efficiency of electron transfer, but this capability of other strains even other species is still in question. In this study, the effects of glucose and maltose on growth and the ability of production riboflavin by *Shewanella xiamenesis* DSMZ 22215 were focused. Different concentration (1, 2, 3, 4, 5 % w/v) of glucose or maltose were supplemented into artificial fermentation media. The growth of *Shewanella xiamenesis* were done with the initial microbial cell about 10^5 CFU ml^{-1} and at 30 °C in the shaker incubator (200 rpm) for 120 hours. Microbial cells, pH, riboflavin concentration and conductivity were monitored during fermentation. The current density was also determined in MFCs. Bacteria grew well in all investigated media reaching about $2.3x10^8$ CFU ml^{-1} after 72 hours. The Monod model was used to determine the growth kinetics of this microbe. Herein, we found that the maximal specific growth rate of glucose substrate was significant higher than of maltose. In details, μ_{max} = 0.14 1 h^{-1}, K_s = 0.9 g l^{-1} (glucose substrate) and μ_{max} = 0.098 1 h^{-1}, K_s = 1.0 g l^{-1} (maltose substrate). Riboflavin concentration was 4.2 nmol ml^{-1} (glucose substrate) and 3.9 nmol ml^{-1} (maltose substrate) after 72 hours.

Keywords: glucose substrate, maltose substrate, riboflavin, conductivity, *Shewanella xiamenensis*

NP1

ASSESSMENT OF GENETIC DIVERSITY OF APRICOT (*PRUNUS ARMENIACA* L.) GENOTYPES USING IPBS RETROTRANSPOSON MARKERS

***Janka BEDŐ* (bedo.janka@gmail.com)[1] – Dezső SURÁNYI (suranyi.dezso@cefrucht.hu)[2] – Anikó VERES (veres.aniko@mkk.szie.hu)[1] – Erzsébet KISS (kiss.erzsebet@mkk.szie.hu)[1]**

[1]*Institute of Genetics, Microbiology and Biotechnology, Szent István University, Páter Károly str 1., 2100 Gödöllő, Hungary*
[2]*NARIC Fruitculture Research Institute Research Stations Cegléd, Szolnoki str 52., 2700 Cegléd, Hungary*

The Hungarian apricot is well known for its quality. This stone fruit can be produced in limited area due to its low adaptive ability. In Hungary, ripening period of apricot starts at the beginning of June and ends at the beginning of August. Furthermore, the flowering of this variety begins early, thus the spring frosts are especially perilous. To increase environmental adaptivity, resistance and fruit quality molecular markers have been introduced for breeding purposes (MAS –marker assisted selection), in case of apricot as well.
The role of DNA-based markers have been grown since breeders can rely on these primers to distinguish varieties, to analyze their origin, where they come from, to evaluate genetic relationships, to predict agronomically important traits (e.g. biotic and abiotic resistance, quality, colour) based on genotypes.
The long terminal repeat (LTR) retrotransposons are highly abundant in the eukaryotic genomes and can be utilized as DNA markers. The basis of iPBS (inter-Primer Binding Site) method is the presence of a tRNA complement as reverse transcriptase primer binding site (PBS) in LTR retrotransposons. Due to their replicative copy-paste transposition they can modify the size and structure of the genome or the function of a DNA-sequence. It has been proven that these primers show high level of polymorphism, so they are suitable to establish DNA-fingerprints without any previous information about the genomic sequence.
Fifteen apricot genotypes have been examined with 25 iPBS retrotransposon markers. Throughout our analysis 15 primers generated polymorphism in our samples.
Our aim is to identify specific iPBS markers which are linked to valuable characteristics and to develop markers applicable for MAS.

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NP2

IN VITRO SOMATIC EMBRYOGENESIS ON ARABIDOPSIS ROOT EXPLANTS I. THE ROLE OF HORMONES

***Péter BENKŐ* (benkopeter@hotmail.com)[2] – *Dóra BERNULA* (bernulad@gmail.com)[1] – *Ildikó DOMONKOS* (domonkos.ildiko@brc.mta.hu)[1] – *Attila FEHÉR* (feher.attila@brc.mta.hu)[1,2] – *Katalin GÉMES* (gemes.katalin@brc.mta.hu)[1,2]**

[1]*Institute of Plant Biology, Biological Research Centre, Hung. Acad. Sci., 62. Temesvari krt, 6726, Szeged, Hungary*
[2]*Department of Plant Biology, University of Szeged, 52. Közép fasor, 6726, Szeged, Hungary*

One of the type of asexual embryogenesis is somatic embryogenesis (SE) during which the genetic program of embryo can be reactivated in differentiated cells. Until now, SE research in Arabidopsis was mostly limited to direct or indirect somatic embryogenesis from immature/mature zygotic embryos. Our aim was to establish a more efficient *in vitro* root culture based experimental system for studying SE in *Arabidopsis*. To induce embryogenesis auxin and cytokinin were used. Auxin induced the formation of lateral root primordia that started to transdifferentiate into shoot meristem in the presence of cytokinin. However, if the induced root explants were transferred onto hormone-free medium in time, the primordial were converted to somatic embryos. To monitor the initiation of the developmental processes, molecular (RT-qPCR) and cellular techniques were used. In this work, we have found that the same treatment failed to induce embryo formation in the case of whole seedlings (roots unremoved from the shoot). However, if shoot-derived auxin transport was inhibited by TIBA (2,4,6-triiodo-benzoic acid), the embryogenic capability of the roots was retained even in whole seedlings. Our results indicate that transient cytokinine treatment in high concentration can induce somatic embryogenesis on Arabidopsis root explants only in the absence of shoot-derived auxin.

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NP3

HOMOGENITY TESTING OF SUNFLOWER (*HELIANTHUS ANNUUS*) SEEDS BY MALDI-TOF MS TECHNIQUE

***Csilla BOJTÉ* (csillabojte1990@gmail.com)[1] – Csaba LOVÁSZ[1] – Eszter TÍMÁR[2] – László LAJKÓ[3] – József LUKÁCS[3] – Adrienn MICSINAI[4] – Szilárd TÓTH[4]**

[1]*Doctoral School of Kerpely Kálmán, University of Debrecen, Böszörményi str. 138. 4032 Debrecen, Hungary*
[2]*WESSLING Hungary Ltd. Anonymus str. 6. 1045 Budapest, Hungary*
[3]*National Food Chain Safety Office, Directorate of Plant Production and Horticulture, Keleti Károly str. 24. 1024 Budapest, Hungary*
[4]*Phytopatent Ltd. Mátray str. 10. 1012 Budapest, Hungary*
[5]*Fleischmann Rudolf Research Intitute Fleischmann str. 4., 3356 Kompolt, Hungary*

The rapid development of mass spectrometry methods have opened up new opportunites for analysis for participants of the agriculture and agricultural biotechnology. Breeders, seed producers or other stakeholders in agricultural production are in need of quick, efficient and reliable information about the plants and plant parts, especially in cases like checking the homogenity in a hybrid seed production lot. Current protocols are based on the investigation of gelchromatographic separation patterns of seed storage proteins, which can be augmented by MALDI-TOF-MS (Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectormetry) methods.

The MALDI TOF MS technique is based on coupling a laser ion source and a time-of-flight mass spectrometer. The energy of the laser emitted is absorbed by the matrix which in turn ionises the target compounds of the sample and thus they can enter the vacuum of the mass spectrometer and finally reach the detector. The obtained mass spectra provide information on the protein and macromolecule profiles of the sample.

Our investigations focused on the storage proteins in sunflower seeds, implementing several different sample preparation techniques. Assessed sample preparation protocols included seeds homogenised with and without the outer shell, crushed and intact. Four extraction methods have been assessed (sodium-chloride solution, acidic sodium-chloride solution, 1-propanol and 2-propanol extraction buffers). Analysis of the obtained spectra was carried out using the Bruker Daltonics flexAnalysis software.

Our results show that based on the intensity and diversity of the obtained spectra, the most effective extraction buffer was the one with 2-propanol. The method when validated with regard to the standard method, can be used in sunflower seed genetic homogenity testing or breed comparison studies.

NP4

NANOSTRUCTURED TITANIUM DIOXIDE AS AN ANTIMICROBIAL AGENT ON GRAPEVINE (*VITIS VINIFERA* L.) LEAVES: A PHYTOTOXICOLOGICAL STUDY

Sakina BOUDERIAS* (sakinabouderiasse@gmail.com)[1,2] – *Péter TESZLÁK (teszlak.peter@pte.hu)[1] – *Gábor JAKAB (jakab@gamma.ttk.pte.hu)[1,2] – *László KŐRÖSI (korosi.laszlo@pte.hu)[1]

[1]*Research Institute for Viticulture and Oenology, University of Pécs, Pázmány P. u. 4, 7634 Pécs, Hungary*
[2]*Department of Plant Biology, University of Pécs, Ifjúság u. 6, 7624 Pécs, Hungary*

In the past two decades, nanostructured materials including colloidal nanocrystals, semiconductor nanoparticles, nanotubes, nanowires and porous materials have received a great attention thanks to their unique physicochemical properties. Titanium dioxide nanoparticles (TiO_2 NPs) excited by UV light can produce different reactive oxygen species (ROS) such as hydroxyl radical, superoxide radical anion and singlet oxygen. This special feature of TiO_2 NPs so-called photocatalytic property can be exploited in numerous fields, especially in water treatment technologies for removing toxic compounds or inactivation various pathogens.

In this work we applied directly TiO_2 NPs on the grapevine (*Vitis vinifera* cv. Cabernet sauvignon) leaves as potential antimicrobial agent. Experiments were performed under field conditions where the plants were exposed to sunlight. Physiological responses, flavonol profile and micro- macroelements of the leaves were studied. We found that TiO_2 NPs with the concentration of 250–3 00 mg kg^{-1} increased the stomatal conductance whereas decreased the photosynthetic rate. Despite of their negative effect on photosynthesis, flavonol profile as a stress sensitive factor showed only minor changes after the foliar exposure as revealed by HPLC-DAD measurements. Elevated level of K, Mg, Ca, P and B were detected in the treated leaves which may be related to the increased stomatal opening. Our results indicate that TiO_2 NPs with the applied concentration can be phytotoxic in the presence of UV irradiation which probably stem from the excess of ROS production of nanoparticles. Consequently, further studies are necessary for determining the parameters which allows safety and efficient field applications of TiO_2 NPs.

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NP5

DETECTION OF NEW, CHERRY INFECTING VIRUSES IN APRICOT IN HUNGARY

Alexandra BÜKKI* (bukki.alexa@gmail.com)[1]– Dániel BARÁTH (barathdani@gmail.com)[1] – Nikoletta CZOTTER (czotter.niki@gmail.com)[1] – Júlia BALÁSSY (sargarigofutty@gmail.com)[1] – Tünde VARGA (varga.tunde07@gmail.com)[1] – Luca SZABÓ (sz.luca.k@gmail.com)[2] – Zoltán KIRILLA (kirilla.zoltan@fruitresearch.naik.hu)[2] – Éva PREININGER (preininger.eva@fruitresearch.naik.hu)[2] – Éva VÁRALLYAY (varallyay.eva@abc.naik.hu)[1*]

[1]*Agricultural Biotechnology Institute, NARIC, Gödöllő, Hungary*
[2]*Fruitculture Research Institute, NARIC, Budapest, Hungary*

Orchards can be infected by several viruses, which may have an important role in causing diseases on apricot. Symptoms range from latent to drastic reduction in vigour and fruit quality and can lead to the death of the tree. Prevention can limited only for control of insect vectors and the elimination of viruses from the propagation materials. In order to prove the virus free status of the propagation material we have to perform diagnostic tests. Disadvantages of traditional virus detection methods (ELISA, RT-PCR and biotests) are that they based on the identified nucleic acid sequence or coat protein of the investigated virus. With new metagenomics approaches we can prove the presence of all viruses present in the investigated plant, including the ones not described on a particular host or country yet. This research aims to evaluate the virus infection status of apricot stock nurseries and isolator houses in our country.

Our group has demonstrated first the presence of Little Cherry Virus 1 (LChV-1) and Cherry Virus A (CVA) from apricot in Hungary by Next-Generation Sequencing (NGS) of small RNAs. Our results were confirmed by RT-PCR, and by Northern blot in the case of CVA. To examine their incidence and the rate of their spread, leaf samples were collected from stock nurseries and isolator houses of NAIK GYKI. After RNA extraction we made pools from the samples and carried out RT-PCR with virus-specific primers designed according to the NGS results.

The monitored stock nurseries and isolator houses were never tested for the infection of LChV-1 and CVA. Results showed that the examined viruses were present in both areas. The presence of viruses in isolator houses, where insect vectors are not present, means that it is possible that the propagation material contained the virus particles thus they were transmitted by grafting or budding. Our research highlights the importance of prevention and the usage of new metagenomics based diagnostic methods during the optimization of the official virus elimination protocols in Hungary.

This work was supported by Hungarian Ministry of Agriculture. Alexandra Bükki is an MSc, Luca Krisztina Szabó and Dániel Baráth are PhD students of Szent István University.

NP6

INVESTIGATION OF DARK MODULATED ENDOPLASMIC RETICULUM (ER) STRESS IN TOMATO PLANTS

***Zalán CZÉKUS* (czekuszalan@gmail.com)[1,2] – Orsolya CSÍKOS (cs.orsolya@mensa.hu)[1] – Dóra HEGEDŰS (hegedorka21@gamil.com)[1] – Péter BORBÉLY (borbely.peter01@gmail.com)[1,2] – Attila ÖRDÖG (aordog@bio.u-szeged.hu)[1] – Péter POÓR (poorpeti@bio.u-szeged.hu)[1]**

[1]*Department of Plant Biology, University of Szeged, Közép fasor 52., 6726 Szeged, Hungary*
[2]*Doctoral School in Biology, Faculty of Science and Informatics, University of Szeged, 6726 Szeged, Hungary*

Light is one of the most important environmental factors, which is required for optimal growth and development or during the stress responses of plants. Dark can alter the light-dependent activation of plant developmental or defence responses, it can induce new signaling and regulation pathways modulated by various signaling molecules such as reactive oxygen species (ROS). Disturbances in the normal functions of the ER e.g. induced by tunicamycin (Tm) lead to the unfolded protein response, which can compensate the damage or trigger cell death. This process can be mediated by ROS, but ROS production can be dependent on the presence or absence of light in plants. Tm induced light dependent changes in ROS homeostasis was examined in the leaves of wild type (WT) and ethylene receptor mutant *Never ripe* (*Nr*) tomatoes. Based on our result, Tm induced superoxide production was higher in those plants, which were kept in dark. In contrast to this, levels of H_2O_2 were higher in the illuminated leaves of wild-type plants after treatment with Tm. Production of ROS was dependent on the active signaling of phytohormone ethylene investigated by *Nr* tomato. The application of the chemical chaperon 4-phenylbutyric acid (PBA) reduced the H_2O_2 production both in the light and dark, but elevated it in *Nr* leaves. The expression of ER stress marker gene, *BiP* was significantly elevated by Tm in the light, but reduced by PBA and dark. Expression levels of *IRE1*α and β were also elevated by the treatment with Tm in the light but did not change under darkness suggesting a specific role of the presence of the light in ER stress response.

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NP7

DESCRIPTION OF GRAPE BERRY (CULTIVAR FURMINT) AND BOTRYTIS *CINEREA* PARAMETERS IN THE PROCESS OF BOTRYTIZATION

***Tamás DANKÓ* (danko.tamas@agrar.mta.hu)[1] – Pál VÁGI (torenia@caesar.elte.hu)[2] – László K. SZABÓ (szabolaszlo0313@gmail.com)[3]– Evelin KÁMÁN-TÓTH (toth.evelin@agrar.mta.hu)[1] – Miklós POGÁNY (pogany.miklos@agrar.mta.hu)[1]**

[1]*Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences; Herman Ottó str 15., 1022 Budapest, Hungary*
[2]*Department of Plant Anatomy, Eötvös Lóránc University Faculty of Sciences; H-Pázmány Péter sétány 1/C., 1117 Budapest, Hungary*
[3]*Faculty of Food Science, Szent István University, Menesi str 44., 1118 Budapest, Hungary*

Botrytized („aszú") wines are produced from ripe grape berries subjected to a unique interaction with the filamentous fungus *Botrytis cinerea*. This special interaction between the berries and *Botrytis* is called noble rot. During noble rot the berries gradually dehydrate and shrivel and the increasing concentration of cellular constituents results in an inhibition of fungal growth. The process of noble rot takes several days and the berries go through four distinct phenotypic stages.

We have developed a high-throughput technique to assess the ratio of living and dead plant cells in the berries and some crucial physical parameters for each phenotypic stage. The level of *Botrytis cinerea* biomass was also simultaneously evaluated by ELISA. Our results enable us to gain insight into the complex relationship between the grape berries and the fungus during noble rot.

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NP8

FAR-RED LIGHT REGULATED CIRCADIAN EXPRESSION OF *CBF*s AND THEIR UPSTREAM SIGNALLING GENES IN BARLEY

***Krisztián GIERCZIK* (gierczik.krisztian@agrar.mta.hu)[1,2] – Gábor GALIBA (galiba.gabor@agrar.mta.hu)[1,2] – Attila VÁGÚJFALVI (vagujfalvi.attila@agrar.mta.hu)[1]**

[1]*Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 2462 Martonvásár, Hungary*
[2]*Festetics Doctoral School, Georgikon Faculty, University of Pannonia, 8360 Keszthely, Hungary*

CBF (C-repeat binding factor) transcription factors are one of the most studied regulators in plants. They play a key regulatory role in the cold acclimation processes through high level expressions in response to low temperature. Recently, more and more publications concluded that, apart from cold, not just the quantity and the duration of the illuminating light, but its spectra also has a pivotal role in the regulation of the *CBF* gene expression. Earlier studies established that the expression patterns of some of these transcription factors follow a circadian rhythm. To understand more deeply this acclimation process, we studied the gene expression patterns of the signal transducing pathways, including the signal perception and the phospholipid signalling pathways, the upstream of the *CBF* gene regulatory hub, and also the influence of the circadian clock. Our analysis revealed that the expression of genes implicated in the phospholipid signalling pathway show a circadian rhythm in the frequently used cereal model species: barley (*Hordeum vulgare* L.). We also demonstrated that from those *CBF* genes which were expressed under non cold inductive condition, only the members of the HvCBF4-phylogenetic subgroup showed a circadian pattern, and also that they were also expressed in the late afternoon or early in the night. We also determined the gene expression changes under supplemental far-red illumination and established that this change in the spectrum led to an earlier and more intense transcript accumulation in several cases. Based on our results, we propose a model to illustrate the effect of the circadian clock and the far-red light on the elements of signalling pathways upstream of the *HvCBF* genes, thus integrating the complex regulation of the early cellular responses, which finally leads to an elevated cold stress tolerance.

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NP9

ANALYSIS OF LONG TERM *IN VITRO* PROPAGATED METHYLOME IN APPLE

***Andrea GULYÁS* (gulyas.andrea@agr.unideb.hu)[1] – Norbert HIDVÉGI (hidvegi.norbert@agr.unideb.hu)[1] – Judit DOBRÁNSZKI (dobranszki@freemail.hu)[1] – Katalin POSTA (posta.katalin@mkk.szie.hu)[2] – Erzsébet KISS (kiss.erzsebet@mkk.szie.hu)[2]**

[1]*IAREF Research Institute of Nyíregyháza, University of Debrecen, 4-6 Westsik Vilmos str., 4400 Nyíregyháza, Hungary*
[2]*Institute of Genetics, Microbiology and Biotechnology, Faculty of Agricultural and Environmental Sciences, Szent István University, 1 Páter Károly str., 2100. Gödöllő, Hungary*

Beyond changes within the DNA sequence various epigenetic modifications in the genome, such as DNA methylation, chromatin methylation and acetylation, siRNA changes, contribute to the actual operation, functionality of a genome due to the modification in the gene expression. It has been described that DNA methylation pattern can be inherited over several generations, and molecular pathways responsible for the DNA methylation pattern may cause the emergence of natural variations (Niederhuth *et al*., 2016). When methylation occurs, the methylation level of DNA cytosine (C) may vary in a wide range among living organisms from different taxonomic groups. For insects, it may be 0–3%, for vertebrates 2–7%, for fish 10% while for plants up to 30% (Adams, 1996; Colot and Rossignol, 1999; Cokus *et al*., 2008). In plants DNA methylation of cytosine may occur linked and not linked to CG, CHG and CHH (where G is guanine, and H may represent any other nucleotides that are not guanine) in which case CHG is a symmetric and CHH an asymmetric sequence. In *Arabidopsis thaliana,* the CG was methylated in 24%, CHG in 6.7% and in CHH 1.7% (Cokus *et al*., 2008), while in maize the methylation of CG, CHG and CHH was 86.4%, 70.9%, and 1.2%, respectively (West *et al*., 2014). For both plant species, methylated and highly methylated (80–100%) levels were observed in CG regions, non-methylated and partially methylated (20–100%) in CHG regions and in CHH regions non-methylated and low methylated (approximately 10%) levels were recorded (Cokus et al., 2008; Lister et al., 2008). The aim of our present research was to determine and examine the epigenome of *Malus* × *domestica* cv.'McIntosh' and 'Húsvéti rozmaring' diploid apple varieties maintained for more than 20 years in tissue culture. Moreover, it aimed at determining if any DNA methylation level differences and changes could occur in the DNA comparing *in vivo*, *in vitro* and acclimatized plants. We were looking for answers to (i) what sort of epigenetic footprint was left within the epigenome of plants due to stress caused by medium containing different plant growth regulators (PGRs) in various concentration during the tissue culturing; (ii) if the DNA methylation pattern recovers to that of the control, *in vivo* plant after acclimatization. Whole-genome bisulfite sequencing (WGBS) was performed for *in vivo*, *in vitro* and acclimatized plant samples of the *Malus* × *domestica.*

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NP10

QUANTITATIVE REAL-TIME PCR ANALYSIS OF THE UTILIZATION OF AN IRON-CONTAINING NANOMATERIAL BY A DICOT MODEL PLANT

Viktória HALASY* (victoriahalasy@gmail.com)[1] – Krisztina KOVÁCS (kkriszti@chem.elte.hu)[2] – Zoltán KLENCSÁR (z.klencsar@esr.hu)[3] – Zoltán HOMONNAY (homonnay@chem.elte.hu)[2] – Ferenc FODOR (ferenc.fodor@ttk.elte.hu)[1] – László TAMÁS (laszlo.tamas@ttk.elte.hu)[1] – Ádám SOLTI (adam.solti@ttk.elte.hu)[1] – Sára PÓLYA (sara.polya@ttk.elte.hu)[1]

[1]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter sétány. 1/C., 1117 Budapest, Hungary
[2]Department of Analytical Chemistry, ELTE Eötvös Loránd University, Pázmány Péter sétány. 1/A., 1117 Budapest, Hungary
[3]Institute of Materials and Environmental Chemistry, Hungarian Academy of Sciences, Magyar Tudósok körútja 2., 1117 Budapest, Hungary

Since plants represent the primary source of iron in human consumption, iron deficiency is among the most common nutritional disorders. Due to suboptimal soil conditions (alkaline pH or high carbonate content) iron can precipitate in the soil that reduces its availability for plants in many agricultural areas. The applications of iron-containing nanoparticles such as nanoferrihydrite (NH) as fertilizer ingredients could be effective to treat iron deficiency of plants. NH is thought to be effective in increasing the available iron content of soils, providing a stable but efficient iron supply even at alkaline pH, similarly to commercial chelates and complexes like Fe-EDTA and Fe-citrate, respectively. Moreover, testing the utilisation of a new substance requires a reliable system. Molecular biological methods contribute testing the utilisation of the nanoparticles in the iron uptake of plants. Quantitative real-time PCR (qRT-PCR) is found appropriate for measuring the gene expression of key components of the iron uptake system. Here we focused on the root iron uptake system in a dicot model plant, cucumber (*Cucumis sativus* L. cv. Joker F1). A key enzyme of this system is the ferric-chelate oxidoreductase (FRO1) – an iron deficiency inducible membrane bound enzyme that is responsible for the reduction of iron at the root surface. We identified the homolog of *Arabidopsis Fro2* gene in cucumber genome. Furthermore, to investigate the bio-utilisation of iron content of NH by cucumber roots, changes in the expression of *CsFro1* were monitored by qRT-PCR upon NH treatment. The results indicate that the expression of *CsFro1* is enhanced by iron deficiency but upon NH treatment it started to decrease and the tendency for decrease has become apparent within thirty minutes. This proves that the utilization of the iron content of NH has been carried out in a very short time frame. Moreover, molecular methods can be successfully used in testing the effects of nanomaterials and thus the results contribute to adjusting the proper dosage of the material, which can successfully cure iron deficiency of plants.

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NP11

OSMOTIC STRESS TOLERANCE EXPERIMENTS ON *IN VITRO* SHOOT CULTURES

Alexandra HANÁSZ (hanasz@agr.unideb.hu) – Katalin MAGYAR-TÁBORI (mtaborik@agr.unideb.hu) – László ZSOMBIK (zsombik@agr.unideb.hu) – Judit DOBRÁNSZKI (dobranszki@freemail.hu)

Research Institute of Nyíregyháza, Debrecen University, Westsik V. road 4-6., 4400 Nyíregyháza, Hungary,

The aim of our research included the selection of winter wheat landraces, wich can be able to adapt to extrem wheather conditions characteristical nowadays. In our experiments, we analyzed the changes of simple morphological parameters of winter wheat landraces exposed to osmotic stress under *in vitro* conditions.

We analyzed the growth of *in vitro* shoot cultures in 12 different winter wheat landreaces on osmotic containing and osmotic-free media. To induce the osmotic stress, we added 5 % polyethylene glicol 600 (PEG, M_w=600) osmotic to the Murashige-Skoog media. The shoot cultures were growth for 7 days under 16 hours photoperiod, at 65 µmol s^{-1} m^{-2} PPF and 22±2 °C. The experimental repetition was eight. On the 7th day we measured the length and the number of viable shoots, the number of roots and the survivor shoots. The genotypes largely affected the parameters of growth, therefore we calculated stress index (SI) for comparing genotypes (SI=growth parameters of PEG treated seedlings/growth parameters of control seedlings x 100).

We found significant differences in the number of shoot and the root lengths SI values. Concerning the shoot lengths, results of the 'Tápiószelei' and the 'Kecskeméti' landraces were significantly the best. Analyzing the number of shoots, the SI values was the highest in the case of the 'Érpataki' and the 'Szajlai' landraces. Considering the same parameters, the 'Stephanus', the 'Abony'cultivars and the 'Nagykállói' landraces showed the lowest results indicating low tolerance against the osmotic stress.

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NP12

IN VITRO PLANT REGENERATION FROM ARABIDOPSIS ROOT EXPLANTS II. THE EFFECT OF POLYAMINES

***Nikolett KASZLER* (kaszler.n@gmail.com)[2] – Dóra BERNULA (bernulad@gmail.com)[1] – Ágnes SZEPESI (szepesia@bio.u-szeged.hu)[2] – Attila FEHÉR (feher.attila@brc.mta.hu)[1,2] – Katalin GÉMES (gemes.katalin@brc.mta.hu)[1,2]**

[1]*Institute of Plant Biology, Biological Research Centre, Hung. Acad. Sci., 62. Temesvari krt, 6726, Szeged, Hungary*
[2]*Department of Plant Biology, Univ. of Szeged, 52. Kozep fasor, 6726, Szeged, Hungary*

Over recent decades, various culture conditions have been established for plant regeneration and utilized for clonal propagation in diverse plant species. A common mode of plant regeneration both in nature and *in vitro* is *de novo* organogenesis in which plant cuttings or explants first form ectopic apical meristems and subsequently develop shoots or roots. The other way of plant regeneration is somatic embryogenesis (SE) whereby the genetic program of embryogenesis can be reactivated in differentiated cells. Polyamines (putrescine, spermidine and spermine) are known to play a vital role during plant regeneration processes. Previous studies have shown that polyamines are important in several physiological processes such as cell division and differentiation, callus formation, regeneration, stress responses and they also influence morphogenesis. In this study, our aim was to compare the role of different polyamines during the initiation of SE or organogenesis. To this end, we used a root culture based regeneration system which was previously established in our laboratory. This system is very useful to compare the initiation steps of organogenesis and SE. Various biochemical and cellular techniques were used to monitor the effect of exogenously applied polyamines. The polyamine content was measured by a HPLC system and the activities of the enzymes involved in polyamine catabolism (polyamine oxidase and diamine oxidase) were determined by spectrophotometry. We have found that exogenously applied spermidine and spermine, but not putrescine strongly improved the regeneration capacity of roots. These preliminary results indicate that exogenous polyamines promote the regeneration in *Arabidopsis thaliana* roots, mainly via the shoot organogenesis pathway.

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NP13

MOLECULAR DIVERSITY ANALYSIS OF PEPPER (*CAPSICUM ANNUUM*) GENOTYPES BASED ON IPBS RETROTRANSPOSON MARKERS

***Zsófia KOVÁCS* (zsoki14@gmail.com)[1] – Gábor CSILLÉRY (csillerygabor48@gmail.com)[2] – Erzsébet KISS (kiss.erzsebet@mkk.szie.hu)[1] – Antal SZŐKE (szoke.antal@mkk.szie.hu)[1] – Anikó VERES (veres.aniko@mkk.szie.hu)[1]**

[1]Szent István University, Institute of Genetics, Microbiology and Biotechnology, H – Páter Károly str 1., 2100 Gödöllő, Hungary
[2]PepGen Ltd., Bartók Béla str 41. 1114 Budapest, Hungary

Pepper (*Capsicum annuum*) is one of the most important spice and vegetable crops of Hungary. Beside other crucial attributes, the breeding work focuses mainly on the improvement of yield, biotic and abiotic stress resistance, berry size and different nutritional values such as capsaicin or high red pigment contents.
To aid the breeding molecular markers have been introduced which can assist plant breeders with invaluable information. With their application, further light is shed on genetic relationships, while also marking attributes important for breeding that drastically shorten breeding times, thus making the selection process easier. In the case of peppers, breeders have many varying marker systems available.
The LTR (long terminal repeat) retrotransposon based iPBS (inter Primer Binding Site) markers are powerful tools in DNA fingerprinting due to their dispersed and ubiquitous presence in the genomes of plants and animals. Since their application is based on the universal presence of a tRNA complement – primer binding site of the reverse transcriptase – such markers can be applied without prior sequence knowledge. LTR retrotransposons employ a so called „copy and paste" replicative transposition mechanism, which causes new genome insertions without the excision of the original element. Such insertions can not only drastically expand the genome size, but they also alter the function of the DNA segment where they get inserted. The pepper genome consists of 81% transposable elements out of which approximately 70,3% attributed to LTR type retrotransposons.
For the preliminary studies peppers deriving from different genetic backgrounds have been selected. Out of the tested 25 iPBS primers, 7 were capable of and showing polymorphism and differentiating genotypes. Primers showing polymorphism were further analyzed to sort out whether the differences are linked to any desirable trait of interest, making them applicable for marker assisted selection.
Furthermore, our aim is to contribute to broadening the integrated genetic map of pepper by locating these markers on the pepper genome.

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NP14

IMPROVING *RALSTONIA*-RESISTANCE IN POTATO BY KNOCKING OUT PPO GENES

***Blanka MONCSEK* (moncsek.blanka@agrar.mta.hu) – Csaba ÉVA (eva.csaba@agrar.mta.hu) – László SÁGI (sagi.laszlo@agrar.mta.hu) – Ervin BALÁZS (balazs.ervin@agrar.mta.hu)**

Applied Genomics Department, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik str. 2, 2462 Martonvásár, Hungary

Ralstonia solanacearum is a quarantine pest of potato. Prevention is still the only way to avoid it. The Gram-negative bacterium infects from the soil through wounded roots and stem and causes wilting of the plants. It mostly spreads with infected tuber and with irrigation. Our purpose is to increase *Ralstonia* resistance in potatoes, primarily in Hungarian cultivars. Plants synthesize phenolic compounds against pathogens. Among these, the chlorogenic acid especially could confer resistance against bacterias and fungi. Literature data show that the suppression of polyphenol oxidase may redirect the phenolic metabolism and increase chlorogenic acid content of potato. Therefore sequencing of PPO genes has begun in our lab, as a prerequisite for designing CRISPR targets. With the use of CRISPR/Cas system PPO-genes could be knocked out so the potato plant cannot be injured by the bacteria, we assume. At first tissue culture and micropropagation of potato cultivars was optimised. In this research we use the model variety Desireé and two Hungarian cultivars Balatoni Rózsa and Botond. To advance their physiological condition MS medium was given for the cultivars without any vitamins. The first steps have also been taken to adapt an Agrobacterium-mediated transformation technique. Various explants have been tried like microtuber, stem and leaves, the last being the most promising. Transgenic nature of the produced plants are tested by genomic DNA PCR using GUS, kanamycin and virB primers. The next part is to edit the cultivars with our PPO targets to get to know if it influences *Ralstonia* resistance.

NP15

THE MECHANISM OF PHOTOSYSTEM II INACTIVATION DURING SULPHUR DEPRIVATION-INDUCED H_2 PRODUCTION IN CHLAMYDOMONAS REINHARDTII

Valéria NAGY (nagy.valeria@brc.mta.hu)[1] – André VIDAL-MEIRELES (meireles.andre@brc.mta.hu)[1] – Anna PODMANICZKI (podmaniczki.anna@brc.mta.hu)[1] – Klára SZENTMIHÁLYI (szentmihalyi.klara @ttk.mta.hu)[2] – Gábor RÁKHELY (rakhely.gabor@brc.mta.hu)[3] – Laura ZSIGMOND[1] – László KOVÁCS (kovacs.laszlo@brc.mta.hu)[1] – Szilvia Z. TÓTH (toth.szilviazita@brc.mta.hu)[1]

[1]Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Temesvári krt. 62., 6726 Szeged, Hungary
[2]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt. 2., 1117 Budapest, Hungary
[3]Department of Biotechnology, University of Szeged, Közép fasor 52., 6726 Szeged, Hungary

Sulphur limitation may limit cell growth and viability in all living organisms. In the green alga, *Chlamydomonas reinhardtii*, sulphur limitation is of particular interest, because it may induce H_2 production lasting for several days, to be exploited as a renewable energy source. Sulphur limitation leads to a large number of physiological changes within the algal cell, including the inactivation of the O_2 evolving photosystem II (PSII), leading to the establishment of hypoxia, essential for the induction of hydrogenase expression and activity. The degradation of PSII has been assumed to be caused by the sulphur-limited turnover of its reaction center protein, PsbA. However, we demonstrate that upon transferring the cells to sulphur-free media, the amount of sulphur per chlorophyll ($a+b$) decreases moderately. On the other hand, ascorbate (Asc) biosynthesis is strongly induced, most probably by $^1O_2$ generated upon sulphur-deprivation. At a high (mM) concentration range, Asc over-reduces the Mn-cluster of the oxygen-evolving complex and the PSII reaction center becomes inactivated in a secondary process, involving the oxidized forms of the primary electron donor Tyr_Z^+ and possibly the reaction center chlorophyll $P680^+$. We also demonstrate that the turnover of the PsbA protein is significantly less affected by sulphur deprivation than that of other photosynthetic subunits, namely CP43 and RbcL. Therefore, our results demonstrate that instead of a limited turnover of PsbA upon sulphur deprivation, the inactivation of PSII is initiated at its donor side, and it is in relation to oxidative stress.

NP16

FUNCTIONAL ANALYSIS OF MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) SIGNALLING IN THE MODEL UNICELLULAR MICROALGA, *CHLAMYDOMONAS REINHARDTII*

***Tímea V. NÁDAI* (nadai.timea@agrar.mta.hu)[1,2] – Balázs KALAPOS (kalapos.balazs@agrar.mta.hu)[1] – Gábor GALIBA (galiba.gabor@agrar.mta.hu)[1] Katerina BISOVA (bisova@alga.cz)[3] – Róbert DÓCZI (doczi.robert@agrar.mta.hu)[1]**

[1]Department of Plant Molecular Biology, Institute of Agriculture, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik u. 2., 2462, Martonvásár, Hungary
[2]Festetics Doctoral School, Georgikon Faculty, University of Pannonia, 8360 Keszthely, Hungary
[3]Centre Algatech, Institute of Microbiology Academy of Scences of the Czech Republic, Opatovicky mlyn, 379 81, Trebon, Czech Republic

The mitogen-activated protein kinase (MAPK) pathways are central regulatory mechanisms in all eukaryotes. Their functions in cell cycle regulation and in stress responses in yeast and human cells are described to great detail. They play key roles in regulating stress responses in plants, yet our knowledge on MAPK signalling in plants is limited in comparison to yeast and mammalian systems. A unicellular plant model offers an efficient experimental system and an evolutionary framework to study MAPK signalling in the plant kingdom. The photosynthetic microalga *Chlamydomonas reinhardtii* is a suitable laboratory model species, and has been utilised to study photosynthesis, lipid biosynthesis or the cell cycle. Moreover, the use of microalgae in biotechnology by exploiting the rich repertoire of algal metabolites as high value products has become a rapidly developing field, which further underscores the importance of studying gene regulatory mechanisms in algae.
Therefore we set out to study MAPK signalling in photosynthetic microalgae. To this end, comparative genomics analysis identified the gene sets encoding MAPKs in a representative set of algal species. Phylogenetic analysis revealed that algal MAPK proteins are highly similar to but are less diversified than MAPKs of flowering plants. Importantly, these results further highlight that MAPKs of the plant kingdom are most related to the cell-cycle-regulatory ERK kinases of the mammalian MAPK types. These findings confirm that the ancestral eukaryotic MAPK was a prototypical ERK-like kinase.
For functional analysis we have cloned selected *Chlamydomonas* MAPK signalling genes and generated various overexpression constructs. Transgenic *Chlamydomonas* lines along with insertion (‘knock-out’) mutants are characterised in terms of cell cycle and stress response regulation.

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NP17

PROTECTIVE EFFECTS OF S-METHYLMETHIONINE-SALICYLATE (MMS) PRETREATMENT IN CASE OF BIOTIC STRESSES OF VITAL CROPS

***Kinga OLÁH* (okinga0820@caesar.elte.hu) – *György BALASSA* (gybalassa@caesar.elte.hu) – *Csilla OLÁH* (olahcsilla5@gmail.com) – *Edit LUDMERSZKI* (ludmerszki.edit@gmail.com) – *Szabolcs RUDNÓY* (rsz@ttk.elte.hu)**

Department of Plant Phisyology and Molecular Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter sétány 1/C, 1117 Budapest, Hungary

Plants, according to their sessile lifestyle, are more vulnerable to environmental impacts, such as biotic and abiotic stresses. *Maize dwarf mosaic virus* (MDMV) and powdery mildew *Blumeria graminis* f. sp. *tritici* are important biotrophic pathogens of maize and wheat, respectively, and responsible for significant decrease in crop yields. Since maize and wheat are among the most vital cereal crops in our country and worldwide, their yield loss can often result in economic problems. Although the resistant crop hybrids could possess effective defense against these pathogens, many frequently cultivated varieties are susceptible and their defense reactions could be strengthened with the use of biologically active compounds.

The biologically active S-methylmethionine-salicylate (MMS) contains two, natural stress-protective component in ratio 1:1, salicylate (SA) and S-methylmethionine (SMM). It is well known that both of these components play an important role in signalization and metabolic changes during biotic stresses. As a signal molecule, SA induces systemic acquired resistance (SAR) against biotic stressors. SMM has a membrane protective effect, plays a role in the sulfur metabolism and also affects the ethylene and polyamine biosynthetic pathways.

In the present work we investigated the effects of exogenous MMS and studied whether this compound can reduce the damage exerted by MDMV and *Blumeria graminis*, in maize and wheat, respectively. PSII maximum quantum efficiency and chlorophyll content were monitored in wheat 3, 6 and 10 days after the fungal infection. In the case of the maize/MDMV interaction, the virus quantity was measured three weeks after the infection, using protein-based (ELISA) and vRNA-based (qRT-PCR) methods.

According to our results, MMS-pretreatment could reduce the negative effects of pathogens through increasing the innate defense capacity of the examined crop species.

NP18

EXAMINATION OF EXPRESSION LEVEL OF ABA INDUCIBLE DEHYDRIN GENES IN CUCUMBER F1 HYBRIDS

(*Cucumis Sativus L.*)

Réka OSZLÁNYI (roszlanyi@gmail.com)[1] – Tímea KATKÓ (timeakatko@gmail.com)[1] – Anita SZEGŐ (szego.anita@ kertk.szie.hu) [1] – Bence SZEMELVEISZ (szemiben@gmail.com)[1] – Noémi KAPPEL (kappel.noemi@kertk.szie.hu)[2] – István PAPP (papp.istvan@kertk.szie.hu)[1]

[1]*Department of Plant Physiology and Plant Biochemistry, Szent István University, Ménesi u 4, 4 1118 Budapest, Hungary*
[2]*Department of Vegetable Growing Szent István University; Ménesi u 44., 1118 Budapest, Hungary*

Cucumber (*Cucumis sativus* L.) is a very sensitive species to water stress and cold which may potentially cause sever decreases in yield. Breeding more resistant varieties represents a great challenge for breeders. According to experiences of growers there is a significant difference between abiotic stress tolerance of open field and greenhouse cultivated hybrid lines. Exploring plant responses to abiotic stresses and their molecular biological background are key issues in plant research. A well-known hormonal modulator of plant stress responses is abscisic acid (ABA). Dehydrins are a subclass of the LEA proteins that may play a role in increasing plant osmotic stress tolerance. They can occur in several tissues of the plants and appear at numerous stages of plant development even independently of stress effects. Induction and expression of these genes have been shown affected by ABA in some stress situations. Our experiments targeted ABA induction of several putative dehydrin genes selected from the cucumber genome. The aim of our studies was to explore if there is any difference in expression levels of these genes among the F1 hybrid lines investigated. We selected three open field cultivated hybrids: 'Szatmár' Szenzáció' and 'Joker' as well as three hybrids cultivated usually in greenhouse 'Americana', 'Oitol' and 'Prior'. The cultivar hybrids were treated externally with ABA solution and leaves samples were collected. mRNA levels of dehydrin genes was tested by RT-PCR and qRT-PCR. Results showed that at least one cucumber DHN gene (*CsDHN1*) was significantly upregulated by ABA application in all cultivar hybrids to different levels. The induced expression of this gene indicates different ABA sensitivities of the varieties, although this did not correspond clearly to the cultivation methods of the hybrid lines. Our results suggest that the *CsDHN1* gene may play a role in ABA dependent stress tolerance responses in cucumber.

NP19

OPTIMISING CRISPR/CAS9 SYSTEM TO PRODUCE GENE-EDITED BARLEY PLANTS

***Réka RUGONFALVI KISS* (rkreka@gmail.com) – Helga ZELENYÁNSZKI (helga.zelenyanszki@ttk.elte.hu) – Sára PÓLYA (sari@polya.hu) – Ádám SOLTI (adam.solti@ttk.elte.hu) – László TAMÁS (laszlo.tamas@ttk.elte.hu)**

Department of Plant Physiology and Moleculer Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter stny. 1/C., 1117 Budapest, Hungary

Today ensuring sufficient crop production to the growing population of humankind is getting urgently important. Creating gene modified crops with the use of the revolutionary CRISPR/Cas9 system could at least partially solve this problem.

In this research a transgenic barley line expressing green fluorescent protein (GFP) was chosen, providing an easy option to visualise the created mutations. The aim of the study was to generate frameshift mutations with the CRISPR/Cas9 system in the gfp gene and to detect the loss of GFP fluorescence.

In our previous work, 120 immature barley embryos were transformed with *A. tumefaciens* bacteria carrying a transformation cassette consisted of *ubi::zcas9* and guide-RNA driven by the wheat U3 promoter. The explants were cultured on medium containing 5 mg/l bialaphos for the whole regeneration process. The concentration of the selection agent may contribute to the failure of plant regeneration, so only the calli were able to analyse. Loss of GFP fluorescence was monitored by multicolour fluorescence imaging system using 370–470 nm excitation filter to detect the autofluorescence of GFP and chlorophylls by 520 and 740 nm emission filters, respectively. Out of the studied 74 calli 70% of them showed significant GFP fluorescence loss. All the calli were frozen for DNA extraction to perform T7 assay followed by sequencing to detect the target mismatches. The results are presented on the poster.

To continue our work the cold inducible promoter of the wheat *wcs120* dehydrin gene is used to drive Cas9 expression. In parallel experiments barley embryos are transformed with CRISPR/Cas9 construct carrying either constitutive or inducible promoter to facilitate the comparison of these two systems. Loss of GFP fluorescence is monitored weekly. A better regeneration and higher gene editing efficiency is expected with the induced Cas9 expression.

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NP20

ESTABLISHMENT OF PHYTOPLASMA AND VIRUS INFECTED *IN VITRO* SHOOT CULTURES OF APRICOT CULTIVARS

***Luca Krisztina SZABÓ* (l.szabo@resinfru.hu)[1] – Nikoletta CZOTTER (czotter.nikoletta@abc.naik.hu)[2] – Dániel BARÁTH (barath.daniel@abc.naik.hu)[2] – Zoltán KIRILLA (z.kirilla@resinfru.hu)[1] – Attila HEGEDŰS (hegedus.attila@kertk.szie.hu)[3] – Éva VÁRALLYAY (varallyay.eva@abc.naik.hu)[2] – Éva PREININGER (e.preininger@resinfru.hu)[1]**

[1]*Research Station of Budatétény, Fruitculture Research Institute, NARIC, Budapest*
[2]*Diagnostic Group, Agricultural Biotechnology Institute, NARIC, Gödöllő*
[3]*Faculty of Horticultural Science, Szent István University, Budapest*

Decline of apricot orchards is a severe problem in Hungarian horticulture and is often thought to be connected to the presence of European stone fruit yellows disease caused by a phytoplasma: *Candidatus Phytoplasma prunorum*. However symptoms of phytoplasmas are well known, emerging symptoms can be altered by the presence of other pathogens (viruses and viroids). Cherry virus A (CVA) and Little cherry virus 1 (LChV1) was detected also in apricot orchards by our group in 2016-2017 by small RNA NGS and RT-PCR. Although their role in symptom development on apricot is elusive, their presence can add new aspects to the apricot tree decline.

In our work we would like to investigate the presence of pathogens and study their transmission in vector free environment and also we would like to test different methods for the production of pathogen free propagation material. For this reason our aim in the current study is to establish and maintain different variants of naturally phytoplasma and virus infected apricots in *in vitro* cultures.

Four *Candidatus Phytoplasma prunorum* phytoplasma infected trees belonging to 'Tom cot' and 'Sherpa' cultivars were used to initiate *in vitro* cultures after phytoplasma detection by nested PCR. The apricot shoots were cultured on modified MS medium after surface sterilization. *In vitro* plants were transplanted to fresh multiplication medium in every four weeks. After 7 and 18 subcultures *in vitro* plants were tested for phytoplasma by nested PCR. After 7 subcultures phytoplasma was still detected from all of the cultures, but after 18 subcultures it could be detected only in one Sherpa line. We would like to use this infected culture to study phytoplasma transmission in experimental conditions. *Catharanthus roseus* -the experimental host and indicator of phytoplasmas- *in vitro* culture was also established for artificial infecting experiments.

CVA and LChV1 was detected in some apricot trees in Hungary last year. To study the elimination of these viruses two apricot varieties: 'Magyar kajszi' and 'Pannónia' were used as explants for *in vitro* culture establishment. After successful *in vitro* culture initiation the plants were micropropagated. After 4 subcultures presence of CVA, LChV1 and also Plum Pox virus (PPV) were confirmed by RT-PCR from the *in vitro* plants. In the future we will apply several *in vitro* therapies on these infected plants to eliminate viruses.

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NP21

ANALYSIS OF LEAF DEVELOPMENTAL ABNORMALITY IN TOMATO CAUSED BY VIRUS INFECTION

***Tamás TÓTH* (toth.tamas@abc.naik.hu)[1] – Anita SÓS-HEGEDŰS (sos.hegedus.anita@abc.naik.hu)[1] – Katalin NEMES (nemes.katalin@agrar.mta.hu)[2] – Péter GYULA (gyula.peter@abc.naik.hu)[1] – Pál SALAMON (salamon.pal@abc.naik.hu)[1] – Katalin SALÁNKI (salanki.katalin@agrar.mta.hu)[2] – György SZITTYA (szittya.gyorgy@abc.naik.hu)[1]**

[1]Epigenetic group, NAIK-MBK, Szent-Györgyi Albert str 4., 2100 Gödöllő, Hungary
[2]Plant Protection Institute, MTA ATK, Herman Ottó str 15., 1022 Budapest, Hungary

Cucumber mosaic virus (CMV) is responsible for many agricultural crop losses worldwide and has one of the broadest host range among plant viruses. CMV has many strains and they can cause different symptoms. Based on the sequence similarities two subgroups are identified, subgroup I strains cause severe while subgroup II strains cause milder symptoms. The main goal of the project is to understand the molecular background of symptom development and how virus strains with similar sequences can cause extremely different symptoms in tomato (*Solanum lycopersicum cv.M82)*. We used two strains which classified into CMV subgroup II : Trk7-CMV which causes mild mosaic and leaf narrowing symptoms and Scl-CMV, which causes severe, shoestring leaves. Scl-CMV infected tomato symptoms resembles to the phenotype of RNA silencing (rdr6) mutant tomato which is unable to produce ta-siRNAs. The product of RDR6 gene is essential in ta-siRNA biogenesis and ta-siRNA pathway is involved in leaf development so we suggested that the virus interferes in leaf development. We studied the elements of this pathway in Scl-CMV and Trk7-CMV infected tomatoes. The amount of ta-siRNAs decreased and ta-siRNAs targeted ARF4 (auxin response factor 4) expression was increased in Scl-CMV infected tomato leaves compared to the Trk7-CMV infected plants and it is correlated with symptom severity. Analysing different reassortants and recombinants of Trk7-CMV and ScL-CMV we found that 3' region of RNA2 responsible for the shoestring symptom development, this region codes for the 2b silencing suppressor. The amino acid sequence of Trk7-CMV and Scl-CMV 2b differs only in three position. Changing of these amino acids one by one may help us to identify which amino acids are responsible for symptom development.

NP22

ANALYSIS OF MITOCHONDRIAL FUNCTIONS IN STRESS RESPONSES

***Mónika VARGA* (monika.varga@brc.mta.hu) – *László SZABADOS* (szabados.laszlo@brc.mta.hu[1] – *Laura ZSIGMOND* (zsigmond.laura@brc.mta.hu)[1]**

Laboratory of Arabidopsis Molecular Genetics; Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Temesvári krt. 62., 6726 Szeged, Hungary

Mitochondria play central role in the efficient provision of energy for eukaryotic cells as well as it is important in production of reactive oxygen species (ROS) which involved in cellular signalling and stress response in plants. Oxidative respiration produces adenosine triphosphate through the mitochondrial electron transport system controlling the energy supply of plant cells. ROS can be produced in the mitochondrial electron transport chain (ETC) under stress, where Complex I and Complex III are the major sites of ROS synthesis. Stabilization of the electron flow in the mitochondrial ETC can protect plants by reducing oxidative damage, control of redox balance and support photosynthesis during stress.

To reveal the importance of genes encoding the mitochondrial proteins in stress responses, we characterized 13 different *Arabidopsis thaliana* T-DNA insertion mutants, in which the mutations are localized in genes encoding the subunits of Complex I and III of the mitochondrial electron transport. When compared to wild type, several mutants showed morphological and physiological changes under various stress conditions. Here we describe the *cyc1.1* and *cyc1.2* mutants in which the mutations disrupted the *CYC1-1* and *CYC1-2* genes. These homologous proteins are members of Cytochrome C1 family. CYC1-1 and CYC1-2 are integral subunits of Complex III, forwarding electrons toward cytochrome c The homologues show morphological differences compared to each other under examined stress conditions. Phenotype of *cyc1.1* is very similar to the wild type under non-stress conditions. Conversely, under oxidative stress conditions, root growth rate is higher in plants containing insertion mutation in *CYC1-1*. Measuring rosette size and chlorophyll content we also found that *cyc1.1* plants are more resistant to oxidative stress. c*yc1.1* is slightly more sensitive to salt stress compared to *cyc1.2* and wild type. In contrast, *cyc1.2* plants have smaller rosettes and show delayed flowering under non-stress conditions compared to wild type and they are able to survive longer under severe salt stress than wild type or *cyc1.1*. Genetic crosses were made to obtain double mutant line by crossing *cyc1.1* and *cyc1.2* but absence of both proteins resulted embryo lethal phenotype. It is documented that some electron transport chain proteins are dual localized in the mitochondria and the chloroplast. It is also suspected in the case of CYC therefore reasonable studying its localization.

Although the studied homologues proteins have very similar structure, our data suggest that they differ from each other in some important attribution influencing stress response therefore it must be beneficial to find out the origin of these differences.

NP23

HEAT ACCLIMATION OF PHOTOSYNTHESIS IN WHEAT GENOTYPES

***Balázs VÉGH* (vegh.balazs@agrar.mta.hu)[1] – *Tihana MARČEK* (tihana.marcek@ptfos.hr)[2] – *Tibor JANDA* (janda.tibor@agrar.mta.hu)[1] – *Éva DARKÓ* (darko.eva@agrar.mta.hu)[1]**

[1]*Department of Plant Physiology, Agricultural Institute, Centre for Agricultural Research of the Hungarian Academy of Sciences, Martonvásár, Hungary*
[2]*Department of Food and Nutrition Research, Faculty of Food Technology Osijek, Croatia*

Photosynthesis is one of the most heat sensitive processes in which both the carbon assimilation and the photosynthetic electron transport are affected. The changes in the function of photosynthetic apparatus can be characterized by the heat sensitivity of plants. Heat acclimation at non-lethal elevated temperatures may induce several metabolic processes providing protection against a subsequent high temperature stress. In this study the effects of heat acclimation were investigated in young wheat plants with different origins through monitoring several photosynthetic processes. In the experiments, seedlings of winter wheat (*Triticum aestivum* L.) genotypes were pre-treated at a moderate (30 °C) temperature (heat priming, HP) then were subjected to a more severe high temperature stress (above 40 °C) and the thermo-tolerance of the photosynthetic apparatus was studied in relation to the non-primed plants (NP). CO_2 assimilation capacity and chlorophyll *a* fluorescence measurements were performed at different temperatures.

The elevated acclimating temperature did not induce either stomatal closure or photoinhibition in any genotypes. Heat tolerant genotypes were able to induce transpiration at acclimating temperature and did not reduce net assimilation. These genotypes could also close stomata faster when they were exposed to severe high temperatures. Heat acclimation could also be detected in various chlorophyll-a fluorescence induction parameters. Heat acclimation resulted in an upward shift in a temperature-dependent decline in the photochemical utilisation of absorbed light energy in HP plants as compared to the NP plants. However, these changes were less genotype-dependent, and less reflected the differences between varieties. The HP plants were better at surviving the extreme high temperature stress. These results indicated that the heat priming effectively could improve the thermo-tolerance of photosynthetic apparatus in wheat seedlings subjected to a later high temperature stress. To differentiate the thermo-tolerance of genotypes, heat sensitivity and heat acclimation indexes were calculated by the use of photosynthetic parameters. They provided a useful tool to differentiate the heat responses of the different wheat genotypes.

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NP24

SMART PLANT GROWTH CHAMBER

***Helga ZELENYÁNSZKI* (helga.zelenyanszki@ttk.elte.hu)**

Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Pázmány Péter stny. 1/C., 1117 Budapest, Hungary

Controlled environment is necessary for healthy model plants, however commercial plant growth chambers are not only expensive but also cost a lot to operate them. Herein, I present the construction of a DIY plant growth chamber controlled by the Internet of things (IoT).

The frame of the chamber is made of galvanized metal, which is combined with polycarbonate sheets. The metal parts serves for stability and the polycarbonate parts for easier attachment of fans and sensors.

The optimal illumination of the plants is supplied by combined LED panels. It contains 70 pcs red (620–630 nm, 655–660 nm) 20 pcs blue (440–460 nm) 8 pcs white (6500–9000 K), one pc UV (390–410 nm) and one pc IR (730 nm) LED diode per panel. The photosynthetic photon flux (PPF) of the two panel is 370.26 µmol/s. LEDs have several advantages over fluorescent lamps, higher efficiency, lower heat emission, longer life span and lower lifetime costs.

The smart plant growth chamber is equipped with humidity-, light- temperature and soil moisture sensors. Arduino and Raspberry Pi devices collect the data from the sensors and control fans to reach optimal humidity and temperature. One of the most important features of this chamber is the automated plant watering system. Its operation is based on the actual soil moisture level. The water pump is only activated if the soil moisture drops below the set point which enable tightly controlled plant watering that adapt to different water demand of different developmental stages of the plants.

The Raspberry Pi device collects and records the data of the sensors by the RasPiViv software that makes possible to monitor the growth parameters via a mobile application. It is also possible to keep an eye on the plants remotely since an 8 mega pixel camera is connected to the raspberry.

The features mentioned above exceed the equipment of the cheaper commercial plant growth chambers for the fraction of the cost.

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A

***Ágoston Miklós*, (-)** *trade@tomtec.hu*
Tomtec Plastics Kft.,

***Anga Csilla*, (-)** -
Richter Gedeon Nyrt.,

***Annus Tamás*, (ÁP1)**
tamasannus@gmail.com
ELTE Természettudományi Kar, Genetika Tanszék

***Antal Botond*, (MP1)**
botond_antal@msn.com
BMGE Vegyészmérnöki és Biomérnöki Kar, ABÉT Tanszék, Biofinomító Kutatócsoport

B

***Balassa György*, (NP17)**
gybalassa@caesar.elte.hu
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék

***Balog József Ágoston*, (GP1)**
balog.jozsef@brc.mta.hu
MTA SZBK, Funkcionális Genomika Laboratórium

***Baráth Dániel*, (NP5, NP20)**
barath.daniel@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Diagnosztikai csoport

***Bedő Janka*, (NP1)**
bedo.janka@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Genetikai, Mikrobiológiai és Biotechnológiai Intézet

***Bedő Soma*, (-)** *bedo12@gmail.com*
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Bedzsó Gabriella*, (NPE3)**
bedzso.gabriella@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Genetikai, Mikrobiológiai és Biotechnológiai Intézet

***Benkő Péter*, (NP2)**
benkopeter@hotmail.com
MTA SZBK, Növénybiológiai Intézet

***Berek-Nagy Péter János*, (NPE1)**
nagyberek92@gmail.com
ELTE Természettudományi Kar, Növényszervezettani Tanszék

***Biró Bálint*, (ÁP2)**
biro.balint@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Mezőgazdasági Biotechnológiai Kutatóintézet

***Boczonádi Imre*, (MP2, MP17)**
boczonadi.imre@science.unideb.hu
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

***Bouderias*, *Sakina* (NP4)**
sakinabouderiasse@gmail.com
PTE Természettudományi Kar, Növénybiológiai Tanszék

***Bodó Kornélia*, (GE1)**
bodo.kornelia@pte.hu
PTE KK, Immunológiai és Biotechnológiai Intézet

***ifj. Bogsch Erik*, (PLE2)**
Richter Gedeon Nyrt.,

***Bojté Csilla*, (NP3)**
csillabojte1990@gmail.com
DE Mezőgazdaság-, Élelmiszertudományi és Környezetgazdálkodási Kar, Wessling Hungary Kft

***Bolgár Evelin*, (-)** -
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Börcsök Dalma*, (ÁE1)**
borcsok.dalma@brc.mta.hu
MTA SZBK, Genetikai Intézet

***Börcsök Judit*, (BP1)**
judit.borcsok27@gmail.com
ELTE Természettudományi Kar, Komplex Rendszerek Fizikája Tanszék

***Böröcz Katalin*, (GP2)**
borocz.katalin@pte.hu
PTE Klinikák, Immunológiai és Biotechnológiai Intézet

***Bősze Zsuzsa*, (PLE1)**
bosze.zsuzsanna@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Mezőgazdasági Biotechnológiai Kutatóintézet

***Bükki Alexandra*, (NP5)**
bukki.alexandra@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Diagnosztikai Csoport

C

***Czékus Zalán*, (NP6)**
czekuszalan@gmail.com
SZTE Természettudományi és Informatikai Kar, Növénybiológiai Tanszék

***Czinkóczky Réka*, (MPE1)**
czreka1@gmail.com
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Czotter Nikoletta*, (NE1, NP5, NP20)**
czotter.nikoletta@abc.naik.hus
Nemzeti- Agrárkutatási és Innovációs Központ, Diagnosztikai Csoport

***Csorba Tibor*, (NE8)**
szaker.henrik.mihaly@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Növénybiotechnológiai Főosztály, Növényi virológiai Csoport

***Csorvási László Ábel*, (-)**
trade@tomtec.hu
Tomtec Plastics Kft.,

***Csuka Pál*, (ME1)**
csuka.pal@mail.bme.hu
BMGE Vegyészmérnöki és Biomérnöki Kar, Szerves Kémia és Technológia Tanszék, Bioorganikus Kutatócsoport

D

***Dankó Tamás*, (NP7)**
danko.tamas@agrar.mta.hu
MTA ATK Növényvédelmi Intézet, Növényvédelmi Intézet

***Demián Emese*, (NE1)**
demian.emese@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Diagnosztikai Csoport

***Dénes Lilla*, (ÁE2)**
denes.lilla@gmail.com
Állatorvostudományi Egyetem, Patológiai Tanszék

***Dóczi Róbert*, (NP16)**
doczi.robert@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényi Molekuláris Biológia Osztály

***Dósa Álmos*, (GP3)**
dosa.almos@vimspectrum.ro
Vim Spectrum Pharmaceuticals,

E

***Erdei Anna Laura*, (-) -**
MTA ATK Növényvédelmi Intézet, Állattani Osztály

***Erdős Gábor*, (BPE1)**
gerdos@caesar.elte.hu
ELTE Természettudományi Kar, Biokémiai Tanszék

***Éva Csaba*, (NE2, NP14)**
eva.csaba@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Alkalmazott Genomikai Osztály

F

***Faragó Dóra*, (NE3)**
farago.dora@brc.mta.hu
MTA SZBK, Arabidopsis Molekuláris Genetikai Csoport / Növénybiológiai Intézet

***Farkas Csilla*, (MP3)**
csilla.farkas@etk.szie.hu
Széchenyi István Egyetem, Élelmiszertudományi Kar, Biomérnöki és Folyamattervezési Kutatási Központ, Sör-és Szeszipari Tanszék

***Farkas Emese*, (GP4)**
farkas.emese@mail.bme.hu
BMGE Vegyészmérnöki és Biomérnöki Kar, Szerves Kémia és Technológia Tanszék

***Fehér Attila*, (NP2, NP12)**
feher.attila@brc.mta.hu
SZTE Természettudományi és Informatikai Kar, Növénybiológiai Tanszék

***Fehér Csaba*, (MP1, MP13)**
csaba_feher@.mail.bme.hu
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Fehér Péter Árpád*, (ÁP3, ÁP4, ÁP10)**
feher.peter@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Genomikai Főosztály

***Fekete Zsófia*, (NPE2)**
fekete.zsofia@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Növényi Szövettenyésztési Csoport

G

***Gabri Evelyn*, (GP5)**
gabri.evelyn@ttk.mta.hu
MTA TTK, Enzimológia Intézet

***Galiba Gábor*, (NP8, NP16)**
galiba.gabor@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényi Molekuláris Biológia Osztály

***Gierczik Krisztián*, (NP8)**
gierczik.krisztian@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényi Molekuláris Biológia Osztály

***Gócza Elen*, (ÁE4, GE4)**
gocza.elen@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Állatbiotechnológia Főosztály

***Gulyás Andrea*, (NP9)**
gulyas.andrea@agr.unideb.hu
Debreceni Egyetem AKIT, Nyíregyházi Kutatóintézet

***Gyarmathy Gergő*, (GE2)**
gyarigeri12@gmail.com
SZTE Természettudományi és Informatikai Kar, SzBK, Növénybiológiai Intézet

H

***Halasy Viktória*, (NP10)**
viktoriahalasy@freemail.hu
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék

***Hamar Renáta*, (-) -**
ELTE Természettudományi Kar, Genetika Tanszék

***Hanász Alexandra*, (NP11)**
hanasz@agr.unideb.hu
Debreceni Egyetem AKIT, Nyíregyházi Kutatóintézet

***Harami Gábor*, (ME2)**
harami@caesar.elte.hu
ELTE Természettudományi Kar, Biokémiai Tanszék

***Heltai Botond István*, (ÁP3, ÁP4, ÁP10)** *heltai.botond@abc.naik.hu*
Nemzeti- Agrárkutatási és Innovációs Központ, Genomikai Főosztály

***Hidvégi Norbert Tibor*, (NP9)**
hidvegi.norbert@agr.unideb.hu
Debreceni Egyetem AKIT, Nyíregyházi Kutatóintézet

***Horváth Ádám István*, (GP6)**
horvath1@caesar.elte.hu
ELTE Természettudományi Kar, Biokémiai Tanszék

***Horváth Brigitta*, (MP4)**
horvath.brigitta920108@gmail.com
DE Mezőgazdaság-, Élelmiszertudományi és Környezetgazdálkodási Kar, Wessling Hungary Kft.

***Horváth Gellért*, (-)** -
Nemzeti- Agrárkutatási és Innovációs Központ, Genetikai Főosztály, Növénygenomika és Növény-Mikroba Interakcio Csoport

***Hriczu Mónika*, (-)** *hriczum@richter.hu*
Richter Gedeon Nyrt.,

I, J

***Izrael Richard*, (GE3)**
izrael.richard@ttk.mta.hu
MTA TTK, Enzimológiai Intézet

***Jakab Ágnes*, (MP2, MP5)**
jakab.agnes@science.unideb.hu
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

K

***Kálmán Nikoletta*, (GP7)**
knikoletta@gmail.com
PTE Általános Orvostudományi Kar,, Biokémiai és Orvosi Kémiai Intézet

***Kanna, Sai Divya*, (MPE2)**
divya.kanna@brc.mta.hu
MTA SZBK, Növénybiológiai Intézet

***Kari András*, (MPE3)**
andraskari@yahoo.com
ELTE Természettudományi Kar, Mikrobiológiai Tanszék

***Kása Eszter*, (ÁP5, ÁP6)**
Kasa.Eszter@mkk.szie.hu
SZIE Mezőgazdaság- és Környezettudományi Kar, Akvakultúra és Környezetbiztonsági Intézet, Halgazdálkodási Tanszék

***Kaszler Nikolett*, (NP12)**
kaszler.n@gmail.com
SZTE Természettudományi és Informatikai Kar, Növénybiológiai Tanszék

***Katkó Tímea*, (NP18)**
katkotimea@gmail.com
Széchenyi István Egyetem, Kertészettudományi Kar, Növényélettan és Növényi Biokémia Tanszék

***Kerekes Adrienn*, (NPE3)**
adrienn.kerekes@gmail.com
Széchenyi István Egyetem, Kertészettudományi Kar, Genetika és Növénynemesítés Tanszék

***Kerekes Erika Beáta*, (MP6)**
kerekeserika88@gmail.com
SZTE Természettudományi és Informatikai Kar, Mikrobiológiai Tanszék

***Király Anita*, (MP7)**
kiraly.anita@science.unideb.hu
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

***Király Gábor*, (GP8)**
kiraly.gabor@science.unideb.hu
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

***Kiss Ádám Ferenc*, (GP5, GP9)**
kiss.adam.ferenc@ttk.mta.hu
MTA TTK, Enzimológiai Intézet

***Kiss Erzsébet*, (NPE3, NP1,NP9, NP13,)** *kiss.erzsebet@mkk.szie.hu*
SZIE Mezőgazdaság- és Környezettudományi Kar, Genetikai, Mikrobiológiai és Biotechnológiai Intézet

***Kocsy Gábor*, (-)**
kocsy.gabor@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényi Molekuláris Biológia Osztály

***Kollár Tímea*, (ÁP5, ÁP6)**
timi.kollar@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Halgazdálkodási Tanszék

***Kovács Szabolcs Cselgő*, (MP8)**
kovszasz@gmail.com
MTA SZBK, Biokémiai Intézet

***Kovács Szilárd*, (BE1)**
kovacssz@brc.hu
MTA SZBK, Növénybiológiai Intézet

***Kovács Szilvia*, (NE4)**
kovacs.szilvia@agr.unideb.hu
DE Mezőgazdaság-, Élelmiszertudományi és Környezetgazdálkodási Kar, Agrárműszerközpont

***Kovács Zsófia*, (MP9)**
zsoki14@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Genetikai, Mikrobiológiai és Biotechnológiai Intézet

***Kovács-Kotogán Alexandra*, (NP13)**
primula15@gmail.com
SZTE Természettudományi és Informatikai Kar, Mikrobiológiai Tanszék

***Kozák Dóra*, (MP10)**
kozakdori@gmail.com
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Kövesi Benjámin*, (ÁP7)**
benjamin.kovesi@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Takarmányozástani Tanszék

***Kurilla Anita*, (ME3)**
kurilla.anita@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, RNS Biológia Csoport

L

***László Anna*, (-)**
laszlo.anna@kertk.szie.hu
Széchenyi István Egyetem, Kertészettudományi Kar, Biometria és Agrárinformatika Tanszék

***Leitner Izabella*, (MPE2)**
leitner.izabella@brc.mta.hu
MTA SZBK, Növénybiológiai Intézet

***Lemaire Bernadett*, (MPE6)**
Bernadett.Lemaire@vizmuvek.hu
Fővárosi Vízművek Zrt.,

***Lenykó-Thegze Andrea*, (NE5)** *lenyko-thegze.andrea@agrar.mta.hu*
MTA ATK Mezőgazdasági Intézet, Génmegőrzési Osztály

M

***Maráz Anna*, (-)**
maraz.anna@etk.szie.hu
Széchenyi István Egyetem, Élelmiszertudományi kar, Mikrobiológiai és Biotechnológiai

***Márialigeti Károly*, (ME8, MPE3)**
marialigeti.karoly@ttk.elte.hu
ELTE Természettudományi Kar, Mikrobiológiai Tanszék

***Mátyás Kinga Klára (Petrovicsné)*, (NPE4, BP2)** *mkinga@georgikon.hu*
Pannon Egyetem Georgikon Kar, Növénytudományi és Biotechnológiai Tanszék

***Mezei Diána*, (ÁP8)**
mezeidiana810@gmail.com
SZTE Természettudományi és Informatikai Kar, Élettani, Szervezettani és Idegtudományi Tanszék

***Mitykó Judit*, (NPE2)**
mityko.judit@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Növényi Szövettenyésztési Csoport

***Molnár Andor*, (ÁPE1)**
andor.molnar@georgikon.hu
Pannon Egyetem Georgikon Kar, Állattudományi Tanszék

***Molnár Zoltán*, (-)**
molnar.zoltan@sze.hu
Széchenyi István Egyetem, Mezőgazdaság- és Élelmiszertudományi Kar, Növénytudományi Tanszék

***Molnár Zsófia*, (GP10)**
molnar.zsofia@mail.bme.hu
BMGE Vegyészmérnöki és Biomérnöki Kar, Szerves Kémia és Technológia Tanszék, Bioorganikus Kutatócsoport

***Moncsek Blanka*, (NE2, NP14)**
moncsek.blanka@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Alkalmazott Genomikai Osztály

N

***Nádai Tímea*, (NP16)**
(nadai.timea@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényi Molekuláris Biológia Osztály

***Nagy Flóra*, (GPE1)**
nagy.flora@mail.bme.hu
BMGE Vegyészmérnöki és Biomérnöki Kar, Szerves Kémia és Technológia Tanszék

***Nagy Lantos Balázs*, (-)**
trade@tomtec.hu
Tomtec Plastics Kft.,

***Nagy Valéria*, (NE6, NP15)**
nagy.valeria@brc.mta.hu
MTA SZBK, Növénybiológiai Intézet

***Nagy Zsófia*, (ME4)**
zsofianagy992@gmail.com
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Németh Áron*, (MPE1, MP10, MP12)**
naron@f-labor.mkt.bme.hu
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Németh Edit*, (NE7)**
nemeth.edit@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényélettani Osztály

***Németh Zoltán Bálint*, (GP11)**
nemeth.b93@gmail.com
ELTE Természettudományi Kar, Biokémiai Tanszék

***Nguyen, Bao Toan*, (MP11, MP19)**
toannguyen1905@gmail.com
Széchenyi István Egyetem, Élelmiszertudományi Kar, Biomérnöki és Folyamattervezési Kutatási Központ, Sör- és Szeszipari Tanszék

O

***Oláh Csilla*, (NP17)**
olahcsilla5@gmail.com
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék

***Oláh Kinga*, (NP17)**
okinga0820@caesar.elte.hu
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék

***Oláh Krisztina*, (MP12)**
olahkriszta95@gmail.com
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

P

***Pajkos Mátyás*, (BE2, BPE1)**
matyaspajkos@caesar.elte.hu
ELTE Természettudományi Kar, Biokémiai Tanszék

***Pákozdi Klaudia*, (MP15)**
pakozdiklaudia94@gmail.com
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

***Pálinkás Hajnalka Laura*, (GE4)**
palinkas.hajnalka@ttk.mta.hu
MTA TTK, Enzimológiai Intézet

***Papp István*, (NP18)**
papp.istvan@kertk.szie.hu
Széchenyi István Egyetem, Kertészettudományi Kar, Növényélettan és Növényi Biokémia Tanszék

***Paszerbovics Bettina*, (MP13)**
betyarka9931@gmail.com
BMGE Vegyészmérnöki és Biomérnöki Kar, Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék

***Pataki Bernadett*, (ÁE3)**
bebe.betti@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Takarmányozástani Tanszék

***Pataki Zoltán Gergő*, (BE3)**
zoltang.pataki@gmail.com
MTA TTK, Enzimológiai Intézet, Membránfehérje Bioinformatika Kutatócsoport

***Pénzes Máté*, (GP12)**
matthew.penzes@gmail.com
ELTE Természettudományi Kar, Biokémiai Tanszék, Motor Farmakológiai Kutatócsoport

***Perjéssy Judit*, (MPE4)**
perjessy.judit@eki.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Biológiai Osztály

***Petrányi Bálint*, (ÁP9)**
balint0705@gmail.com
Állatorvostudományi Egyetem, Szülészeti és Szaporodásbiológiai Tanszék

***Pham, Minh Tuan*, (MP14)**
phamminhtuan1508@gmail.com
Széchenyi István Egyetem, Élelmiszertudományi Kar, Biomérnöki és Folyamattervezési Kutatási Központ, Sör- és Szeszipari Tanszék

***Pintér Tímea*, (ÁPE2)**
timea.pinter90@gmail.com
Nemzeti- Agrárkutatási és Innovációs Központ,

***Podmaniczki Anna*, (NE6, NP15)**
podmaniczki.anna@brc.mta.hu
MTA SZBK, Növénybiológiai Intézet

R

***Rudnóy Szabolcs*, (NP17)**
rsz@ttk.elte.hu
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék

***Rugonfalvi Kiss Réka*, (NP19)**
rkreka@gmail.com
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék

S

***Salamon Pál*, (GP3, NP21)**
salamonpal@uni.sapientia.ro
PTE Természettudományi Kar, KDI

***Schiller Mátyás*, (BPE2)**
matyasschiller@gmail.com
Nemzeti- Agrárkutatási és Innovációs Központ, Mezőgazdasági Biotechnológiai Kutatóintézet

***Somoskői Bence*, (ÁP9)**
somoskoi.bence@univet.hu
Állatorvostudományi Egyetem, Szülészeti és Szaporodásbiológiai Tanszék

Sz

***Szabó Luca Krisztina*, (NP5, NP20)**
sz.luca.k@gmail.com
Nemzeti- Agrárkutatási és Innovációs Központ,

***Szabó Zsuzsa*, (MPE5, MP15)**
szabo.zsuzsa@science.unideb.hu
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

***Szaker Henrik Mihály*, (NE8)**
szaker.henrik.mihaly@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Növénybiotechnológiai Főosztály

***Szentgyörgyi Anna*, (MPE6)**
Anna.Szentgyorgyi@vizmuvek.hu
Fővárosi Vízművek Zrt., Central Wastewater Treatment Plant

***Szentgyörgyi Flóra*, (ME5)**
floraszentgyorgyi5@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Környezetbiztonsági és Környezettoxikológiai Tanszék

***Szentpéteri Viktor*, (MP16)**
szviki213@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Genetikai, Mikrobiológiai és Biotechnológiai Intézet, Mikrobiológiai és Környezettoxikológiai Csoport

***Szepesi Kinga*, (ÁP3, ÁP4, ÁP10)**
szepesi.kinga@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Alkalmazott Vad- és Haszonállat Genomika

***Szerényi Dóra*, (ÁPE3)**
dori.szerenyi94@gmail.com
SZIE Mezőgazdaság- és Környezettudományi Kar, Halgazdálkodási Tanszék, Akvakultúra és Környezetbiztonsági Intézet

***Szikora Bence*, (GPE2)**
szykora17@gmail.com
ELTE Természettudományi Kar, Immunológia Tanszék

T

***Tajti Judit*, (NE9)**
tajti.judit@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényélettani Osztály

***Takács Piroska*, (MPE7)**
tpiroska94@gmail.com
Pannon Egyetem Mérnöki Kar, Biomérnöki, Membrántechnológiai és Energetikai Kutatóintézet

***Tálas László*, (MP17)**
talas88laszlo@gmail.com
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

***Tamás László*, (NE2, NP10, NP19)**
tamasl@caesar.elte.hu
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék

***Temesváry-Kis Fanni*, (ME6)**
f.temesvary@gmail.com
MTA TTK, Enzimológiai Intézet

***Tóth Adrienn*, (ME7)**
toth.adrienn@etk.szie.hu
Széchenyi István Egyetem, Élelmiszertudományi kar, Hűtő- és Állatitermék Technológiai Tanszék

***Tóth Dániel Tamás*, (MP18)**
7danieltoth7@gmail.com
DE Természettudományi és Technológiai Kar, Mikrobiális Biotechnológiai és Sejtbiológiai Tanszék

***Tóth Fanni*, (ME8)**
fannytoth@ttk.elte.hu
ELTE Természettudományi Kar, Mikrobiológiai Tanszék

***Tóth Liliána*, (ME9)**
toth.liliana88@gmail.com
MTA SZBK, Növénybiológiai Intézet

***Tóth Máté*, (-)** -
Nemzeti- Agrárkutatási és Innovációs Központ, NFB

***Tóth Roland Imre*, (ÁE4)**
toth.toland.imre@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Állatbiotechnológia, AES Labor

***Tóth Tamás*, (NP21)**
toth.tamas@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Epigenetika Csoport

***Tóth Tímea*, (BE4)** *totht@brc.hu*
MTA SZBK, Biokémiai Intézet

***Török Dóra*, (ÁP9)**
Állatorvostudományi Egyetem, Szülészeti és Szaporodásbiológiai Tanszék és Klinika

***Tran, Thi Mai Anh*, (MP11, MP19)**
tranthimaianh_2807@yahoo.com
Széchenyi István Egyetem, Élelmiszertudományi Kar, Biomérnöki és Folyamattervezési Kutatási Központ, Sör- és Szeszipari Tanszék

***Truong, Hoang Duy*, (MP20)**
truonghoangduy@iuh.edu.vn
Széchenyi István Egyetem, Élelmiszertudományi Kar, Biomérnöki és Folyamattervezési Kutatási Központ, Sör- és Szeszipari Tanszék

***Turáni Melinda*, (GE5)**
femystra@gmail.com
DE Természettudományi és Technológiai Kar, Biotechnológiai és Mikrobiológiai Tanszék

V

***Vadászi Henrietta*, (GE6)**
vadasziheni@caesar.elte.hu
ELTE Természettudományi Kar, Biokémiai Tanszék

***Várallyay Éva*, (NE1, NP5, NP20)**
Varallyay.eva@abc.naik.hu
Nemzeti- Agrárkutatási és Innovációs Központ, Diagnosztikai Csoport

***Varga Julia*, (BE3, BP3)**
varga.julia@ttk.mta.hu
MTA TTK, Enzimológiai Intézet, Membránfehérje Bioinformatika Kutatócsoport

***Varga Mónika*, (NP22)**
monika.varga@brc.mta.hu
MTA SZBK, Növénybiológiai Intézet

***Vass Imre*, (PLE3)**
vass.imre@brc.mta.hu
MTA SZBK, Növénybiológiai Intézet

***Végh Balázs*, (NP23)**
vegh.balazs@agrar.mta.hu
MTA ATK Mezőgazdasági Intézet, Növényélettani Osztály

***Virág Eszter*, (NPE4, BP2)**
virag@georgikon.hu
Pannon Egyetem Georgikon Kar, Növénytudományi és Biotechnológiai Tanszék

W, Z

***Wirth Roland*, (ME10, ÁP8)**
wirth@bio.u-szeged.hu
SZTE Természettudományi és Informatikai Kar, Biotechnológiai Tanszék

***Zelenyánszki Helga*, (NP19, NP24)**
helga.zelenyanszki@ttk.elte.hu
ELTE Természettudományi Kar, Növényélettani és Molekuláris Növénybiológiai Tanszék