

added, finally filled 70% ethanol up to the mark. The absorbance was measured after 60 min from the reaction beginning. Conclusion On the differential absorption spectrum, two maxima were detected at wavelengths of 391 ± 2 and 438 ± 1 nm, which made it possible to determine two groups of flavonoids, in terms of apigenin and quercetin. The content of flavonoids in terms of quercetin was $0,171 \pm 0,005\%$, apigenin content was equal $0,866 \pm 0,010$. The publication has been prepared with the support of the “RUDN University Program 5-100”

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Flavonoids and anthocyanins content diversity in rye forms

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Rye is a cereal traditional for Northern and Eastern Europe with very good resistance to abiotic stresses and ability to grow on poor soils. Because of relatively recent domestication and obligate cross-pollination rye still possesses a great diversity of gene variations leading to variations in content of secondary metabolites. In our study we evaluated anthocyanins and flavonoids content in grains of 24 rye forms and inbred lines (Peterhof collection of rye) by HPLC-ESI-MS. There were 4 types of grain colours: colourless, yellow, green, brown and violet. Only in green and violet seeds anthocyanins were detected: mostly, cyanidin and peonidin as aglycones and rutinoside and glucoside as sugar residues. Variations of anthocyanins content are considerable even in cases of closely related rye forms and forms with the same phenotype. Variability of detected flavonoids and other secondary metabolites correlates with the grain colour: the least in colourless seeds and yellow-seeded forms, the greatest in violet seeds. The most interesting differences in flavonoids content were revealed for 5 lines (vi1, vi2, vi3, vi4 and vi6) which carry recessive mutations, leading to anthocyaninless phenotype. The differences between lines in content of flavonoids, including colorless precursors of anthocyanins, allowed us to suggest candidate genes for vi mutations. Our results confirmed wide variability of flavonoids and anthocyanins content in rye. These substances are involved in plant pigmentation, UV filtration and also are a valuable elements of food with anti-inflammatory, anti-cancer activities. Wide variability existing in rye is a good source for breeding of specialized rye cultivars as well as a genetic reserve of the valuable traits to be used in related cereals, including wheat. This study was supported by RFBR (grant No 19-016-00205) and state budget (project “Genetics and breeding of rye on the base of natural hereditary diversity”).

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Genetic diversity of CAD genes in *Linum usitatissimum* L.

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Flax (*Linum usitatissimum* L.) is an important crop that is used for production of seed and fiber. The quality of flax fiber is determined by many factors, and lignin content is an essential characteristic,

which is important for the use of fiber in textile and composite productions. Cinnamyl alcohol dehydrogenase (CAD) is involved in the control of lignin formation, therefore the study of *CAD* genes is necessary for understanding of lignin biosynthesis and breeding of improved cultivars. Polymorphism of *CAD* genes was estimated in 288 flax cultivars and lines with lignin content varied from 2% to 7.5% (Institute for Flax, Torzhok, Russia). DNA was extracted from pools of at least 40 seedlings for each cultivar and line using CTAB protocol. Primers were designed for amplification of full sequence of *CAD* genes and their probable promoter region using overlapping DNA fragments with 450–500 bp in length. DNA libraries for high-throughput sequencing were obtained by two successive polymerase chain reactions according to the Illumina recommendations for 16S metagenomic sequencing library preparation with modifications. Evrogen (Russia) reagents were used in preparation of DNA libraries, whose quality and concentration were evaluated by Qubit 2.0 fluorometer (Life Technologies) and Agilent 2100 Bioanalyzer (Agilent Technologies). High-throughput sequencing was performed on MiSeq Illumina with 300 + 300 read length. The pipeline was developed for bioinformatics analysis of the sequencing data. The average coverage of *CAD* genes for an individual sample was over 100x. Polymorphism of the genes within studied flax cultivars and lines was assessed. Obtained results contribute to evaluation of genetic diversity of flax and determination of association between *CAD* allelic variants and lignin content in flax fibers. This study was funded by the Presidium of the Russian Academy of Sciences, Program No. 41 «Biodiversity of natural systems and biological resources of Russia».

Natural networks and systems

P-29-001

Identification of unique molecular signature of browning in human primary adipocytes from deep and subcutaneous neck fat

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There are two types of thermogenic adipocytes, classical brown and beige (BAT) which are UCP1-positive dissipating energy as heat. BAT markers have been well studied in rodents but detailed molecular studies are still lacking in humans where BAT is interspersed at several sites and may serve as a target of anti-obesity therapies. Our study aims to identify the unique signature of browning in human primary adipocytes from the different anatomical location by analyzing global gene expression patterns. Preadipocytes were obtained from subcutaneous (SC) and deep neck (DN) and differentiated to white and brown adipocytes. We analyzed differential gene expressions by total RNA sequencing, molecular pathways by KEGG Mapper, genetic constraint by ExAC and verified several genes of interest associated with adipocytes browning. We identified 37 genes which are closely clustered to UCP1. Out of those 13 genes have been already described to play a role in thermogenesis (CIDEA, CKMT1A/B), while the roles of the others are still unclear (ANO5, FAM151a). Several pathways were represented, such as retinoic acid biosynthesis which was upregulated (CPT1, CYP261B), while extracellular matrix organization pathways were among the downregulated ones (COL, ITGF). Mitochondrial creatine kinases, CKMT1a/b, are reported to play role in UCP1-independent thermogenesis; UCP1 and CKMT1a were expressed higher in DN, as compared to SC adipocytes and this was verified by RT-qPCR. Several

transporters were expressed higher in DN, such as transporter of amino acids (SLC7A10), glutamate (SLC25A18) and pyruvate (SLC16A7). Our data proves that progenitors from DN fat can be differentiated to browning adipocytes at a greater extent than SC ones. We have started to investigate revealed molecular elements not linked yet to browning by deleting, inhibiting or over-expressing them. This work is supported by the GINOP-2.3.2-15-2016-00006 project co-financed by the European Union

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S2B (Specific-Specific Betweenness): a novel method to prioritize cross-disease associated genes and its appliance in motor neuron degeneration as case of study

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Cell functions are accomplished by the interaction of numerous components on biological networks. In the same way, patho-phenotypes are the result of molecular alterations that cannot be deciphered through reductionist approaches. Indeed, the discovery of new associations between genes and diseases is crucial for the understanding of pathological mechanisms and for the establishment of new therapeutic targets. It is commonly assumed that proteins that physically interact are more likely involved in similar functions. Likewise, proteins that interact with disease-genes are also suspected to be involved in the same disease. Thus, we conjecture that diseases with similar phenotypes are triggered by alterations in proteins localized in a close neighborhood. We present a novel network-based method called S2B (Specific-Specific Betweenness) for disease-associated genes prioritization able to identify proteins linking two disease phenotypes. S2B was applied to Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy diseases, both characterized by motor neuron degeneration. By using artificial disease modules, we saw that proteins with higher S2B scores are more likely part of the disease modules overlap. Interestingly, S2B method was robust to interactomic artificial noise and disease-associated gene list size incompleteness. S2B candidates are enriched in functions associated to motor neuron degeneration and pinpoint interesting pathways that might bring novel cross-disease mechanistic hypotheses. Additionally, several S2B candidates were already associated to other mental or neuro-muscular disorders. These results were recently published in a peer-review journal and S2B function, disease-gene associations and Protein-Protein Interaction (PPI) data is publicly available. Work supported by BioSys-PhD programme by FCT(PT) (PD/BD/128109/2016) and EU Joint Programme supported through FR/ANR, GE/BMBF, PT/FCT and SP/ISCIH (UID/MULTI/04046/2013 JPND-CD/0002/2013).

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Natural yeast-like fungi strains producing potential antibacterial agents

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Lack of strict control of antimicrobial therapeutic and veterinary agents' usage has resulted in spread of antibiotic resistance among pathogenic bacteria forcing the humankind to expand

spectra of new antibacterial drugs. Natural compounds with the efficacy verified as a result of natural selection till now constitute a source of material for further optimization in laboratory conditions. Our study is aimed at finding and investigation of low-molecular weight antimicrobial compounds, which are produced by the strains of the unique collection of yeast and yeast-like fungi. This collection, containing more than 2500 strains, was formed by B.F. Yarovoy and V.P. Stepanova during several expeditions to extreme regions of Russia: the Kuril Islands, the Kamchatka Peninsula and Sakhalin Island. The diversity of species from extreme natural areas is under-investigated, and our research may contribute to the discovery of previously unknown antimicrobial agents. We perform the analysis of yeast and yeast-like fungi culture liquids applying high-throughput bacterial system that expresses specific reporter fluorescent proteins in the presence of substances impairing translation or inducing SOS response in the analyzed mixture. In our experiment, we use fresh culture of bacterial strain with the test system and analyze two fluorescence signals of reporter proteins (ex/em wavelengths: 553/574 nm and 588/633 nm) normalized to the bacterial culture growth in the presence of the culture liquids samples to evaluate the presence of potential antimicrobials. We have analyzed 600 samples from the collection and identified samples inhibiting bacterial cell growth, as well as enhancing the expression of SOS response reporter proteins. No samples inhibiting translation were found pointing to the fact that this mechanism of combating bacteria is not characteristic for yeast and yeast-like fungi. This work is supported by the RFBR grant 17-00-00368.

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Tick-borne pathogens in sympatric areas of *D. reticulatus* and *I. ricinus* ticks in Latvia

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Ticks and transmitted pathogens are important in human and animal health, and they are examples of the ecological complexity of parasitic associations in a landscape. These natural networks are highly structured, and the recent studies indicate that ticks and hosts interact along the shared environmental gradient, while pathogens are linked to groups of phylogenetically close reservoirs. In this study, we analysed the presence of two epidemically-important pathogens, Tick-borne encephalitis virus (TBEV) and *Borrelia*, in sympatric areas of *I. ricinus* and *D. reticulatus* ticks which were recently appeared in several regions of Baltic States. In total, 1960 ticks were collected, geolocated and analysed. RNA and DNA were isolated, and the samples were screened for the presence of TBEV and *Borrelia* using molecular RT-PCR and PCR methods. The results showed that 21.9% of *I. ricinus* and 2.6% of *D. reticulatus* ticks were *Borrelia*-positive; this difference was statistically significant ($P < 0.0001$). By contrast, TBEV was detected in similar proportions of *I. ricinus* and *D. reticulatus* ticks: 0.9% and 1.1%, respectively ($P > 0.05$). The availability of suitable vertebrate hosts and environment facilitate the establishment of foci of ticks and pathogens. *D. reticulatus* tick, which is spreading in Latvia from the South, is a generalist tick with a wide host range similarly to *I. ricinus*. It seems that TBEV could pervade between sympatric tick species through feeding on the same animals indicating existence of strong interaction and transmission networks. This is also dangerous factor for other coinfection outbreak. On the other hand, the distinct difference in *Borrelia* prevalence indicate the complexity of such interaction. Another possibility is that zoonotic potential and/or a general life-cycle of *D. reticulatus* ticks is less suitable for *Borrelia* and has to be addressed in further studies.