

DISSERTATION SUMMARIES

Microbial production, purification and structural elucidation as well as biological activity of ophiobolins

Ottó Bencsik

Department of Microbiology, University of Szeged, Szeged, Hungary

Ophiobolins are sesterterpene-type secondary metabolites produced by filamentous fungi belonging to the genera *Bipolaris*, *Cochliobolus*, *Drechslera* and *Aspergillus*. Until now 28 ophiobolin analogues have been described and assigned into 15 subgroups based on their characteristic structure in the carbon skeleton. The best known member of this family of compounds is ophiobolin A which has several biological activities such as antimicrobial, cytotoxic, nematocid or calmodulin antagonist.

In our work, initially an isocratic HPLC method was developed and optimized for the detection of the different ophiobolin compounds. The chromatographic parameters of the analysis were determined and the method was validated.

After that the ophiobolin A production abilities of numerous *Bipolaris* and *Cochliobolus* isolates representing 23 different species are characterized with the optimized HPLC method. Six of the tested isolates produced remarkable amounts of ophiobolin A (>1 mg/g [dry weight]). The ophiobolin secretion kinetics of the examined *Ascomycetes* were determined during the whole cultivation procedure. The strains aggregated into the following four groups based on their production abilities: I. the ophiobolin A production showed one maximum level in the range of 5-8 days; II. strains showed also one maximum level at days 5-7, however in case of these microorganisms strong decreasing tendencies were observed after the maximum production level; III. the production had two maximum level during the cultivation period at 3-5 days and 9-10 days; IV. strains did not show any ophiobolin A production under the applied cultivation conditions.

With the selected isolates the fermentation were carried out at larger scales to gain higher amount ophiobolin compounds for the further purifications. For this purposes a preparative HPLC method was also developed, which was combined with a foregoing and cost effective Solid Phase Extraction pre-cleanup procedures. Using our new preparative method we have successfully cleaned up three potential ophiobolin analogues from a *B. oryzae* strain. One of them was identified as ophiobolin A using the available analytical standard compounds (Sigma). The purities of our batch was determined by HPLC, and proved to be 95% for ophiobolin A, and above 94% for the two other compounds. The mass spectrometric examinations indicated that the isolated secondary metabolites have ophiobolin-like fragmentation patterns, and the recorded m/z values suggested their structures, however the identification of their identity requires the applications of further structure-determination methods.

During the investigations of biological activities of the purified compounds, agar diffusion and *in vitro* antagonism tests were used. Their antimicrobial activity were determined against a number of microorganisms and in some cases it showed remarkable antimicrobial activity.

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Supervisors: Tamás Papp, András Szekeres
E-mail: bencsikotto@gmail.com

Phenotypic heterogeneity provides evolutionary advantage under high level of stress

Zoltán Bódi

Csaba Pál Laboratory, Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

Isogenic individuals within a population generally show a certain level of phenotypic variability. This can be explained by the different nature of promoters which fluctuate between active and inactive status. If the switching time is long enough, then at a given time point a certain gene is active in specific cells, while this certain gene is inactive in other cells of the population, resulting a phenotypically heterogeneous population. The role of this phenomenon in evolutionary processes is highly debated and needs to be explained.

To shed light on the possible role of heterogeneity in evolution, two isogenic strains with significantly different heterogeneity of gene expression of a GFP fused efflux pump (Pdr5p) were established, by transforming two synthetic genomic constructs into the same yeast (*Δpdr5*, *Saccharomyces cerevisiae*) background. The high heterogeneous (HH) strain and the low heterogeneous (LH) strain have similar mean expression level of *PDR5-GFP*, while the coefficient of variation is different. Pdr5p is a good candidate to examine gene expression heterogeneity, since it provides resistance against a well-known antifungal agent, fluconazole.

Under low level of stress, the high heterogeneity of a population provides no advantage, however under high level of stress it can be

beneficial. This beneficial effect can be explained by the extensive size of the surviving sub-population in the high heterogeneous population. Evolutionary experiments were carried out in the presence of the antifungal agent, fluconazole. The ancestral strains were cultivated in parallel cultures in 96 well plates. 10^5 cells were serially transferred into fresh medium in the adaptation experiment using a constant level of fluconazole. In contrast, 10^7 cells were serially transferred in the adaptation experiment using a gradually increasing level of fluconazole.

After 100 generations, there was no difference in the evolutionary adaptation rate of the different heterogeneous strains, which suggests a heterogeneity-independent adaptation. The high heterogeneity provides an advantage when the population faces a higher selective pressure: the survival subpopulation is greater which provides an increased chance of accumulation of beneficial mutations. The bistable system remained the same after the evolutionary experiment; therefore, the acquired resistance of the HH strain is presumably caused by adaptive beneficial mutations.

We suggest that these beneficial mutations interact with the synthetic construct. The mean expression did not change, but the coefficient of variation increased after 100 generations. For the first time, our results provide experimental evidence that phenotypic heterogeneity of an isogenic population can contribute to adaptive advantage under a high level of stress. In sharp contrast, under a low level of stress this enormous advantage vanished.

Supervisor: Csaba Pál
E-mail: bodi.zoltan@brc.mta.hu

The role of ATAC histone acetyltransferase complex on steroidogenic gene expression

Barbara Nikolett Borsos

Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary

The *Sf-1* (steroidogenic factor 1) plays an important role in steroidogenic gene expression and also in the adrenogonadal development. *Sf-1* and similarly its *Drosophila* orthologue the *ftz-f1* transcription factor belongs to the nuclear hormone receptor family. The transcriptional activity of *Sf-1/ftz-f1* is controlled by posttranslational modifications. Phosphorylation at Ser203 and acetylation by GCN5 and p300 enhance *Sf-1* function.

The *Sf-1* shows tissue specific expression (adrenal cortex, testis, ovary, hypophysis, ventromedial hypothalamus, skin and spleen) and its mutation, absence or in some cases, overexpression can lead to tumor formation.

Recently we have reported that the lack-of-function mutations of the GCN5 histone acetyltransferase (HAT)-containing ATAC complex influence steroid biosynthesis. In contrast, the lack of the other GCN5-containing HAT complex, SAGA has only a mild effect on steroid biosynthesis. The mechanism by which ATAC affects steroid synthesis, however, remains to be discerned. The two most probable scenarios could be that ATAC influences the transcription of genes involved in steroid hormone biosynthesis directly by histone acetylation at their promoters, or that it acetylates FTZ-F1/SF1 and by this regulates the transcription of steroid converting gene indirectly.

We demonstrated that *Halloween* gene expression is detectable and modified by protein acetylation in S2 insect cells. We found that the stability of FTZ-F1 was increased after treatment of TSA (histone deacetylase inhibitor). Furthermore, we established that the overexpression of *ftz-f1* significantly increases the expression of *Halloween* genes in the *Drosophila* S2 embryonic cell line.

We performed chromatin immunoprecipitation experiments to answer whether histone acetylation has a role in steroid hormone biosynthesis. We found that H4K5 acetylation can be observed at the regulatory regions of *disembodied (dib)* and *shade (shd)* *Halloween* genes, while we did not detect H3K9 acetylation at any regions of these genes. In contrast to that, H3K9 acetylation can be observed at the initiator region of the mammalian *Cyp11a1* gene, while H4K5 acetylation can be detected at its promoter and initiator regions.

Based on our findings we conclude that the ATAC HAT complex plays a role in *Drosophila* steroid hormone biosynthesis through histone acetylation. To provide further proofs to this conclusion we continue our studies with the aim to detect the presence of the ATAC complex at the regulatory regions of the *Cyp11a1* gene.

Supervisor: Imre Miklós Boros
E-mail: riddles86@gmail.com

Characterization of different BRCA1 and BRCA2 variants and their interaction with the DNA damage tolerance pathway

Márton Zsolt Enyedi

Mutagenesis and Carcinogenesis Research Group, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

BRCA1 and BRCA2 proteins are involved in control of homologous recombination and double-strand break (DSB) repair in response to DNA damage. DSBs are generated naturally when replication forks encounter blocking lesions. Stalled replication forks can activate the DNA damage tolerance pathway that takes the replication machinery through the damaged site. In other case the replication fork can be rescued by recombination dependent mechanisms, which, however, have a potential for DNA rearrangements: nonhomologous end-joining and homologous recombination. In normal cells a delicate balance of damage bypass and homologous recombination can ensure cell survival and at the same time effectively prevent increased mutagenesis. However, mutation in genes affecting one of these pathways results in high degree of mutagenesis and frequent gross chromosomal rearrangements leading to cancer.

Mutations in *BRCA1* and *BRCA2* account for 20-40% of families with hereditary susceptibility to breast and ovarian cancer. Such mutations are located throughout the genes and typically result in premature translation termination. Structural and functional changes of mutated proteins caused by different *BRCA1* mutations are not identical and can lead to various phenotypes of cancers (genotype-phenotype correlations). For this reason, clinical presentations, outcome and response to treatment of tumours can differ significantly depending on the type of mutations. Therefore, there is currently a need to study the genotype-phenotype correlation among different mutations in *BRCA1* and *BRCA2* genes.

To face this challenge we developed a new method for next generation sequencing of *BRCA1* and *BRCA2* genes. The methodology relies on a multiplex PCR amplification of the two genes combined with enzymatic fragment library preparation. A training set of samples was used to optimize and to validate the performance of the workflow. The method was successfully validated being suitable for the detection of mutations, small insertions and deletions specific for the hungarian population. We also plan to characterize the unclassified and newly discovered mutations in sensitivity and mutagenesis assays. For this we use *BRCA1* and *BRCA2* mutant cell lines, in which we knock down the expression of DNA damage tolerance genes, and examine their sensitivity to various DNA damaging agents. Gaining more insight into the interaction of BRCA1 and BRCA2 with other players of DNA repair is important for understanding the molecular basis of genome stability and carcinogenesis.

Supervisor: Lajos Haracska
E-mail: enyedi@brc.hu

Regulation of gene expression by cis acting chromatin elements. Investigation of long-range promoter-enhancer interactions in the *Ubx* domain in *Drosophila*

Dávid Farkas

Laboratory of Chromatin Structure and Gene Expression Research, Developmental Genetics Unit, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

In the post-genomic era, one of the main challenges facing biology is answering the question of how different cell types and cell lineages, deriving from the zygote during development, utilize the very same genome differently. Part of the answer must be sought at the level of chromatin structure. The alteration of the chromatin structure is a substantial process of epigenetic regulation of gene expression. In *Drosophila* the *bithorax complex* (BX-C) is an exquisitely convenient model system to study epigenetic regulation.

In *Drosophila* the POLYCOMB group proteins are responsible for maintaining inactive chromatin conformation of numerous key regulator genes. The mechanism of repression and the *Drosophila* POLYCOMB group proteins are evolutionally conserved. The long-term silencing effect is amounted to the condensation of the target gene's chromatin structure. The *Polycomb* regulation depends on special DNA sequences called Polycomb Response Elements (PREs). The PREs are able to interact with each other over large distances; they were mostly studied in transgenic constructs. With a new gene conversion method developed in our laboratory, we are able to modify and study PREs *in situ*. We focus on the well-known *bx*d PRE.

Our aims: 1. to investigate the enhancer sets localized in the proximal and distal subdomain of the functionally divided *bx*d cis-regulatory region; 2. to study the effects of a built in boundary region and the separation of the PRE on the *Ubx* promoter and on the reporter gene; 3. to identify sequences on the homolog chromosome that affects the expression level of the integrated *Gal4-VPI6* reporter gene and investigate the role of the *bx*d PRE in this *cis-trans* communication.

Supervisors: László Sipos, Izabella Bajusz
E-mail: farkas.david@brc.mta.hu

Isolation, taxonomy and ecophysiological characterization of endophytic fungi from *Ambrosia artemisiifolia*

Mária Fehér

Department of Microbiology, University of Szeged, Szeged, Hungary, Department of Ecology, University of Szeged, Szeged, Hungary

Ambrosia artemisiifolia (common ragweed) causes a serious health and environmental problems all over Hungary. Therefore the knowledge of its ecology, life-style and spreading is essential. Common ragweed can live with endophytic fungi in mutualistic relationship, without they causes negative symptoms to ragweed, although these fungi belong to plant pathogen genera such as *Alternaria*, *Fusarium*, *Mucor* and *Penicillium*. The advantage of them is that can help the host plant to uptake nutrients such as phosphorus and nitrogen, and secrete toxic alkaloids to protect the host plants against herbivores.

The goals of this research were to isolate and identify endophytic fungi from roots, stems and leaves of common ragweed deriving from typical sandy soil habitat types of Southern Great Hungarian Plain: grassland, field and abandoned field. Furthermore we described the most common endophytic fungal genera, which could help the life of the *Ambrosia* plants, from different habitats (field, abandoned field, lawn and roadsides). In addition we characterized the fungal isolates, measured their secreted enzymes: cellobiohydrolase, xylanase, β -1,3-glucanase, laccase, β -glucosidase, trypsin, chymotrypsin, cellulase, exochitinase, lipase and pectinase.

Endophytic fungi were isolated from symptomless *Ambrosia* roots, flowers and seeds from different habitats in Szolnok and near Maros in 2010, Kiskundorozsma and Ásotthalom in 2011. We made culturing from roots, leaves and flowers of common ragweed after surface sterilization and described the fungal colonies (105 colonies) at least in genus level. Most frequently *Penicillium*, *Mucor* and *Fusarium* species were isolated.

For the molecular identification, total DNA was extracted from the growing cultures belonging to distinct filamentous fungal genera. The fungal strains were identified by morphological characters and by ITS sequence analysis.

We sequenced the ITS region of 23 fungal strains from different genera and the following species were identified: *Absidia repens*, *Alternaria alternata*, *Fusarium chlamydosporum*, *Fusarium oxysporum*, *Fusarium redolens*, *Fusarium solani*, *Fusarium subglutinans*, *Meyerozyma guilliermondii*, *Mucor circinelloides*, *Penicillium aurantiogriseum*, *Pithomyces chartarum* - teleomorph: *Leptosphaerulina chartarum*.

In summary we can say that *Alternaria* and *Fusarium* species were the most abundant in flowers and seeds also, not only in the roots. *Alternaria* spores, linked to any part of the plant: adhered and germinated, could be seen by microscopical investigations. We identified *Alternaria* from *Ambrosia* seeds after aggressive surface sterilization, at first time. The activity of extracellular enzymes (laccase, cellobiohydrolase, exochitinase, β -1,3-glucanase, β -glucosidase, trypsin and lipase) activity of the fungal strains (*Alternaria*, *Fusarium*, *Penicillium* and *Mucor* genus) were also examined copper sulphate containing glucose minimal liquid medium. We experienced more increasing activity of enzyme activities in the case of the isolates belonging to the *Alternaria* and *Penicillium* genera. We ascertained that the endophytic fungal isolates could secrete cellobiohydrolase, xylanase, β -1,3-glucanase, laccase, β -glucosidase, trypsin, chymotrypsin, cellulase, exochitinase, lipase and pectinase enzymes.

Our data can contribute to more knowledge of endophytic fungi, and can help to the development of biocontrol techniques, which can solve the environmental friendly reduction of *Ambrosia artemisiifolia*.

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Supervisors: László Körmöczi, László Manczinger
E-mail: Feher.Maria@stud.u-szeged.hu

The molecular mechanism of sulfanilic acid biodegradation

Botond Hegedűs

Department of Biotechnology, University of Szeged, Szeged, Hungary

Sulfanilic acid (SA) is produced by the industry in large amount and – as a consequence of careless handling - it often appears in the wastes and environment. Because of its toxic properties, it should be eliminated from the natural milieu. Biodegradation is a natural environmentally sound solution for detoxification of our ecosystems.

Novosphingobium subarcticum SAI, a strain isolated from a contaminated industrial area, is a strictly aerobic chemoheterotrophic bacterium with astonishing metabolic versatility. Out of numerous aromatic compounds it can utilize sulfanilic acid as a sole carbon, nitrogen and sulfur source. Our previous studies identified the enzymes involved in the degradation of sulfanilic acid. The enzymes taking part in the

first hydroxylation reaction might be part of a membrane associated protein complex, which would be a notable advantage if we consider that they are participating in the bioconversion of a highly toxic substrate.

Recently, we sequenced the genome of this strain by new generation genome sequencers. Analysis of the genome sequence revealed that the sulfanilic acid catabolic genes are found in separate operons. Further studies on the promoters of these operons disclosed a well-regulated system which is sensitive to the nutrition, growth conditions.

Whole cell transcriptional analyses have been recently performed, which confirmed the previous findings. Additionally, several new SA inducible genes were also identified, which are likely related to the sulfanilic acid metabolism: their gene products might participate in the uptake of sulfanilic acid, in the increased export of the sulfate released from sulfanilic acid. Moreover, elevated expression of iron transport proteins were also observed, which might be a concomitant of highly expressed iron containing enzymes.

Our results suggest a well-regulated complex pathway for degradation of sulfanilic acid, in which the essential step is made by a sensitive membrane associated complex responsible for the coupled uptake and conversion of the substrate.

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Supervisor: Gábor Rákhely
E-mail: rakhely@brc.hu

Selenium- and zinc-induced stress responses in *Arabidopsis thaliana* and *Pisum sativum*. Possibilities of biofortification

Nóra Lehotai

Department of Plant Biology, University of Szeged, Szeged, Hungary

Cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), mercury (Hg) and arsenic (As) are worldwide the main contaminant metals. Their long-term deposition in soil can lead to accumulation, transport and biotoxicity because of their mobility and bioavailability. Some plants are able to take up heavy metals from soils offering possibilities for phytoremediation. However, most of the plants are sensitive to the excess of heavy metals. Zinc is an essential element for plants, animals and humans and plays necessary role in e.g. the enzyme activation, protein synthesis and carbohydrate, nucleic acid and lipid metabolism. Compared to zinc as a heavy metal, selenium (Se) is a metalloid element with large similarities to sulphur. Selenite and selenate are the main inorganic Se species present in soil and are easily processed or transferred. Selenium's necessity for plants is still questionable, on the other hand, for humans and animals it is a crucial trace element.

The main goal of this study was to investigate the short- and long-term effects of Zn and Se treatments on *Arabidopsis* morphology and the involvement of the hormonal and signalling system in this process. Modern agriculture must pay attention to the correct essential nutrient intake, since plant-based foods should be sources of all natural minerals, which are necessary for animals and humans. Therefore, also biofortification studies were carried out using pea plants.

In *Arabidopsis*, higher Se concentrations reduced primary root development, which can be an adaptation process of the plant. This reorientation of means from development for protection mechanisms ensures a better survival. Exposure of selenium disturbs protein synthesis, directly leading to cell death in the primary root meristem and growth inhibition. The hormonal balance of the root system is also affected by selenium. The Se-induced hydrogen peroxid (H_2O_2) can reduce auxin-responsive gene expression during early development, while nitric oxide (NO) inhibits auxin transport in older roots and the decrease of root auxin level results in growth inhibition. Cytokinin responsive gene expression was also enhanced by Se, which lead to growth inhibition. The selenite-induced enhancement of ethylene biosynthesis may cause cell death resulting growth hindrance. There is no regulatory link between ethylene and NO under Se exposure. The optimal level of H_2O_2 is necessary for Se tolerance and NO overproduction in *Arabidopsis* roots ensures Se tolerance.

In pea plants, results showed that selenium and zinc caused very different alterations in the development and morphology of pea plants: since Se inhibited the growth, brought forward and shortened the flowering period, Zn treatment proved to be advantageous with the increase of morphological parameters, resulted in the same long flowering period and crop production as it was observed by the control. The enzymatic background reflects to different adaptation strategies as response for the added treatments. Both selenium and zinc were effective in the stimulation of pesticide-dependent glutathione S-transferases and altogether, the shoot system was more sensitive, the activity of the antioxidant defense system was elevated. ICP results present an improved uptake of selenium and zinc in the new seeds, however the accumulation of selenium was more pronounced. Both elements accumulated in the root system at the highest level, furthermore in the case of Se this accumulation was ten times higher compared to the shoot data.

Based on the results, we could point out the effect of Se/Zn excess on the development of *Arabidopsis* and pea plants. The results can contribute to the better understanding of the hormonal and signalling background mechanisms of stress-induced morphogenic responses. Our biofortification studies on the food plant, pea may offer a solution against nutrient deficiencies, since the accumulation of the added treatments did happen and were presented in the crop.

Supervisors: Zsuzsanna Kolbert, László Erdei
E-mail: lehotai.nora@gmail.com

Examination of melanoma tumor-host relationship

Annamária Marton

Laboratory of Cytokine Research, Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

Metastatic melanoma (MM) is an especially aggressive skin cancer. The mechanism of its rapid metastasis formation, high genetic variability and effective immune escape mechanisms are not explained yet. The NF- κ B signal pathway plays a complex and major role in malignant diseases. Up-regulated NF- κ B activity is frequently detected in various tumors, and it is implicated in many facets of the malignant behavior, including tumor immune escape, invasion, angiogenesis and metastasis, and chemotherapy resistance. Chemotherapy might further increase the elevated NF- κ B activity of tumor cells, thus protecting them from chemotherapy-induced cell death. Among the potential mediators, tumor-derived exosomes might also contribute to tumor escape.

Our first hypothesis was that NF- κ B inhibitor vanillin analogs might have an anti-tumor effect, and they may also synergize with anti-cancer drugs. Blocking of the NF- κ B signal pathway by vanillin analogs might have a complex anti-tumor effect. In the first step of our project, we have tested the *in vitro* cytotoxicity and NF- κ B inhibitory effect of a panel of vanillin analogs on the B16 mouse melanoma cell line. Based on the results, we have selected the most promising analog, ortho-vanillin (QL7), for the *in vivo* experiments. Testing the second hypothesis, we explored the interaction of melanoma cell derived exosomes (mcd) and their microenvironment. We investigated how mcd-exosomes influence CD4+ T cell proliferation induced by bone marrow derived dendritic cells. We quantified the NF- κ B activation in mature macrophages stimulated with mcd-exosomes, then we analyzed their cytokine/chemokine profile.

In *in vitro* cytotoxicity assay we found that the vanilloid analogue QL7 reduces cell proliferation after 48h incubation. Treating the cells with QL7 plus doxorubicin induced cell proliferation, while the doxorubicin-induced by 6 h NF- κ B activation was suppressed by QL7. In animal experiments we found that the QL7 administered together with cyclophosphamide reduces the primary tumor size. We observed that mcd-exosomes help the maturation of dendritic cells, and enhance T cell proliferation induced by the treated dendritic cells. The exosomes also activated macrophages, as measured by NF- κ B activation. The cytokine and chemokine profile of macrophages treated with mcd showed marked differences from those induced by either LPS or IL-4.

Our results suggest that 1) NF- κ B inhibitor vanillins have an anti-tumor effect, and 2) exosomes may play a role in the tumor progression and metastasis via supporting tumor immune escape mechanisms.

Supervisors: Csaba Vizler, Krisztina Buzás
E-mail: csaba.vizler@brc.mta.hu; kr.buzas@gmail.com

Characterization and identification of the molecular interaction partners of an actin regulating protein

Ede Migh

Laboratory of Actin Cytoskeleton Regulation, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

The formin proteins are an important and evolutionarily well conserved class of actin binding proteins with essential biological functions, including cell division, cell migration and organelle transport. In these processes the best understood molecular role of formins is to promote the nucleation and elongation of unbranched actin filaments, although some formins have also been implicated in the regulation of microtubules. We have previously shown that the single *Drosophila* DAAM ortholog, dDAAM, is involved in multiple aspects of tracheal development and axonal growth regulation, however the molecular mechanisms underlying these morphogenetic functions remain to be uncovered. To gain a better understanding of the molecular functions of dDAAM, we aim to identify the protein interaction partners of dDAAM with biochemical and genetic methods. The biochemical interaction partners are aimed to be identified by affinity chromatography. To this end, we created a dDAAM-Flag fusion protein by tagging the *dDAAM* gene *in situ* by site specific mutagenesis. The purification of the dDAAM containing protein complex is carried out from the adult head that is easy to isolate and, based on our former observations, dDAAM is highly enriched in the brain.

Besides the interaction partners, we are also interested in the functional characterization of dDAAM. During the investigation of the *dDAAM^{Flag}* mutant strain we revealed the existence of a novel dDAAM isoform (dDAAM-PD) that is absent from the brain but enriched in muscles. Because *dDAAM* plays an important role in sarcomere formation, it is of main interest to understand the functional properties of dDAAM-PD that appears to be the major muscle isoform. To this end, we created *dDAAM-PD* specific overexpression and RNAi tools that will hopefully allow us to determine how this isoform contributes to the development of muscles and other tissues.

Supervisor: József Mihály
E-mail: migh.ede@brc.mta.hu

HMG-CoA reductases of *Mucor circinelloides*

Gábor Nagy

Department of Microbiology, University of Szeged, Szeged, Hungary

Mucor circinelloides is a carotene producing zygomycete, which is used as a model organism in the study of carotenoid biosynthesis. Carotenoids and other important isoprenoids of the fungal cell (such as ergosterol and the prenyl group of certain proteins) are synthesized in the acetate-mevalonate pathway. The central step in the pathway is the conversion of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonic acid catalysed by the HMG-CoA reductase enzyme.

The *M. circinelloides* genome contains three different HMG-CoA reductase genes (named as *hmgR1*, *hmgR2* and *hmgR3*), which were cloned using the sequence data available in the genome database of the fungus (<http://genome.jgi-psf.org/Mucci2/Mucci2.home.html>). We used the genes in gene expression studies to investigate their function. Relative transcription levels of the three genes during the life cycle and under different cultivation conditions (aerobic/anaerobic growth, different carbon sources, different temperature and salt stress) were analyzed by quantitative real-time PCR (qPCR). In these studies, *hmgR1* showed low relative transcription levels under all conditions, while *hmgR2* showed high transcription levels under all aerobic conditions. Under anaerobic condition, transcription of *hmgR3* increased significantly.

We built three different expression vectors (pNG1, pNG2 and pNG3 containing the genes *hmgR1*, *hmgR2* and *hmgR3*, respectively) and used PEG mediated protoplast transformation to elevate the copy number of the genes. The carotenoid production and the sensitivity of statins changed after elevating the copy number of the genes. Enhanced expression of *hmgR2* increased the amount of the ergosterol in the transformants. Elevated copy number of *hmgR3* affected the carotene production and the sensitivity of statins in the highest degree among the different types of transformants.

We used antisense RNA (asRNA) mediated gene silencing to investigate other function of each gene. Three different vectors (pAS1, pAS2 and pAS3) were built containing antisense DNA fragments of *hmgR1*, *hmgR2* and *hmgR3*, respectively between the promoter and terminal region of glyceraldehyde-3-phosphate dehydrogenase. After PEG mediated protoplast transformation, transformants were isolated (MS12-pAS1, MS12-pAS2 and MS12-pAS3). Macro- and micromorphology, carotene and ergosterol content and the growth rate were examined in the resulting transformants. Growth rate, germination of spores and ergosterol content decreased in the transformants MS12-pAS3. Moreover, transformants showed altered morphology with swollen, frequently branching hyphae indicating a possible role of *hmgR3* in the mycelial development. In the MS12-pAS2 transformants, ergosterol content also decreased, but the morphology did not change.

Our results suggest that *hmgR2* may play an important role in the general isoprenoid metabolism and highly expresses under aerobic conditions. According to the transformation and qPCR studies, *hmgR3* seems to have role in the mycelial development, carotene biosynthesis and may be necessary for the sensing of the oxygen concentration of the environment. Moreover *hmgR3* may necessary to the germination of sporangiospores and apoptotic processes.

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Supervisor: Tamás Papp
E-mail: nagygab86@gmail.com

TAF10 proteins indicate structural and functional links between histone acetyltransferase and basal transcription factor complexes

Zoltán Gábor Páhi

Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary

TATA-binding protein associated factors (TAFs) have been identified as subunits of the TFIID basal transcription factor complex required for RNA polymerase II initiation. More recent studies indicate that TAFs are also present in histone acetyl transferase complexes, which regulate transcription initiation and the organization of the chromatin structure. This observation raises the possibility of complex „transmutation” by which due to changes in subunit composition one type of multiprotein complex is converted to an other type as transcription initiation is progressing.

Of the two *Drosophila* GCN5 histone acetyltransferase (HAT)-containing complexes SAGA and ATAC, TAF10 subunits are present in the former while they are missing from the latter. Despite that we found that the gene expression alterations in *taf10* mutants are very similar to those observed in ATAC subunit (Ada2a, Ada3) mutants. First, we aimed to find out whether only *taf10* mutants have similar gene expression alterations to ATAC mutants or other TAFs mutants also show the ATAC specific gene expression patterns. For this we studied the gene expression pattern of *Drosophila* stocks in which *taf5*, *taf10* or *taf8* was downregulated by RNAi.

We have recently shown that *Halloween* genes, which are expressed in the prothoracic gland and regulate ecdysone synthesis are

regulated by the ATAC HAT complex. As a result of ecdysone synthesis failure ATAC mutants arrest development at the larval-prepupal transition though they do not present any evident defect during larval development. We silenced *taf5*, *taf8*, *taf10* genes specifically in the ring gland where ATAC-regulated ecdysone synthesis occurs at late larva stage and observed developmental arrest before the prepupal transition, while there was no effect detectable on the other larval developmental stages. This phenotype is similar to that seen in ATAC mutants. 20-hydroxyecdysone feeding rescue TAF mutant L3 stage larvae and they reach pupa stage. These data indicate that TAFs influence the ecdysone synthesis similarly as it was observed in ATAC-specific *Ada2a* mutants. Furthermore, decreased expression of TAF proteins in the wing discs results in notched wing phenotype, which suggests that similarly to ATAC, TAF proteins also play role in apoptosis induction.

Our data suggest a functional interconnection between the ATAC HAT complex and the basal transcription factor TFIID. By further studies we aim to elucidate the details of the structural and functional interrelationship of the two complexes.

Supervisor: Imre Miklós Boros
E-mail: pahizol@gmail.com

The role of black *Aspergillus* species in food safety and human health as potential mycotoxin producers and opportunistic human pathogens

Gyöngyi Szigeti

Department of Microbiology, University of Szeged, Szeged, Hungary

Aspergillus is among the economically most important fungal genera. Species belonging to section *Nigri* (black Aspergilli) of this genus play an important role as human pathogens and mycotoxin producers, and in the food and biotechnological industries due to their ability to produce hydrolytic enzymes (lipases, amylases) as well as organic acids (citric acid, gluconic acid).

Aspergilli are one of the more difficult groups concerning classification and identification. New molecular approaches have shown that there is a high biodiversity, and the different species are difficult to be recognized based solely on their phenotypic characters. One of our aims was the production of a robust genus-wide phylogeny based on 6 gene sequences to get insight into the evolutionary relationships within this economically important genus. According to former studies, section *Nigri* was suggested to belong to subgenus *Circumdati*; however, our data indicate that sections *Cremeri* and *Nigri* are unrelated to subgenus *Circumdati*, and possibly represent new subgenera.

Black Aspergilli are commonly found as soil organisms decomposing dead plant residues, as postharvest contaminants, and as pathogens of several crops including grapes, almond and onion. Black Aspergilli are of concern not only for their ability to destroy several agronomically important food crops, but also due to their ability to produce several mycotoxins including ochratoxins and fumonisins. In our work we isolated black Aspergilli from raisins, onions, figs and dates and identified the strains at the species level by comparing their partial calmodulin gene sequences. Various black *Aspergillus* were isolated from raisins and figs, while only *A. awamori* and *A. tubingensis* were identified on onions and dates, respectively. Fumonisin contamination of the samples was examined by HPLC-MS/MS technique. Fumonisin were detected in all products in varying quantities. Several fumonisin isomers have been identified for the first time in black Aspergilli.

Nowadays *Aspergillus* species cause human infections more frequently. 92 clinical isolates from The Netherlands were assigned at the species level using sequence analysis of part of the calmodulin gene. 55 *A. tubingensis*, 21 *A. acidus*, 14 *A. niger* and 2 *A. awamori* isolates were detected. Antifungal susceptibility tests of the isolates are in progress. *Aspergillus* is also considered to be the predominant causative organism of otomycosis, with *Aspergillus niger* as the most frequently described species. We analysed black Aspergilli isolated from otomycosis cases in Iran and Hungary. The results indicate that *A. niger* is not the only black *Aspergillus* species involved in otomycosis cases: *A. awamori* and *A. tubingensis* are also able to cause ear infections. Antifungal susceptibility tests revealed that all isolates were highly susceptible to terbinafine, while exhibited moderate susceptibilities against amphotericin B and ketoconazole. *A. niger* and *A. awamori* were found to have higher MICs for ketoconazole than *A. tubingensis*.

Regarding the population structure of black Aspergilli, only limited data are available. We started to examine the structure of various black *Aspergillus* populations using molecular methods. Regarding the distribution of the mating type genes in different species, close to 1:1 ratios were observed in *A. niger* and *A. tubingensis* populations regardless of the origin of the isolates. However, most *A. awamori* isolates were found to carry the MAT1 idiomorph. Analysis of the genetic variability of the isolates by UP-PCR analysis is in progress.

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Supervisor: János Varga
E-mail: szigie@gmail.com

Aligned alteration of enteric neurons, smooth muscle cells and inflammatory markers involved in stricture formation in a rat model of Crohn's disease

Petra Talapka

Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary

Crohn's disease (CD) is a multifactorial, relapsing disorder with chronic inflammation involving all layers of the gut wall. The development of obstructive strictures associated with CD causes major complications in patients. Because no effective therapies are available to prevent stricturing we wanted to gain a better understanding of its pathogenesis by developing a rat model suitable to investigate the involvement of the enteric neurons, the intestinal smooth muscle cells (SMCs) and different inflammatory markers in the intestinal stricturing.

Colitis was induced by an enema of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 10 mg) in 25% ethanol. Tissue samples were taken from control, as well as once, twice and three times treated rats from the inflamed segment, and also proximal and distal to the inflamed segment of the colon in different time points between 2 and 120 days. Quantitative features of myenteric neurons were investigated after HuC/D immunohistochemistry. The expression of multiple inflammatory markers was determined by RT-PCR. The strictures were studied by transmission electron microscopy.

The number of myenteric neurons decreased significantly in all three colonic segments in the acute phase of inflammation. However, 8 days after the TNBS treatments no further changes in the neuronal number was detected until the end of the investigation. Strictures developed at 60th day after the first TNBS treatment and the frequency of strictures increased until day 120th. Thickened muscle layers, expanded intercellular spaces and matrix deposition characterized the strictures. Loosed SMCs with the morphological sign of apoptosis was frequently seen, while enteric ganglia were morphologically intact. HO-1 mRNA was upregulated in all samples from the TNBS-treated rats in the acute phase of the inflammation, and the HO-1 level remained high until day 120th. The increased activity of MMP9 after repeated treatments referred to severe local tissue injury. TGF- β 2, but not TGF- β 3 was expressed in each tissue samples from the rats with colitis. This expression profile of TGF- β isoforms is characteristic to CD.

The repeated induction of TNBS colitis enhanced intestinal stricturing making this rat model suitable to investigate its pathogenesis. Our preliminary findings indicate that aligned alteration of enteric neurons, smooth muscle cells (SMCs) and different inflammatory markers have a critical role in the development of intestinal strictures. After repeating TNBS treatments, decreased extension of mucosal inflammation was observed when compared to rats treated with TNBS only once. Therefore, a preconditioning effect of repeated TNBS treatment was suggested. Based on the evaluation of quantitative properties of the enteric neurons seemed that this preconditioning did not evolve in the enteric neurons. Ultrastructural morphometry revealed an increased amount of extracellular matrix deposition and increased number of SMCs with proapoptotic markers. Consequently, the distance between SMCs and myenteric ganglia increased, which might be responsible for the default innervation of SCMs and the formation of intestinal strictures.

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Supervisors: Éva Fekete; Mária Bagyánszki
E-mail: talapka.petra@gmail.com

Characterization of the innate and adaptive immune responses induced by the opportunistic human pathogen *Candida parapsilosis*

Adél Tóth

EMBO Candida Group, Department of Microbiology, University of Szeged, Szeged, Hungary

The genus *Candida* comprises more than one hundred species, of which less than 20 have been associated with human infections. Depending on the age group and geographical region, *C. parapsilosis* is the second or third most common species after *C. albicans* and *C. glabrata* causing invasive candidiasis. Although in recent years there has been a great progress in the understanding of immune responses induced by *C. albicans*, little is known about the immunity against *C. parapsilosis*.

During our study, we examined the innate and adaptive immune responses induced by *C. albicans* and *C. parapsilosis*. Firstly, we compared the cytokine responses evoked by *C. albicans* and *C. parapsilosis* using an *in vitro* model of human peripheral blood mononuclear cells (PBMCs). PBMCs were stimulated with heat killed *C. albicans* or *C. parapsilosis*, and the cytokine production was measured by enzyme-linked immunosorbent assay (ELISA). *C. parapsilosis* induced similar quantities of TNF α and IL-6, and slightly lower amounts of IL-1 β in human PBMCs compared to *C. albicans*. However, stimulation of PBMCs with *C. parapsilosis* resulted in higher IL-10 and lower IFN γ production compared to *C. albicans*, indicating a skewed T helper cell response. Furthermore, *C. parapsilosis* induced much lower IL-17 and IL-22 production compared to *C. albicans*. Following intracellular cytokine staining, flow cytometric analysis confirmed that the decreased production of IL-17 and IL-22 was in line with a lower number of IL-17 producing cells. Blocking of the three classical

MAP kinases (p38, ERK, JNK) resulted in decreased cytokine production after stimulating PBMCs with *C. albicans* or *C. parapsilosis*, indicating that these kinases are all involved in the signal-transduction following the recognition of the two *Candida* species. However, cytokine levels indicated that there are certain differences in the signal-transduction following the immune sensing of *C. albicans* and *C. parapsilosis* by PBMCs. Additionally, decreased cytokine production following the inhibition of Dectin-1 revealed that this receptor plays a role in the recognition of both *C. albicans* and *C. parapsilosis*. To further elucidate the immune responses evoked by *C. parapsilosis*, we examined the interactions of this pathogen with human primary monocytes-derived macrophages. As secreted fungal lipases have been shown to play an important role in pathogenesis, we compared the response of human macrophages to a wild type (wt) as well as a lipase deficient (*lip⁻*) *C. parapsilosis* strain that has been previously established in our lab. When co-cultured with macrophages, both strains induced a significant increase in the expression of TNF α , IL-1 β , IL-6, IL-8 and PTGS-2 (prostaglandin-endoperoxide synthase 2) genes in host cells after 12 hours, as determined by quantitative real-time PCR. Notably, macrophages stimulated with lipase mutant *C. parapsilosis* showed at least two-fold higher expression of these pro-inflammatory mediators compared to those infected with lipase-producing (wt) *C. parapsilosis*. Additionally, we examined the phagocytosis of wt and *lip⁻* *C. parapsilosis* strains by human PBMC-derived macrophages using quantitative imaging flow cytometry. We found that although after 2 hours both strains were phagocytosed to the same extent by host cells, the rate of internalization and phagolysosome fusion was higher in case of *lip⁻* *C. parapsilosis*. These findings confirm the role of fungal lipases as important virulence factors during *C. parapsilosis* infection and support the hypothesis that these microbial compounds have anti-inflammatory potential. Taken together, our results contribute to the better understanding of the immune response induced by *C. parapsilosis*, and highlight the role of fungal lipases during host-pathogen interactions.

Supervisor: Attila Gácsér
E-mail: toth.adel.86@gmail.com

Investigating the structure and the mechanism of action of *Neosartorya fischeri* antifungal protein

Máté Virágh

Department of Microbiology, University of Szeged, Szeged, Hungary

Today there is a substantial demand for new antimicrobial compounds because of the increasing number of fungal infections. The defensin-like antimicrobial peptides produced by filamentous fungi are interesting in this respect, because they can inhibit the growth of several filamentous fungi. Different defensin-like antimicrobial miniproteins have been isolated from seven ascomycetous filamentous fungal species (*Aspergillus clavatus*, *Aspergillus giganteus*, *Aspergillus niger*, *Fusarium polyphialidicum*, *Neosartorya fischeri*, *Penicillium chrysogenum*, *Penicillium nalgiovense*). Until now the *Penicillium chrysogenum* antifungal protein (PAF) and the *Aspergillus giganteus* antifungal protein (AFP) have been intensively studied. Although AFP and PAF generate similar symptoms in the susceptible organisms, they have different mode of actions. AFP disturbs the cell wall biosynthesis by specific inhibition of chitin synthases, and PAF evokes programmed cell death via G-protein signal transduction pathway. The *Neosartorya fischeri* antifungal protein (NFAP) consists of 57 amino acid residues and has a calculated molecular mass of 6625.5 Da and a pI of 8.93. Our *in silico* structure modelling revealed that NFAP contains five antiparallel β -strands connected by three loops, and showing a β -barrel topology in general, which is stabilized by three intramolecular disulfide bridges. Previously we demonstrated that NFAP effectively inhibits the growth of numerous filamentous fungi including human and plant pathogens and the model organism, *Aspergillus nidulans*. As in the case of similar proteins, the high yield production of NFAP is not resolved despite the available knowledge of the nature of its 5'-upstream transcriptional regulation elements in response to environmental signals and stress. For the future investigation it would be important that NFAP could be producible in a non-sensitive, easily fermentable, "generally recognized as safe" fungus. On the other hand the understanding the exact antimicrobial effect and the mode of action of NFAP are essential for its future practical applications.

For these reasons, we carried out the heterologous expression of the *nfap* gene in *Pichia pastoris* KM71H by using the pPICZ α A vector. After purification, the final yield of the hNFAP from 1000 ml ferment broth was 2.4 \pm 0.2 mg, which is twofold amount compared to the native producer, *Neosartoria fischeri* NRRL 1881. N-terminal sequencing experiments revealed that the first 5 amino acid residues of the purified heterologous protein is LEYKG (which corresponds well to the determined amino acid sequence of the native NFAP) and the different ion chromatograms from the mass spectrometry correspond six out of all peptides found by analysis of mass lists. Based on the signal dispersion of the amide region (6-10 ppm), it is proven that the protein exists in folded state. Tertiary structure determination needs further NMR investigations using isotope-labelled NFAP. The antifungal activity of the hNFAP was the same as described in the case of NFAP. Based on our previous studies, it seems that the antifungal mechanism of NFAP differs from what was described in the case of AFP and PAF. For revealing the exact mechanism of action of NFAP fluorescent microscopic investigations and antifungal susceptibility assays on *Aspergillus nidulans* mutants in protein kinase C/mitogen-activated protein kinase and cAMP/protein kinase A signalling pathway are in progress.

Our results provide basis for further applied research, *e.g.* developing new antimicrobial peptides in therapy, pest control and food preservation.

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Supervisors: Csaba Vágvölgyi, László Galgóczy
E-mail: viragh.mate@gmail.com

Opening of the blood-brain barrier for drug delivery to the brain: the effects of tesimalifene and short-chain alkylglycerols on brain endothelial cells

Fruzsina Walter

Laboratory of Molecular Neurobiology, Institute of Biophysics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary

The blood-brain barrier (BBB) forms a dynamic interface between the blood and the brain. It selectively regulates the transcellular and paracellular transport of molecules and passage of cells between the blood and the central nervous system. The BBB restricts drug penetration to the brain preventing effective treatment of several neurological diseases. Therefore it is an increasing need to find new ways to improve drug delivery to the brain. Brain capillary endothelial cells constitute the anatomical and functional basis of the BBB. One of the strategies to increase drug delivery to the brain is changing cerebral endothelial functions by opening the BBB through the modification of the paracellular or the transendothelial transport pathways.

In this study two agents, tesimalifene and short-chain alkylglycerols (AGs) were selected for detailed examination. Tesimalifene, a tamoxifen-related compound, has chemopotentiating properties in experimental and in clinical cancer studies. Treatment with tesimalifene caused temporary, acute CNS side-effects in patients indicating the opening of the BBB. Previous studies from our laboratory have shown that tesimalifene increases the permeability of the BBB in rats. Intraarterial injection of short-chain AGs, such as 1-*O*-pentylglycerol and 2-*O*-hexyldiglycerol, open the BBB and increase the delivery of molecules to rodent brain parenchyma *in vivo*. The mechanism underlying AG and tesimalifene-mediated modification of BBB permeability is still unknown. The aim of the present study was to test the direct effects of tesimalifene and AGs on barrier properties of cultured brain microvascular endothelial cells, a model of the BBB.

The triple co-culture BBB model was constructed on cell culture inserts using primary rat brain endothelial cells, rat cerebral glial cells and rat pericytes. Barrier integrity of the BBB endothelial monolayers was analyzed by transendothelial electrical resistance and permeability measurements. In addition to functional assays, toxicity tests, immunostainings for junctional proteins and freeze fracture electron microscopy were performed.

Short-term tesimalifene and AG treatment decreased the resistance of endothelial monolayers, and increased the permeability for fluorescein, a marker of paracellular flux. Tesimalifene also enhanced the transcellular transport of albumin. These short-term changes were accompanied by changes in cell morphology and immunostaining for junctional proteins. AG and tesimalifene-mediated increase in brain endothelial permeability was reversible. Short-term treatments did not alter the viability of brain endothelial cells. Tesimalifene did not affect the functions of P-glycoprotein, but decreased the activity of the multidrug resistance associated protein-1 and the production of nitric oxide in endothelial cells. Tesimalifene also altered the mRNA expression of several tight junction proteins measured by a custom Taqman gene array.

Our data support previous clinical observations and the results of animal experiments, and clearly indicate that AGs and tesimalifene increase the permeability of the BBB by directly acting on brain endothelial cell functions. Tesimalifene and AGs are promising adjuvants in the transient opening of the BBB for clinical use, especially for treatment of brain tumors.

Supervisor: Mária A. Deli
E-mail: walter.fruzsina@brc.mta.hu

Metagenomics of biogas producing microorganisms

Roland Wirth

Department of Biotechnology, University of Szeged, Szeged, Hungary

The production of renewable energy carriers is currently receiving increasing attention worldwide. Biogas is a promising technology as its production may combine the treatment of various organic wastes with the generation of energy. Biogas can be converted to heat and/or electricity, and its purified derivative, biomethane, is suitable for every function for which fossil natural gas is used today. The degradation

of organic materials by a microbial community is carried out under anaerobic conditions. The composition of this microbial consortium depends on various factors. A clear understanding of the organization and behavior of this multifarious community is crucial for optimization of their performance and attainment of the stable operation of the process. Classical microbiological methods are principally based on studies of isolated pure strains of microbes, and hence are of little help when the goal is elucidation of the relationships among members in this complex microbial consortium. The development of high-throughput sequencing technologies has opened up new avenues for such investigations. The „next generation” sequencing methods employ various chemical reactions for the rapid determination of DNA sequences. Huge databases and sophisticated bioinformatics are prepared to analyze the results. This metagenomic approach allows the real-time study of live consortia in various environments through identification of the members and/or determination of the relative abundances of particular physiological functions.

The aims of the study were to determine the possibility of applying the next-generation sequencing technologies for the characterization of the microbial consortium in different biogas fermenters and to find a novel substrate to replace expensive maize silage, which is commonly used to feed biogas fermenters today.

First the microbial composition of maize silage fed fermenter with an extremely parallel SOLiD™ type short-read DNA sequencing platform was determined. The results showed the members of the Firmicutes and Bacteroides phyla played the most important role in the hydrolysis of the plant biomass and in the secondary fermentation. In particular, many *Clostridium* species were identified, which possess cellulolytic and H₂-producing activities, both properties being apparently essential for the efficient degradation of the biomass. In the Archaea domain, Methanomicrobiales is the most abundant order that uses CO₂ as a carbon source and H₂ as an electron donor for methanogenesis. The results demonstrate the importance of the metabolism of hydrogen beside acetate within the biogas producing microbial community.

Unfortunately maize silage is an expensive substrate for biogas production. Therefore biogas power plants are looking for more economical substrates. Algae are promising candidates. Algae can produce biohydrogen and the remaining alga biomass is a good substrate for biogas fermentation. Thus more renewable energy can be obtained through the combination of these two technologies. We tested an algal mixture consisting of *Scenedesmus* and *Chlamydomonas* species. These algae contain large amount of starch that is a good substrate for the microbial community inhabiting the biogas fermentor. It was found that, depending on the starch content and the algal cell wall destruction methods, a higher concentration of biomethane as produced from microalgae relative to corn silage. Microbial composition of alga-fed fermenters were examined with the Ion Torrent™ next generation DNA sequencing platform. The result showed that the composition of microbial community was significantly changed in the microalgae fed fermenters relative to maize silage fed technologies. Instead of the Firmicutes and Bacteroides the Proteobacteria phylum dominated the Bacteria and in the Archaea domain the acetotrophic Methanosarcinales represented the overwhelming majority.

Supervisor: Kornél L. Kovács
E-mail: roland.w@freemail.hu