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dedicated to the 55th Anniversary of the M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences and the 80th Anniversary of Professor Yuri Ovchinnikov

September 15–19, 2014; Moscow, Russia

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RUSSIAN BIOCHEMICAL SOCIETY
"PARK-MEDIA"

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Increase of DNA-hydrolyzing properties of Ig G is likely to be associated with great number of extracellular DNA. Detection of high values of proteolytic activity may be associated with occurring in serum of blood of patients of large number of destructed or damaged proteins.

Support by Grant of RSF № 14-15-00480 «The search for biomarkers of socially significant endogenous mental disorders» 2014-2016.

Searching bacteriocin-like peptides in cell culture of *lactobacillus plantarum* 8PA-3

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There is a great interest last years to probiotic lactic-acid bacteria which are, because of their non-hazardous, high enzymatic and antimicrobial activity, in the focus of the interest of fundamental scientific investigations. Bacteriocins are bacterial antimicrobial peptides and proteins. Bacreriocins – bacterial antimicrobial proteins and peptides can be considered to be potential antimicrobial and antifungal agents which regulate bacterial population growth and provide colonization stability of human body. Sequencing of genome of commercial probiotic strain *Lactobacillus plantarum* 8PA-3 found a locus that can be responsible for synthesis of two bacteriocins EF and NC8.

For choosing an optimal method of purifying fractions of bacteiocins literature data analysis was performed. Two methods of purifying bacteriocins mentioned in this work are Mota-Meira's method (Mota-Meira M. et al., 1997) and Todorov's method (Todorov S.D. et al., 2004)

Using of two methods mentioned above let us purify fractions of low molecular weight cationic peptides with molecular weights between 1 and 10 kDa which possess antnimicrobial activity against broad range of test microorganisms (*E. coli*, *L. monocytogenes*, *St. epidermidis*, *C. albicans*). During LC-MS analysis of bacteriocins fractions peptides with molecular weights between 2946.7–6280.4 kDa and 1535.9–4845.8 kDA were determined although during MW analysis no correlations between purified peptides and predicted bacteriocins EF and NC8 were found. The lack of correlation could be consequence of peptide degradation or modification during purifying or aggregation of bacteriocins with peptides from growth medium.

X-ray study of the red fluorescent protein from a lancelet. Chromophore covalently bound to a nearby tyrosine

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In recent decades green fluorescent protein (GFP) and related fluorescent proteins (FPs) established themselves as efficient noninvasive molecular instruments in cell biology and biomedicine that are widely used for visualization and monitoring of internal processes within living cells and whole organisms. Design of new advanced fluorescent biomarkers is strongly supported by X-ray studies which provide valuable information on the structure-spectral relationships. The key property of GFP-like proteins is their ability to form chromophore autocatalytically by posttranslational modification of the internal amino acid triad. Green-emitting FPs can be further modified into red-emitting FPs.

This study presents the results of an X-ray crystallographic and biochemical study of the wild-type FPs: green laGFP and red laRFP from the lancelet *Branchiostoma lanceolatum* (Chordata). Lancelet FPs are evolutionarily distant from extensively-studied cnidarian FPs (~20% sequence homology) and remain poorly characterized. It was shown that laGFP is characterized by narrow excitation and emission peaks which are typical for green FPs ($\lambda_{ex}/\lambda_{em}$ ~502/511 nm). Freshly purified laRFP demonstrates green fluorescence ($\lambda_{ex}/\lambda_{em}$ ~ 506/522 nm) which within several days slowly converts into red fluorescence ($\lambda_{ex}/\lambda_{em}$ ~521/592 nm). The structure of laRFP (the only known red FP outside phylum Cnidaria) has revealed three unique features:

1) unusual red chromophore-forming sequence Gly58-Tyr59-Gly60

2) presence of Gln211 instead of strictly conserved catalytic Glu crucial for the red chromophore biosynthesis.

3) absence of posttranslational modifications typical for the previously reported red chromophores and presence of an unusual covalent bond between C^{β}-atom of the chromophore Tyr59 and hydroxyl of the proximal Tyr62. The impact of this covalent bond on red fluorescence emission of laRFP and its large Stokes shift (~ 70 nm) was further proved by extensive structure-based site-directed mutagenesis. We also performed stepwise mutagenesis aimed at the conversion of green laGFP to red-emitting variant by introduction of limited number of mutations in the chromophore-surrounding area. Based on the data above the mechanism of formation of the red chromophore from the intermediate green state has been proposed.

Four-locus analysis of the genetic polymorphism of enniatin-producing *Fusarium* species from different regions of Russia

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Fusarium fungi are worldwide distributed agricultural pathogens. In addition to harvest quality and quantity decrease, Fusarium head blight (FHB) infection leads to accumulation of dangerous mycotoxins in grain and its products. One of the major types of mycotoxins produced by *Fusarium* species are enniatins – cyclic peptides which possess antimicrobial, insecticidal, and phytotoxic activities. The main producers of enniatins are *Fusarium avenaceum*, *Fusarium tricinctum*, *Fusarium acuminatum*, *Fusarium torulosum*.

Nowadays, *Fusarium* taxonomy studies take into account data of the polymorphism of DNA sequences which allow estimating the rate of similarity between species and clearing the evolution history of the genus in whole. However, the number of highly polymorphic DNA sites with known sequence is relatively small and searching of novel variable loci proved to be a very relevant and important goal.

In this study partial phosphate permease gene (*PHO*) sequences have been determined for four enniatins-producing *Fusarium* species: *F. avenaceum*, *F. tricinctum*, *F. acuminatum*, *F. torulosum* for the first time. Phylogenetic analysis of 27 strains of those species based on comparison of partial sequences of *PHO*, translation elongation factor 1 alpha (*TEF1α*), beta-tubulin (*β-TUB*) and enniatins synthase genes demonstrated that the *PHO* gene possesses the highest rate of both inter- and intraspecific variability among them. A dendrogram based on the analysis of *PHO* sequences has revealed the presence of individual clusters of each of species studied. Moreover, *F. avenaceum* strains were divided into 5 separated clusters which appeared to be independent on their geographic origin and host plant. The polymorphism of *PHO* sequences have also been used to design primers and hydrolysis (TaqMan) probe to perform the highly specific identification of all four enniatins-producing *Fusarium* species studied.

Development of pilot-scale biotechnologies for production of analgesic peptide toxins for preclinical studies

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The feature that unifies most diseases is inflammation that is usually accompanied by pain syndrome; therefore, pain relief is one of the key objectives of the medicine that strives to comfort the ill. The nature, location and etiology of pain vary from case to case, thus necessitating the search for new analgesics with a high specific activity.

Earlier the Laboratory of Neuroreceptors and Neuroregulators (Institute of Bioorganic Chemistry of the RAS) was the first to extract and characterize Purotoxin-1 (PT1) from the *Geolycosa sp.* venom and APHC3 from the sea anemone *Heteractis crispa*. Both toxins demonstrated a strongly pronounced analgesic effect and a high selectivity: PT1 in respect to the P2X3-receptor (isoform purinoceptor P2X), and APHC3 in respect to the TPRV1 channel. Therefore, it was reasonable to proceed with the development of the efficient technology for the above referenced toxins production for preclinical studies and, eventually, clinical trials.