agrobacterium carrying the vectors pCXSN-Ch100mb or pCXSN-Ch140mb, almost complete attenuation of the GFP fluorescence was observed. At the same time, qPCR analysis confirmed that there were no changes in GFP expression in all samples, indicating that the amount GFP was only regulated post-translationally. Thus, we have created the system which allows us to perform the selective degradation of plant proteins via the UPS. Supported by RFBR #15-04-09365.

P.07-041-Tue
The new ability of mycelial fungi *Aspergillus nidulans* to acetylate 11alpha-hydroxyprogesterone

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It is known that biotechnological transformation of steroids using enzyme systems of microorganisms is often the only possible method to modify the molecule in the industrial production of steroid drugs. Mycelial fungus *Aspergillus nidulans* is one of the most well-known eukaryotic model systems. Despite this *A. nidulans* strains have been little studied as steroid-transforming microorganisms. We studied the ability of the *A. nidulans* VKPM F-1069 wild type strain (syn. FGSC A4, ATCC 3863, 12996, 26451, CBS 112.46, NRRL 194) and its auxotroph strain - *A. nidulans* 031 with argB2 and pyrG89 mutations (syn. FP-308.1; AN031; CBS 129193) to transform progesterone (PG) for the first time. It has been established that PG acetoxylation is a two-stage process. In the first stage, the hydroxylation of PG occurs into 11alpha position, then the formed 11alpha-hydroxy-PG is modified into 11alpha-acetoxy-PG. Thus, the acetylated product can be obtained both from PG and from 11alpha-hydroxy-PG. It was shown that argB2 and pyrG89 mutations had no effect on the regio- and stereospecificity of hydroxylation and the ability of the strain to acetylate 11alpha-hydroxy-PG, but influence the ratio of final products and provides an increase in 11alpha-acetoxy-PG. The formation of products was confirmed by the ^{1}H NMR, ^{13}C NMR, 2DNMR, high-resolution mass spectrometry analysis. For 11alpha-acetoxy-PG these characteristics were compared with those for 11alpha-acetoxy-PG chemically synthesized from 11alpha-hydroxy-PG. The potential of mycelial fungi, incl. genus *Aspergillus*, for the biocatalytic esterification of 11alpha-hydroxy-PG, wasn't previously described in available literature. These data can serve as a basis for development of the medical drugs synthesis technology, alternative to chemical synthesis, where *A. nidulans* strains can be used as biocatalysts for the biotechnological modification of steroids. This study was supported by a Grant of RFBR No. 18-34-00653.

P.07-042-Wed
Study of some metals effect on α-amylase activity of *Bacillus amyloliquefaciens* MDC 1974 strain

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α-Amylase (EC 3.2.1.1; α-(1,4)-d-glucan glucanohydrolase) is an endo-type enzyme that hydrolyzes starch by cleaving α-1,4-glucosidic linkages in a random fashion. Most of the α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity, and stability. α-Amylase has potential application in a wide number of industrial processes and can be produced by different species of microorganisms. Among them, those from *Bacillus* have been intensively studied and are important in industry. The aim of the work was to study the effect of some metal ions on purified to homogeneity α-amylase activity of *Bacillus amyloliquefaciens* MDC 1974 strain (GenBank, MF953984.1). The reaction for determination of effect of different metal ions on enzyme activity was carried out in the reaction medium containing 0.5% starch, 0.5 mM $CaCl_2$, 50 mM HEPES, pH 7.2 and 5 mM salts of metals, at temperature 60°C. The duration of reactions was 20 min. All metals were used in the chloride or sulfate forms. The amount of reduced groups was determinate by Dinitrosalicylic acid (DNS) method. Obtained results indicated that 80% of α-amylase activity was lost when the enzyme was incubated with 10 mM EDTA, thus, indicating the enzyme was a metalloenzyme. The enzyme activity was not retained after incubation with 10 mM $CaCl_2$. It is shown that Cd^{2+} (residual activity – 39.7%) and Cu^{2+} (residual activity – 43.7%) had strong inhibitory effect, while Mg^{2+} (115.4%), Fe^{2+} (129.4%) and Fe^{3+} (166.9%) enhanced the enzyme activity in studied concentrations. On the other hand, K^{+}, Na^{+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Ba^{2+}, Pb^{2+}, Co^{2+} did not show any effect on α-amylase activity. Thus, Cd^{2+} and Cu^{2+} are strong inhibitors of studied α-amylase.

P.07-043-Mon
Study of the influence of cells-producers of prokaryotic and eukaryotic origin on the functional activity of the transgenic GDNF

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The aim of this research is to study the effect of prokaryotic and eukaryotic cells on the functional activity of the transgenic GDNF. The study was conducted on PC12 cells, whole and dissociated root dorsal ganglion and neuroblastoma SH-SY5Y. Cell cultures were cultivated in the presence of modified forms of the neurotrophic factor GDNF: GDNF with pro-sequence, GDNF with deletion prepro-sequence mGDNF. Pro - and mGDNF was expressed in two different systems: prokaryotic *E. coli* and eukaryotic - HEK293. The number of neural processes during using the mGDNF derived from transgenic HEK293/mGDNF is greater than during the cultivation in the presence of the mGDNF expressed in *E. coli*. But proGDNF that was synthesized in the prokaryotic system showed better results compared to proGDNF that was synthesized in the eukaryotic system. In general a greater number of sprouts were observed during cultivation in the presence of the mGDNF expressed in the

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eukaryotic system compared to other forms of protein. A significant increase of β3-tubulin expression in the case of cultivation in the presence of mGDNF was confirmed by qPCR. As a result of the action of MPP toxin, the number of living cells was higher during co-cultivation with proGDNF obtained from *E. coli*. An isolated from transgenic eukaryotic cells factor, which showed a good result in maintaining the viability of cells, was mGDNF. The results of the study indicate that proGDNF is necessary to maintain cell viability, at the same time GDNF has increased neuroinductor properties. Significant neuroinductor properties of the mGDNF ensure its use if the population of neural cells reduction is necessary, for example, in cases of traumatic injuries or neurodegenerative diseases. Cells-producers are important for the formation of the studied forms' biological activity of a neurotrophic factor. And, evidently, it is preferable to use GDNF, expressed from eukaryotic cells, due to its higher neuroinductor properties.

P.07-044-Tue
A novel approach for fractional photodynamic therapy utilizing a photosensitizer with an additional 2-pyridone module

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Photodynamic therapy (PDT) is considered a promising approach for the treatment of several types of cancer. Generation of cytotoxic singlet oxygen, which has a short lifetime, increases the probability of selective action. Singlet oxygen generation in a photosensitized manner within cancer tissues during PDT is self-limiting, as the hypoxic conditions within cancers is further diminished during the process. In order to minimize photoinduced hypoxia, the light may be introduced intermittently (fractional PDT) to allow time for the replenishment of tissue oxygen. However, such applications extend the time required for effective therapy. In the current study, we show that a photosensitizer with an additional 2-pyridone module for trapping singlet oxygen would be useful in fractional PDT. Therefore, the endoperoxide of 2-pyridone is generated along with singlet oxygen in the light cycle. The endoperoxide undergoes thermal cycloreversion to produce singlet oxygen in the dark cycle, regenerating the 2-pyridone module. The photodynamic process can thus continue in the dark as well as in the light cycles. We validated this working principle *in vitro* with HeLa (human cervical cancer cell line) cell culture studies with cell viability / cytotoxicity assays (MTT). HeLa cells were incubated with varying concentrations of the compound and illuminated with a red light source (λ = 655 nm LED array, 324 μmol/m^2/s photon flux) for 10 min in every one hour, which was repeated 24 times. It was found that even low doses of this novel drug results in a significant decrease of the HeLa cell viability (CC_{50} value: 8.6 nM). Since both intrinsic tumor hypoxia and PDT-induced hypoxia are considerable issues against the wider applicability of PDT, such bifunctional photodynamic therapy agents may prove to be very promising in the future.

P.07-045-Wed
Multimeric expression of acid-cleaved alpha-fetoprotein receptor binding peptide

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Alpha-fetoprotein (AFP) and its receptors (RECAF) are well known tumor-related biomarkers used in cancer diagnostics and tumor-targeted drug delivery systems. RECAF is known to be expressed by the majority of tumor cells, while its expression is downregulated in non-fetal normally functionalized cells. It is common knowledge that the part of AFP, namely KQEFLIN heptapeptide is essential for RECAF binding. In this study, genetic constructs encoding recombinant peptide multimers, consisting of as few as 1 and as high as 14 extended 23 amino acid RECAF-binding sites (PWGVALQTM*KQEFLIN*LVKQKPQITD) of native AFP linked via Asp-Pro cleavage sites have been prepared and cloned in pET 28a+ plasmid via NcoI and XhoI first, SalI and XhoI 2–14th repeat. The expression analysis of transfected *E. coli* BL21(DE3) strains revealed an increase in productivity upon expression from 2 to 5 monomers, respectively. Strains transformed with 6 and more repeats were found to express mostly 3 membered multimer. The most stable multimers were purified and cleaved at Asp-Pro sites to monomers with formic, acetic and hydrochloric acids in different concentration, time, pH and temperature regimes both with chaotropic agent or not. The cleavage process was analyzed by Tricine SDS-PAGE, RP-HPLC and optimized for each multimer. The most effective cleavage agent was found to be ≥70% formic acid, however, it induce several monomer modifications, which cause difficulties during purification. The major products of formic acid hydrolysis were purified. Further, it is planned to characterize all the products by MALDI-TOF MS and both by cellular and cell free RECAF binding assays to evaluate its potency as vector for tumor-targeted drug delivery systems or and an affinity ligand for RECAF purification.

P.07-046-Mon
Enhancing 3-hydroxypropionic acid production in combination with sugar supply engineering by cell surface-display and metabolic engineering of *Schizosaccharomyces pombe*

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3-hydroxypropionic acid (3HP) is the important chemical for building bio-sustainable society. Here, we describe metabolic engineering of fission yeast *Schizosaccharomyces pombe* for 3HP production via malonyl-CoA pathway from glucose and cellobiose. Genes encoding malonyl-CoA reductase (MCR) of Chloroflexus aurantiacus was dissected into two functionally distinct fragments (MCR-C and MCR-N) and the activity between MCR-C and MCR-N was balanced. To increase the cellular supply of malonyl-CoA and acetyl-CoA, we introduced genes encoding endogenous aldehyde dehydrogenase (atd1), acetyl-CoA synthase from *Salmonella enterica* (acsSE*), and endogenous pantothenate kinase (ptk1). The resultant strain produced 1.0 g/L of 3HP from 50 g/L consumed glucose. We also engineered sugar supply by displaying beta-glucosidase on its cell surface. When 50 g/L of cellobiose was used, the engineered strain efficiently consumed cellobiose and produced 3HP at 3.5 g/L. Finally, this