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The role of alternative histone usage in differentiated cell and tissue functions

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Histones play an essential role in the function of both dividing and post-mitotic cells through the regulation of chromatin related processes, such as replication, repair and gene expression. Any defect that affects the amount or structure of histone proteins can lead to cell death or to failures in division. Although several degenerative diseases and cancer types have been associated with histone dysfunctions, there are still many open questions about histone functions. In the focus of our interest is the role of replication indepent (RI) histones.

The *His4r* is the gene of an alternative form of the core histone *H4* in *Drosophila*. The gene is located outside the canonical histone cluster, it is present in the genome in one copy as typical alternative histones are, transcribed independently to the time of DNA replication, and has a regular eukaryotic gene structure. Unlike canonical histones, *His4r* contains an intron and the mRNA transcribed from it is polyadenylated. Nonetheless, the amino acid sequence of His4r protein is identical to that of the canonical H4, what poses the intriguing question that what could be the functional role of His4r.

In order to make possible distinquishing His4r from its identical canonical H4 counterpart we have created a transgenic *Drosophila* line in which the *His4r* gene is modified to produce His4r with a 3xFlag tag. Using this *Drosophila* line we analysed His4r expression in different tissues at several stages of fly development, and found that the ubiquitous expression of His4r becomes cell type-specific during neuronal development. Based on promoter analysis of *His4r*, and expression data from experiments of our own and others, we hypothesize that His4r may play a role in neuronal differentiation and environmental stress responses. To confirm this theory, we performed ChIP-seq experiment and found that His4r indeed showed an increased amount at inducible genes and genes controling neuronal differentiation. This genomic distribution raises the possibility that His4r may also play a role in the development of transcriptional memory. Experiments to verify this assumption are in progress.

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Agricultural recycling of spent mushroom compost following microbiological processing

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Besides spawn and mushroom compost, casing material with proper quality is also an important requisite of economical production of champignons (*Agaricus bisporus*). In mushroom growing houses, the compost colonized by the mushroom mycelia is covered by the casing material, the role of which is primarily to ensure the conditions for fruiting body formation and provide high water content. The nutrient-rich mushroom compost has low water retaining capacity, which is compensated by the casing material. The depletion of peat mines in Hungary and other European countries, as well as the environmental problems arising from peat mining lead to an emerging need for the development of alternative solutions for the production of high-quality casing materials. Due to its high fiber, as well as P, K, and N content, harvested mushroom compost may serve as an excellent raw material for the development of casing materials. The recycling of spent mushroom compost has been attempted in some cases by vermicomposting, and its microbiological re-composting might also be a possible alternative. The purpose of our work was the selection and characterization of fungal and bacterial strains that could be used for the controlled transformation of spent mushroom compost, thus recycling it as casing material.

A total of 32 bacterial and 23 fungal strains were isolated from samples deriving from experimental re-composting process

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of spent mushroom compost. Species identification of the bacterial isolates, performed by sequence analysis of a fragment of the *gyrA* gene amplified by the gyrA-F and gyrA-R primers revealed *Bacillus subtilis* (3), *B. licheniformis* (8) and *B. safenesis* (1), *Bacillus* sp. (1). The *Lysinibacillus fusiformis* (2), *L. macroides* (3), *Lysinibacillus* sp. (1), *Ochrobactrum anthropi* (1), *Alcaligenes faecalis* (1), *Lactococcus lactis* (1), *Lactobacillus paraplantarum* (1), *Microbacterium* sp. (1) strains performed by EUB primers (Eub 341-F and Eub 1060-R). while fungi were identified by ITS 1-4 (internal transcribed spacer) region as *Mortierella wolfii* (6), *Mucor circinelloides* (4), *Fusarium solani* (1), *Geotrichum candidum* (4), *Geotrichum* sp. (1), *Lichtheimia ramosa* (1), *Penicillium griseofulvum* (1), *Trichosporon asahii* (1), *Rhodotorula mucilaginosa* (1) and *Trichoderma harzianum* species complex (1). *Aureobasidium pullulans* (3) strains were previously isolated and deposited in a strain collection. The *Bacillus* and *Aureobasidium* strains were subjected to ecophysiological characterization and extracellular enzyme activity assays as well as seed germination experiments. The examined *B. subtilis*, *B. licheniformis* and *A. pullulans* strains were found to produce high lipase, protease and cellulase activities. The spent mushroom compost is being tested for use as casing layer in mushroom cultivation, as well as a plant growth medium. The application of the re-composted spent mushroom compost as casing resulted in 509 g white button mushroom crop, while the maximum mushroom yield using peat was 402 g in a potted cultivation experiment. Based on our results we conclude that the re-composted spent mushroom compost may be a suitable raw material for the production of casing layer for mushroom cultivation, at it may even contribute to increasing mushroom yield.

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Sigma-1 receptor agonist Fluvoxamine engages anti-inflammatory actions and decrease oxidative stress in TNBS-induced experimental colitis

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Inflammatory bowel disease (IBD) is a serious health issue of the gastrointestinal tract, which is manifested mainly in prolonged gut inflammation and an imbalance between free radicals and antioxidants. Considering the autoimmune nature of the disease complete recovery is still not possible, however several treatment options exist to alleviate its symptoms. Sigma-1 receptor (σ1R) is originally described by Martin et al. as an opioid receptor. Now it is suggested based on ligand binding affinity measurements that σ1R seems to possess a unique receptor class. σ1R is located in the mitochondria-associated endoplasmic reticulum membrane and it is responsible for Ca^{2+} homeostasis. Furthermore, it seems that the activation of the receptor has anti-inflammatory and antioxidant properties.

Therefore, our study aimed to investigate the effects of the activation of σ1R in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced experimental colitis. To this end, a single intracolonic (i.c.) TNBS administration was used in Wistar-Harlan rats to induce inflammation in the colon. Then to clarify the effects of σ1R activation in this inflammatory condition, i.c. administered fluvoxamine (FLV) was used as a σ1R agonist and BD1063 as an antagonist. A combination of the two ligands was also administered as a co-treatment for further clarification. Our radioligand binding studies with [^{3}H](+)-pentazocine ligand clearly showed the existence of the receptor in the colon and FLV treatment significantly increased the maximum binding capacity of σ1R compared to TNBS. Furthermore, FLV significantly reduced the colonic damage which effect was abolished by the co-treatment with BD1063 antagonist. Additionally, σ1R agonist FLV alleviated pro-inflammatory markers, such as myeloperoxidase (MPO) activity and interleukin-6 (IL-6), which effects were abolished by the administration of BD1063 antagonist. Furthermore, FLV was shown to decrease pro-oxidant factors, such as 3-nitrotyrosine (3-NT), and significantly increased glutathione (GSH) and peroxiredoxin-1 (PRDX-1) antioxidants. Taken together, the activation of σ1R through FLV administration seems to be a promising therapeutic approach against experimental IBD.

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Microtubule organizing centers contain testis-specific γ-TuRC proteins in spermatids of *Drosophila melanogaster*

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Centrosomes are the most studied Microtubule-Organizing Center (MTOC) in animal cells, they consist of a pair of centrioles surrounded by an amorphous pericentriolar material. Gamma Tubulin Ring Complex (γ-TuRC) is known as the main player for microtubules nucleation in MTOC. Proteins involved in the formation and the development of MTOC in *Drosophila* are conserved among eukaryotes. Mutations of many of MTOC proteins are known to cause diseases in human. γ-TuRC was studied in many *Drosophila* tissues but not in late spermatogenesis. Since later stages of *Drosophila* spermatogenesis are controlled mainly by testis-specific proteins, MTOC formation and cellular remodelling is an open question. Our aim was to study the γ-TuRC distribution and function during late stages of *Drosophila* spermatogenesis. γ-tubulin exists in two complexes in *Drosophila*: γ-tubulin Small complex (γ-TuSC) and γ-TuRC. γ-TuSC is smaller composed of two γ-tubulin and two Grip proteins: Grip84 and Grip91, while γ-TuRC composed of multiple γ-TuSC in addition of three or four γ-TuRC binding proteins: Grip128, Grip163, Grip75 and Grip71. We conducted phylogenetic analysis for the γ-TuRC component proteins, and we identified three testis-specific γ-TuRC proteins. γ-TuSC represented by t-Grip84 and t-Grip91 a paralogue of Grip84 and Grip91 respectively, the third one is t-Grip128 a paralogue of Grip128. This suggests the existence of testis-specific γ-TuRC (t-γ-TuRC). We analyzed the phenotype of the mutants of t-γ-TuRC genes. We found that t-Grip84 mutant is male sterile, t-Grip91 mutant is male semi-sterile and t-Grip128 mutant fertility is normal. We made transgenic lines and checked the localization of the tagged version of t-γ-TuRC proteins. They localize to the centriole adjunct after meiosis, and also to the nuclear tip during nuclear elongation and transfer to the surface of the mitochondria during cyst elongation. We analyzed the interactions of the t-γ-TuRC proteins biochemically and proved binding to γ-tubulin and Mzt1 and also the complex members to each other. Our results provided the first proof of the existence and essentiality of t-γ-TuRC, which can lead us to understand better the molecular composition of the different MTOCs during the late stages of spermatogenesis.

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Exploring the potential of genetically modified bacteriophages against multidrug resistant (MDR), highly pathogenic bacterial species

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The rapid spread of antibiotic resistant pathogenic bacteria represents an ever-growing challenge around the globe. It is expected to become the leading cause of deaths in the near future. Fortunately, there are many promising alternatives under excessive development. One such direction is the application of bacteriophages (phages). Phages are bacterial viruses and are prime candidates as therapeutic agents. My main focus is to develop techniques to genetically modify phages to extend their contribution in the fight against MDR bacteria.

Both the direct, and indirect applicability of phages are addressed in my research. In the latter case, we developed a method to rapidly construct hybrid bacteriophage particles to serve as effective DNA delivery agents. These particles were then used to deliver metagenomic libraries into pathogenic species to mimic horizontal gene transfer, the main driving force behind the spread of antibiotic resistance. The novelty of this approach is the successful involvement of clinically relevant pathogenic species into such investigations. Our method can therefore shed light on previously unknown factors that shape the spread of antibiotic resistance. The next step is to extend the direct therapeutic applicability of bacteriophages using genome engineering techniques. The primary aim is the creation of phage cocktails consisting diverse sets of synthetic bacteriophages with enhanced features for clinical applications. To achieve this goal, we will build artificial phages using viral parts that are nec-

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essary for their propagation and has therapeutic benefits. Our first step is to seek bacteriophages with therapeutic potential. To this end we isolated phages from hospital sewage targeting a set of MDR pathogenic strains. We have discovered dozens of novel phages, many of which we have sequenced and characterized, revealing therapeutically useful components that can be used to construct synthetic phages. Lastly, we are going to apply targeted mutagenesis using our own technique to further optimize the features of synthetic phages. The end goal of this research is to put the generated phage cocktails into practice.

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Mapping of symbiotic peptides of *Amorpha fruticosa*

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Nitrogen is a vital element for the development of living organisms thus plants, which can be introduced into the soil with artificial or natural fertilizers, the efficiency of this method is unsatisfactory and quite polluting. An obvious alternative would be the atmospheric nitrogen, but this cannot be utilized directly by plants due to its strong triple bond, it is converted by the diazotrophic bacteria into a form that can be taken up by plants. This includes the genus paraphyletic group of *Rhizobium bacteria*; whose members form a unique symbiotic organ with plants from Fabaceae family: the nodule.

A. fruticosa is also a species of the Fabaceae family. It moved from North America to Europe, where it spread rapidly. In Hungary, one of the typical shrub plants on the riverbanks of the Tisza, the intoxicating scent of bright purple flowers is one of the favourites of honeybees. At the beginning of our work, we collected wild plants and soil samples from its root zone, and then the plants were later grown under laboratory conditions. The symbiotic bacterium was re-isolated from wild-type nodules by excavation. The recovery and maintenance of the unknown organism proved to be a particularly difficult task, as only a negligible amount of information can be found in the literature. Due to the described and more detailed knowledge, we considered it very important to create the most accurate picture possible. Transmission and scanning electron microscopy sections as well as light and confocal microscopic images, demonstrated the nodule specific transformation of symbiotic bacteria to bacteroids, suggesting that this plant also expresses nodule-specific cysteine rich peptides like *Medicago truncatula* and its analogs, the NCR-like peptides. The intranodular conversion of nitrogen-fixing bacteroids, has evolved at least five times independently during evolution, but in case of *A. fruticosa* is perhaps the oldest or sixth form of choice, so the symbiosis chosen by us can play a key role in getting to know the process more extensively.

The efficiency of nitrogen fixation was confirmed by acetylene reduction experiments and compared to alfalfa in the absence of a reference. In general, the efficiency of 10-20-day old nodules is the highest. Knowing the 16S rRNA sequence, the symbiotic bacterium *Mesorhizobium amorpha* was probable.

Compounds produced by *A. fruticosa*, which are promising molecules for the treatment of diabetes, or their ability to improve soil through nitrogen fixation, reveal that this invasive, aggressive, and indestructible weed has untapped potential.

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The effect of acute adenosine treatment on the blood-brain barrier tightness

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Sleep restriction increases blood-brain barrier (BBB) permeability in mice to fluorescein, that is completely restored after 24 hours of sleep recovery. Adenosine, a potent somnogenic molecule, is accumulated during sleep loss and may regulate BBB permeability. The blockade of A2A adenosine receptors reverted the BBB dysfunction induced by sleep restriction in rats. Our

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aim was to investigate the presence of adenosine receptors in the cells of the BBB and the effect of acute adenosine treatment on BBB culture models and on BBB permeability in rats.

The mRNA expression of A1, A2A, A2B and A3 adenosine receptors were examined by qPCR. The kinetics of cell response was followed by real-time impedance measurement (RTCA-SP, Agilent). Primary rat brain endothelial cells in monoculture, in co-culture with glial cells, or with brain pericytes and glial cells were treated with adenosine, the A2A receptor agonist NECA and antagonist SCH-58261. The integrity of the barrier was tested by electrical resistance (TEER) measurements across the culture model of the BBB and permeability measurements performed both on cultures and in Wistar rats.

We verified that rat brain endothelial cells expressed mRNA for A2A and A2B receptors, but not for A1 and A3 receptors. Rat brain pericytes expressed A1, A2A and A2B, while astroglial cells expressed all four types of receptors. We demonstrated statistically significant increase in impedance values in both adenosine and NECA treatment groups in the first 2 hours, and no damage of brain endothelial cells was observed until 24 hours. Adenosine treatment from the luminal side of brain endothelial cells tightened the barrier and decreased the fluorescein permeability in monoculture and co-culture BBB models. Correspondingly, in Wistar rats treated with intracardiac adenosine injection BBB permeability was decreased for fluorescein and albumin. Adenosine treatment on the abluminal side increased the permeability in the triple co-culture model containing pericytes but not in monoculture and co-culture with glial cells. Adenosine given to the cerebrospinal fluid also increased BBB permeability. A2A receptor antagonist inhibited the effects of adenosine and NECA in culture and animal models.

We demonstrated the presence of adenosine receptors on cultured brain endothelial cells, pericytes and astroglial cells. Acute adenosine treatment from the luminal side acting directly on brain endothelial cells tightened the barrier in BBB culture models and in rats, which was blocked by an inhibitor of adenosine A2 receptors. We also revealed that brain pericytes may mediate the barrier opening effect of acute adenosine treatment from the abluminal side.

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Surfactin production profiling of different *Bacillus* strains isolated from vegetable rhizospheres

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Surfactins are cyclic lipopeptides consisting of a β-hydroxy fatty acid and a peptide ring of seven amino acids linked together by a lactone bridge, forming the cyclic structure of the peptide chain. They possess a high variability in their fatty acid chain lengths and amino acid sequences, bearing numerous variants and isoforms. These compounds are produced mainly by *Bacillus* species and possess numerous biological effects such as antibacterial, antifungal and antiviral activities. Different *Bacillus* species and strains produce distinct variants of surfactins in various ratios, thus affecting the biological and environmental characteristics of the varying ferment broths. Owing to their surface effect, the research of surfactins in therapeutical, environmental and agricultural applications is also a subject of increasing interest.

For their surfactin production profiling, several *Bacillus* strains isolated from vegetable rhizospheres were identified by GC-FID technique. Then a fast and easily evaluable HPLC-HESI-MS method was developed using SIM/SRM mode to achieve the simultaneous quantitative and qualitative characterizations of the extracted ferment broths. Altogether the production profiles of 25 different *Bacillus* species and strains isolated from vegetable rhizospheres were acquired based on the developed methods. More than half of the examined *Bacillus* strains produced surfactins and the MS^2 spectra analyses of their sodiated precursor ions revealed a total of 29 surfactin variants and homologues. Certain surfactins were occurred with extremely high number of peaks with different retention times, suggesting the large numbers of variations in the branching of their fatty acid chains. Results supported the conclusions of our former studies stating that the appearance of previously rarely encountered group of surfactins with methyl esterified aspartic acid in their fifth amino acid position may be encountered in considerable numbers and the fatty acid chain lengths to vary between 12 and 18 carbon atoms.

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Detection, elimination and damaging effect of singlet oxygen in the photosynthetic apparatus of plants and unicellular microalgae

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Singlet oxygen ($^1O_2$) is a highly reactive oxygen species and is produced in photosynthetic organisms under high light stress. $^1O_2$ is involved in the process of photoinhibition; however, the exact mechanism of action of $^1O_2$ is still unclear, and this is due to the lack of suitable methods to detect singlet oxygen intracellularly. The damaging effects of $^1O_2$ are also debated, and the main question of this debate is whether $^1O_2$ can directly damage the Photosystem II (PSII) complex or it can inhibit only the protein synthesis dependent repair of PSII.

Our aim was the detection of singlet oxygen, characterization of its role in photodamage of PSII and its elimination to prevent its damaging effects. $^1O_2$ was detected by various methods; electron paramagnetic resonance, fluorescence probing by Singlet Oxygen Sensor Green (SOSG), and oxygen uptake due to chemical trapping in vitro and in isolated thylakoids. Protoplasts were prepared to investigate the intracellularly produced $^1O_2$ by using intracellular $^1O_2$ labeling dye SOSG, as these fluorophores are impermeable to intact cells due to the presence of a cell wall. Our data demonstrated for the first time that SOSG is penetrated to the protoplasts of *Symbiodinium* sp., and therefore protoplasts are amenable to investigate singlet oxygen signaling in *Symbiodinium* sp. To eliminate the $^1O_{2,}$ proline was found to be a quencher of singlet oxygen in vitro via a physical mechanism with a bimolecular quenching. To study the damaging effects of $^1O_{2,}$ we employed a multiwell plate-based screening method combined with chlorophyll fluorescence imaging to characterize the effect of externally produced $^1O_2$ on the photosynthetic activity of isolated thylakoid membranes and intact *Chlorella sorokiniana* cells. The results show that the externally produced $^1O_2$ by the photosensitization reactions of rose bengal damages Photosystem II both in isolated thylakoid membranes and in intact cells in a concentration-dependent manner, indicating that $^1O_2$ plays a significant role in photodamage of Photosystem II.

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Involvement of polyamine metabolism in plant developmental and stress responses

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Homeostasis of reactive oxygen species (ROS) is controlled by low molecular weight compounds, such as polyamines (PAs). Polyamine oxidases (PAO) participate in the regulation of PA homeostasis, mediating their oxidation/ back-conversion. Products of PAO-mediated enzymatic action include H_2O_2. Studies involving overexpression or downregulation of PAO indicate that H_2O_2 derived from PA catabolism is important in stress responses. Evidence also suggests that, parallel with ROS, production of reactive nitrogen species (RNS) and particularly nitric oxide (NO) also occurs during developmental processes and exposure of plants to different abiotic stresses. Considering the function of PA, PAO and NO in plant developmental and stress responses, and peroxisomal localization of certain PAO and peroxisomal accumulation of NO, regulatory role of PA metabolism, mainly the role of PAO, on NO generation was speculated. To monitor the effect of PAOs during abiotic stress responses and developmental processes *Arabidopsis thaliana* different mutant lines was used. During salt stress ROS production was increased in S-AtPAO3 plants. Interestingly, inhibition of NADPH oxidase resulted a decrease in ROS production of S-AtPAO3 roots. NO production was also affected by PA catabolism however not AtPAO3 but AtPAO2 was involved in this process. Involvement of AtPAO2 in heat stress responses was also investigated through the regulation of NO production by NR. Based on these results a possible cross talk between peroxisomal PAOs, NADPH oxidases, ROS and NO in salt and heat stress responses of *Arabidopsis* could be supposed.

To study the involvement of PA metabolism in pollen germination and pollen tube elongation in vitro pollen germination technique was used. Putrescine had a somewhat positive effect on pollen tube emergence, but negatively regulated its further elongation; spermidine enhanced both processes, while spermine had negative effect on pollen germination but did not in-

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fluenced pollen tube growth. Our data indicated that PAs regulate pollen germination primarily via regulating the ROS level, while tube elongation primarily influencing the NO level. Our results further supported the involvement of PAs in the regulation of pollen germination and elongation affecting ROS and/or NO levels in a polyamine- and cellular-region-specific way.

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Role of jasmonic acid in chitosan-induced plant defence responses

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The efficiency of plant defence reactions against pathogens is highly dependent on environmental factors, such as the availability of light. Among microbe-associated molecular patterns (MAMPs) one of the most-commonly-used is the fungal cell wall derivative chitosan (CHT) which is able to effectively induce plant defence responses. Plant hormones, especially jasmonic acid (JA) plays a crucial role in the regulation of these processes. However, the role of JA in the CHT-induced defence responses has not been clarified yet.

Therefore, the aim of our work was to examine the JA- and light-dependence of the CHT-induced defence responses by using JA-insensitive (*jai-1*) tomato plants which were kept in the light or under darkness after elicitor treatments. Moreover, our work also focuses on the development of systemic responses in the distal leaves from the treated ones which is also less studied. Stomatal closure is one of the first defence responses activated upon biotic stress stimuli. We found, that CHT induced significant stomatal closure in both genotypes locally as well as systemically, however it was inhibited in the absence of light. Production of superoxide radical was elevated upon CHT treatment in both wild type and *jai-1* plants locally and systemically, however, it was not observable in the dark. Significant change nor in hydrogen peroxide neither nitric oxide production was observable. CHT treatment significantly elevated the relative transcript level of *SlDEF9* locally in the leaves of wild type plants which was more significant under darkness but it was not induced in the *jai-1* mutants. Expression of the ethylene response gene *SlERF1* was significantly decreased after CHT treatment Interestingly, the relative transcript accumulation of *SlPR1* was also induced by CHT only in the wild type plants both locally and systemically. Our results suggest a potential fine-tuning role of JA in the CHT-induced defence responses which are also under light-dependent regulation.

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Molecular investigation of DNA transposons as therapeutic gene delivery devices

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Gene therapy procedures offer tremendous promise for many serious diseases for which effective conventional therapy is not available. Initially, viral vectors were used for gene therapy to deliver the desired genetic information into the cell. DNA transposon systems have emerged as new players on the palette and *Sleeping Beauty* (SB) and *piggyBac* (PB) DNA transposons have since been used in clinical trials.

Our research is aimed at assessing the risk factors of these new gene therapeutic devices. For comparison, we treated the mouse model of the human Tyrosinemia type I disease with liver-targeted gene delivery using both the SB100 and the hyperPB systems, and then vector insertion sites were identified from the treated livers. To this end, we developed a novel next-generation

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sequencing procedure that allows us to locate large number of integration sites and explore hitherto unknown features of the operation of the systems. With our next-generation sequencing method, we could observe the so-called hot regions, where a significant amount of vector integrations is clustered. We have studied these hot regions further, having explored some of the chromatin-level factors contributing to their formation. The hot regions are important for the safety of gene therapy protocols, because the given gene delivery vectors integrate into them with high frequency. Therefore, we examined the genomic positions of the SB and PB hot regions to assess whether they could pose a risk of carcinogenesis when disturbed by vector integrations. In addition, specific qPCR and RT-qPCR assays were used to better characterize the process of DNA transposon-mediated gene delivery. We identified the average therapeutic gene dose in the affected organs and determined the kinetics of transposase expression and transposon excision product accumulation. These measurements helped us to reveal another hitherto unrecognized risk factor of DNA transposon-mediated therapeutic gene delivery. Using a variety of molecular biological methods, we have assessed the consequences and risk factors of the application of hyperactive SB and PB transposon systems for gene therapy. In the light of our results, we can make safety-enhancing recommendations for the development of clinical gene delivery protocols. Our conclusions will be applicable to other transposon-based gene therapy protocols.

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Investigating the effects of deubiquitinases on mutant Huntingtin induced pathology

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Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder associated with the gradual degeneration of neurons. The disease is caused by the expansion of a CAG repeat in the *huntingtin* gene, which leads to the production of mutant Huntingtin (mHtt) protein that due to misfolding can easily form aggregates. The degradation of misfolded proteins is performed by the ubiquitin-proteosome system (UPS). In HD proteosomes and ubiquitins are sequestered to aggregates and the UPS is unable to fulfill its function. mHtt is polyubiquitinated but it is not degraded efficiently that eventually might lead to depletion of the free monoubiquitin pool. The removal of ubiquitin from proteins is performed by deubiquitinase enzymes (DUBs), which also play an important role in the maturation of monoubiquitin (mUb), and in the regulation of the half-life and activity of proteins.

Therefore, we investigated the effect of DUBs on the pathomechanism of HD. We performed a comprehensive genetic screen using 39 DUB mutant strains to study their effect on viability, longevity, motor activity, and neurodegeneration in a *Drosophila* model of HD (HD flies). We identified *CG4603,* a gene encoding an OTU family DUB, that ameliorated HD phenotypes if overexpressed. Analyzing mUb levels we found that while the level of mUb decreased with age in HD flies, it did not change with age in HD flies overexpressing CG4603. We examined the effect of CG4603 on mHtt aggregation, however, we found no difference in the number, size, and size distribution of aggregates compared to control animals. As CG4603 is involved in UPR processes we analyzed the expression of endoplasmic reticulum (ER) stress genes *Hsc70-3* and *fic*. Hsc70-3 is the major ER chaperone whose activation is regulated by another ER stress protein, named fic. The expression of *Hsc70-3* and *fic* genes appears to be reduced due to overexpression of *CG4603*. Our results show that CG4603 influences the ER stress response and may have an effect on maintaining the free monoubiquitin pool. To facilitate the study of the role of CG4603 in the pathogenesis of HD we cloned *CG4603*, *Hsc-70-3* and *fic* genes with FLAG-tag.

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Fatty acid components of Armillaria species

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Fatty acids are important cellular components that are highly variable in fungi. The fatty acid composition could help to clarify the taxonomy of the plant pathogen *Armillaria* species. Furthermore, this could be useful as a diagnostic tool in the protection of our forests. This study investigated the fatty acid components extracted from *Armillaria* species. *Armillaria* species are plant-pathogenic fungi causing white rot, a severe destructive disease, on a wide range of hosts. *Armillaria* root disease has negatively impacted the health of trees and the whole forest productivity.

The Sherlock Chromatographic Analysis System (CAS) have been applied to identify and quantify the fatty acid components in *Armillaria* genus. *Armillaria* genus there are approximately 400 species form these *A. mellea*, *A. cepistipes*, *A. ostoyae*, and *A. gallica* were selected in our study. For the optimization of the culture condition, the strains were cultured in various medium during 30 days. After that, the mycelia were collected, lyophilized and the fatty acids were extracted and derivatized under various conditions to form fatty acid methyl esters (FAMEs). The final FAME extracts were run via gas chromatography controlled by CAS.

It could be concluded that culturing in potato-dextrose broth at 25^0C during for 30 days was the optimized condition of *Armillaria*-growth. The extraction-choosing process was followed the MIDI instruction started with 10 mg of lyophilized mycelia and 30 minutes of saponification. The analyzed results showed that the *Armillaria* species shared similar fatty acid profiles with minor differences. The 18:2 w6c shared 55-65% of fatty acid components is the largest composition. The 16:0 ranges from 17% to 19% of fatty acid components. The 12:0 is around 4% in *A. mellea* and *A. ostoyae*, and 8% in *A. cepistipes* and *A. gallica*, respectively. Other fatty acids were identified with trace.

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Role of survival factor genes in the pathogenicity processes of *Mucor circinelloides*

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Mucor circinelloides is a filamentous fungus belonging to the order Mucorales. Several members of this fungal group may cause frequently fatal invasive infections called as mucormycoses.

Survival factor protein (SVF) plays a crucial role in the protection of cells from oxidative and other stresses (e.g. cold stress) in *Saccharomyces cerevisiae*. Furthermore, this protein participates in the sphingolipid biosynthesis of the cell membrane. Sphingolipid signalling performs an important role in the control of various crucial cellular processes. Transcriptomic studies showed the upregulation of the encoding genes in several human pathogenic fungi during the host-pathogen interactions. However, the function and regulation of the SVF protein and the encoding gene(s) are still quite unknown in mucormycosis-causing fungi.

In the *Mucor circinelloides* genome, two hypothetical *svf* genes were identified and named as *Svf1* and *Svf2*. We have studied the expression of the genes after culturing the fungus under different conditions by real-time quantitative reverse transcription PCR. Using the CRISPR/Cas9 technique, single gene disruption mutants were constructed for each gene and we have started the characterization of the resulting strains. Macromorphology and sensitivity to different stressor chemicals (e.g., acetate, H_2O_2, Congo red and Calcofluor white) were tested. Mutants showed altered characteristics compared to the original strain suggesting that the cellular integrity may be damaged in the mutants. Pathogenicity of the mutants was also examined in alternative *Drosophila melanogaster* model and a decreased virulence was detected. Moreover, we studied the pathogenicity in *Galleria mellonella* model too and we could observe increasing virulence. We also carried out susceptibility test of our strains against various antifungal drugs (e.g. different azoles, amphotericin B). Next to these investigations we studied the differences of carotene volume after various (e.g. H_2O_2, posaconazole, TritonX-100, SDS, calcofluor white, Congo red) treatments.

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Lipid-protein interaction in photosynthetic membranes

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Cold stress is one of the major limiting environmental factor of plant growth. Overwintering plants have high plasticity at multiple points during their life cycle to adapt cooling of ambient temperature from seed germination. Cold acclimation in the vegetative stage is the main object of our research. Because of their longer growing period winter cereals out-yield the spring counterparts by 30-40%. The cold acclimation process is controlled apart from cold temperature, by the circadian clock, day length. It also depends on both the intensity and the spectral composition of light. The changes in the lipid membrane composition are important part of cols acclimation process. However, lipidome of plants has been recently studied extensively to elucidate the cold acclimation process mainly on the model plant *Arabidopsis*, and only scarcely in cereals such as winter barley. Moreover, to elucidate the possible lipid background of the light quality induced pre-hardening the process according to our best knowledge has not been studied, yet. Based on our results the white light + decreased red: far-red light ratio (WL+FR) and white light enridged blue light affected both (WL+BL) the lipid content and quality in the leaves of barley (*Hordeum vulgare* ssp. vulgare 'Nure') plants. Applying electrospray ionization triple quadrupole mass spectrometry, we can determine how the membrane lipid classes are quantitatively and qualitatively altering under different temperature and light conditions. We have seen a remarkable change at the HexCer, Lyso PC, MGDG, and MGDG/DGDG. Those are increased as a result of WL+FR and WL+BL treatment. PC/PE decreased in the leaf samples with lowering the temperature from 15 to 5°C in WL+FR but increased at WT+BL. The expression of MGD2, DGD, NC, LLO2, LOC, and AD3 genes increased significantly as a function of WL+FR at 15 °C after 1 day. We suggest that the light quality-induced freezing tolerance should be considered as an important part of the pre-hardening phase. In summary, the lipidome changes induced by cold acclimation and light regime well correlate with the freezing tolerance.

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Morphological and molecular studies of diabetes-related intestinal dysfunction in the myenteric ganglia and their microenvironment of streptozotocin-induced diabetic rats

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In type 1 diabetes (T1D), thickened endothelial basement membrane (BM) of mesenteric capillaries has been described. Our first aim was to study the thickness of BM surrounding myenteric ganglia in different gut segments in a T1D rat model. Our second aim was to quantify the expression of matrix metalloproteinase-9 (MMP9) and tissue inhibitor of metalloproteinase 1 (TIMP1), which are essential in the breakdown of extracellular matrix molecules. Gastrointestinal (GI) symptoms are common in diabetic patients; therefore, we have studied the proportion of serotonin-immunoreactive (5-HT-IR) neurons, which are crucial in the regulation of GI motility.

Samples were taken from the duodenum, ileum and colon of diabetic, insulin-treated diabetic and control rats ten weeks after the onset of streptozotocin-induced hyperglycaemia. The thickness of BM was measured by electron microscopic morphometry. Expressional changes of MMP9 and TIMP1 were evaluated by post-embedding immunohistochemistry in the myenteric ganglia, capillary endothelium and intestinal smooth muscle. Myenteric whole-mount preparations were immunostained with anti-5-HT and pan-neuronal anti-HuCD markers.

In diabetic rats, the BM surrounding the myenteric ganglia was significantly thicker in the ileum, but it remained unchanged in the duodenum compared to controls. In the diabetics, the MMP9 expression and the MMP9/TIMP1 ratio decreased significantly in the myenteric ganglia of ileum, but not in the duodenum compared to controls. In the ileal endothelium and smooth muscle, the expression of MMP9 was also lower in diabetic rats. Immediate insulin treatment had region-specific effects on diabetic alterations. The proportion of myenteric 5-HT-IR neurons enhanced in T1D in a region-specific manner. The im-

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mediate insulin replacement prevents the hyperglycaemia-induced higher amount of 5-HT-IR neurons and restores that to the control level in each investigated gut segments.

These hyperglycaemia-related alterations may play an important role in the development of GI symptoms in T1D.

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Analysis of structure and catalytic mechanism of type VI sulfide:quinone oxidoreductase enzymes

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Although sulfide is toxic at and above certain concentrations, it plays various important physiological roles in microorganisms and higher eukaryotes as electron donor, energy source and signal molecule. Sulfide:quinone oxidoreductases (Sqr) are ancient membrane-bound flavoproteins catalyzing sulfide oxidation coupled to quinone reduction. Based on phylogenetic analysis, Sqr enzymes are classified into six groups (type I-VI). *Thiocapsa roseopersicina* is a photosynthetic, purple sulfur bacterium which possesses a type VI Sqr enzyme (SqrF). Catalytic properties of the purified recombinant SqrF differed from other characterized Sqrs indicating a distinct catalytic mechanism in type VI Sqr enzymes. In our former studies, based on the biochemical analysis of the cysteine mutant enzymes, a model of sulfide oxidation mechanism of SqrF was suggested.

The aims of our studies were the identification and characterization of additional functional amino acids in the active centre of SqrF participating in the quinone binding and catalysis of the quinone reduction phase of the catalytic process. Based on sequence and structural alignments, Val331, Ile333 and Phe366 residues seemed to be involved in the formation of the quinone substrate binding pocket in the *T. roseopersicina* SqrF enzyme. These residues were replaced by site-directed mutagenesis and the mutant SqrF variants were biochemically characterized. Moreover, *in silico* approaches were applied for modeling the enzyme-substrate interactions. The experimental results revealed that all these amino acids play a role in the interaction between the protein and quinone molecules. Hydrophobic stacking interaction between the redox head group of quinone and the benzene ring of Phe366 residue is the most crucial component in the proper binding of the electron acceptor in the active site. Further site-directed mutagenesis experiments of a highly conserved amino acid, Glu163 located in the active centre near the FAD cofactor demonstrated that this residue has an essential role in the catalysis of sulfide oxidation. In the presented work, novel amino acids involved in the complex redox process catalysed by the SqrF enzyme have been identified. According to these results, our catalytic model established for SqrF enzymes was further improved.

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Characterization of potential agricultural bioeffector *Trichoderma* strains isolated from soils in the Hungary-Serbia cross border region

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Certain *Trichoderma* strains are commonly used as agricultural agents due to their beneficial effects to both plants and soil. In general, the consortia have one or two strains of these filamentous fungi and they usually play biocontrol and plant residue degrading roles. Several harmful *Trichoderma* species were also described. Some of them produce toxins, cause diseases in mushroom cultivation or could be opportunistic human pathogens. Therefore the selection of agricultural bioeffector strains should be performed with great care.

Our goal was to isolate, identify and characterize *Trichoderma* strains potentially applicable for agricultural purposes. We

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isolated 48 *Trichoderma* strains from various agricultural soils in the Hungary-Serbia cross border region. The isolated strains were identified based on sequence analysis of a fragment of the translation elongation factor 1α (*tef1α*) gene. The identification is also important to exclude strains that belong to potentially harmful *Trichoderma* species such as *Trichoderma brevicompactum*, *T. longibrachiatum* or *T. aggressivum*. Cellulolytic enzyme activities was also measured. Based on the molecular identification and enzyme activity tests, 10 strains were selected. The 10 selected strains underwent a series of examinations to determine their pH and temperature optima and their tolerance to heavy metals and low water activity. The resistance to several fungicides was also tested to investigate the possibilities of combined application with antifungal agents. Biocontrol indices (BCI) were also examined in parallel with chitin degrading enzyme activity tests. Both experiments are good indicators of biocontrol abilities against filamentous fungal plant pathogens. We did not find strong correlations between the BCI values and the chitin degrading enzyme activities of the tested strains, although most of the strains showed promising antagonistic abilities against fungal plant pathogens. Plant growth promotion was also tested in greenhouse experiments on maize and alfalfa. Certain strains showed slight plant growth promotion in greenhouse experiments on maize and alfalfa seedlings, although the measured parameters were not significantly different from those of the untreated control.

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Predicting the spread of antibiotic resistance genes using functional metagenomic screens in pathogenic bacteria

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Horizontal gene transfer plays an important role in the development of multidrug resistance. In the present antibiotic resistant crisis, besides examining genomic mutations, it would be highly important to consider this evolutionary process in drug development.

Functional metagenomic screens has the potential to identify antibiotic resistance genes (ARGs) in different environments that are potential candidates for horizontal gene transfer. These screens, however, are usually performed in a single non-pathogenic host, therefore being insufficient to reveal the whole resistome of an environment and lacking clinical relevance. My work aimed to extend functional selections to non-model pathogenic bacteria to capture the resistome of three different environments. I constructed small-insert metagenomic libraries from microbial DNA of the human gut, contaminated soil samples and from the genomic DNA of 73 pathogenic bacteria isolated from hospitals. I introduced and expressed these libraries in the model organism *Escherichia coli* and in three pathogens: *Shigella sonnei*, *Klebsiella pneumoniae* and *Salmonella enterica*. I performed selection experiments in the presence of 6 newly developed antibiotics in comparison with their 7 older counterparts of the same antibiotic class. Overall, in the screens I identified 86 known ARGs, out of which only 50% is coming from the screens in *E. coli*, showing the need for multi-host selections. I also found that the majority of ARGs identified in all four hosts are known to be horizontally transferred in nature according to literature databases. This shows the predictive force of the screens. Moreover, according to my results, some resistance gene classes seem to be specific to certain hosts. Finally, as the number of already known resistance genes is not lower for new antibiotics compared to old ones, my work highlights the need for a more thorough resistance testing pipeline.

In sum, I showed that by the application of multi-host functional metagenomics we can gain a better coverage of the environmental resistomes and aid antibiotic development.

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Mesr4: a novel positive regulator of germline differentiation

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Adult stem cells divide continuously. One of the daughter cells remains in stem cell state and the other starts to differentiate. The main question of stem cell biology is how these cells know if they have to remain in stem cell state or they have to start their differentiation program. To investigate this fundamental biological phenomenon, the *Drosophila* ovary provides a perfect model system. The ovarian germline stem cells (GSCs) reside in a special microenvironment called stem cell niche. In the niche, the GSCs are surrounded by somatic cells secreting the Dpp signalling molecule which inhibits the expression of the main differentiation gene *bag of marbles* (*bam*) in the GSCs. After the division of the GSCs, one of the daughter cells is displaced from the niche, the negative Dpp signal cannot reach it, and becomes a pre-cystoblast (pre-CB). At this stage, initiation of bam expression promotes the differentiation into a cystoblast (CB). While the negative regulation of GSC differentiation is very well known, the positive factors are barely known.

Previous observations suggested that the nuclear protein, MesR4 is required for GSC differentiation. Cell biological analysis of the MesR4 depleted ovaries revealed that Mesr4 is required for the last step of pre-CB to CB transition in a cell autonomous manner. By a series of epistasis experiments and transcriptional analysis, we demonstrated that MesR4 positively regulates the transcription of bam. The Mesr4 protein contains eight C2H2 type zinc finger domains, a plant homeodomain finger, a nuclear localisation signal and an AAA-ATPase domain. We showed that the plant homeodomain finger and the AAA-ATPase domain are dispensable for the proper of Mesr4 function in the ovary suggesting that the DNA binding activity of the C2H2 type zinc fingers is essential for *bam* regulation. Based on our results, we propose the hypothesis that MesR4 directly binds the regulatory regions of the *bam* gene and act as a transcriptional activator. Our results indicate, that the lack the inhibitory Dpp signal is not sufficient, but an additional positive signal is required to complete the differentiation program in the GSCs.

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Complex examination of bacterial strains with plant growth-promoting and biocontrol potential

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The application of plant growth-promoting bacteria (PGPB) and biocontrol agents in agricultural systems are promising strategies for the sustainable enhancement crop productivity. However, the effectiveness of PGPB *in vivo* is often limited by adverse environmental conditions like salinity or drought, as well as and the presence of xenobiotics, such as heavy metals or pesticides. Our work aimed the isolation of PGPB and their complex characterisation including tolerance to different abiotic stress factors.

Thirty strains were isolated from an agricultural soil sample previously exposed to the herbicide glyphosate on solid minimal medium amended with glyphosate as the sole carbon and nitrogen source (1 mg/ml). Ten strains showing the highest growth rate and different colony morphology were selected for the further studies. Based on the sequence analysis of fragments of the 16S rRNA and *rpoB* genes 7 strains were identified as *Pseudomonas resinovorans*, 2 as *Ensifer adhaerens*, and a single isolate as *Ochrobactrum anthropi,* which was excluded from the further assays due to its opportunistic human pathogenic nature. Among the 9 remaining strains, *E. adhaerens* SZMC 25856 and *P. resinovorans* SZMC 25875 could enhance root and total length of tomato seedlings significantly ($p<0.05$). Eight isolates showed remarkable siderophore-producing potential (12.66-36.44%), and 5 strains were able to synthesize the phytohormone indole-3-acetic acid (0.19-0.81 μg/ml). All strains tolerated Al, Cu, Fe, Mn, and Pb ions at 0.1 mM, and 14 different pesticides at 25 μg/ml concentration. They resisted also salinity (6.3-12.5 g/l NaCl) and drought (125 g/l polyethylene glycol 6000), and preferred pH 7.00-7.96. All *P. resinovorans* isolates could antagonize *Agrobacterium tumefaciens* and *A. vitis* (updated scientific name *Allorhizobium vitis*), the pathogens of grape, cherry, and walnut.

Our findings propose the possibility of applying the isolated strains, particularly *E. adhaerens* SZMC 25856 and *P. resinovorans*

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SZMC 25875 as plant growth-promoting and biocontrol purposes even under adverse environmental conditions.

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A simple *E. coli* system for studying sequence-specific DNA binding of proteins

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Sequence-specific DNA-protein interactions play essential roles in many biological processes. During characterization of DNA binding proteins, it is often important to test whether the protein can bind to a particular DNA sequence, and to determine how alterations in the protein or in the DNA affect the binding strength or specificity. The drawback of the available methods is that they either require purified proteins or protein fusions. We have developed a simple *in vivo* technique, which can detect binding of a protein to its target site without the requirement of creating protein fusions. The method called I-Block assay detects inhibition of the *E. coli lacI* gene transcription by the tested protein bound to its target site inserted next to the *lacI* promoter. The method was tested with two zinc finger proteins, with the λ phage repressor and with CRISPR-dCas9, but is expected to be generally applicable to all sequence-specific DNA binding proteins for which the target site is known (*Nucleic Acids Res.* 2020, 18;48(5):e28).

The I-Block assay uses β-galactosidase activity as readout and can, in its current form, process reactions involving the protein of interest and one or a few potential target sequences. Our current work aims to develop a high throughput version of the I-Block assay, which can select from a complex sequence library the best binding target sequence for a protein, or the best binding protein variant for a target sequence. To this end we first tested whether β-galactosidase production can be used as a selective phenotype on agar plates containing the non-inducing β-galactosidase substrate phenyl-β-D-galactopyranoside (P-gal) as the only carbon source. Unfortunately, the growth difference between the β-galactosidase producing and non-producing colonies was not large enough to reliably identify binding-positive clones. We now work on an alternative approach, in which the β-galactosidase gene is replaced by an antibiotic resistance gene allowing binding positive clones to be selected by virtue of their acquired antibiotic resistance.

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