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Mycoplasma HU proteins restore growth deficit of *E. coli* with double genetic knockout of hupA and hupB genes

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Omnipresent in bacteria, DNA-binding histone-like HU proteins (HUs) are nucleoid-associated proteins (NAPs) involved in DNA supercoiling, nucleoid compaction, modulation of DNA-dependent transaction and bacterial adaptation to stress conditions. The first small molecule inhibitor of *M. tuberculosis* HU developed by crystal structure-based drug design demonstrated antibacterial activity. The antimycoplasmic activity of HU inhibitors is supposed to be more profound, as the absence of HUs is lethal for organisms where they are the only NAPs available, e.g. Mollicutes. High genome plasticity of Mollicutes resulting in high diversity of their protein sequences, requires conducting additional in vitro and in vivo assessments of the abilities of newly designed inhibitors to affect target-proteins independently of their sequence and structural variations. In this study, mycoplasma HU proteins: HUSpm from *S. melliferum* - an insect parasite infecting honeybees and HUMgal from *M. gallisepticum* - a poultry pathogen causing severe respiratory disease in chicken and turkeys, were produced under control of arabinose promoter in *E. coli* cells with double knockout of the *hupA* and *hupB* genes coding heterodimeric *E. coli* HU. An electromobility shift assay performed with synthetic oligonucleotide duplexes detected that DNA-binding activities in cell-free extracts of *E. coli* expressing both mycoplasma HUs are similar to those of purified recombinant proteins. Arabinose induction of either HUSpm or HUMgal expression in a slow growing knockout $hupA^{-}/B^{-}$ *E. coli* cells restores the wild-type growth. These findings indicate that both HUs execute all essential functions of histone-like HU proteins. We suggest that *E. coli* cell-based complementation system represents a simple in vivo test for rapid HU inhibitor evaluation not requiring cultivation of the infectious bacteria. This work was supported by the Russian Science Foundation grant 15-14-00063P.

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Efficient DNA-free nanoparticle mediated genome editing of potato using CRISPR-Cas9 RNP complex

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Due to its simplicity, practicality and efficiency CRISPR–Cas9 is rapidly developing as the emerging technology for genome editing on various eukaryotic systems including higher plants and animals. Here we describe new approach for delivery of pre-assembled Cas9–sgRNA ribonucleoproteins into dissected potato (Solanum tuberosum, cv Chicago) shoot apical meristems by chitosan nanoparticles using vacuum infiltration and regeneration of plants with edited alleles. As a model gene for genome editing, we have selected a gene encoding coilin, a major protein of Cajal bodies(CBs). In additional to its traditional role in assembly of CBs, coilin also participates in biotic (virus attack) and abiotic (high salinity) plant stress responses. Using this method of delivery, we have demonstrated DNA-free genome editing and recovery of potato plants with mutated (disrupted) coilin gene. The generated edited plants exhibit high level of resistance to potato virus Y and significant tolerance to osmotic and salt stress. This work opens up a new avenue for practical application of CRISPR-Cas9 technology to produce genome edited (transgene-free) potato plants and other crops. The present work was performed with the financial support from the Russian Science Foundation (grant No. 16-16-04019).

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Development of potentiometric micro-sized urea biosensor based on urease-dextran complex

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Enzymatic sensors for the potentiometric determination of urea belong to the best known class of biosensors. Although potentiometric urea biosensors emphasising on better sensitivity or higher response range are reported, not much effort has been made in resolving the drawbacks of enzyme instability, difficulty in storage and handling, and fragility of the immobilization matrix. These drawbacks can be overcome by enhancing enzyme stability with neutral biopolymers for development of biosensor. Therefore in this study, development of micro-sized potentiometric urea biosensor has been purposed by using modified urease enzyme with dextran, immobilized on ammonium selective electrode surface via glutaraldehyde as a crosslinking agent. The potentiometric micro sized urea biosensor by enhanced stability against environmental conditions using urease-dextran complexes will not include inner reference electrode and inner reference solution. Potentiometric performance of urea biosensor based on urease-dextran complex (selectivity constants, linear working range, determination of limit, response time, pH working range, temperature affect, reproducibility, storage stability) is examined with a computer-controlled measurement system and results are compared with other biosensors based on unmodified urease. The urea biosensor prepared by using urease-dextran complex showed more effective performance than unmodified urease ones.

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Identification of major bee venom allergen Api m 1 IgE epitopes and characterization of their corresponding mimotopes

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Immunotherapy with bee venom extracts is associated with high rate of side effects including severe systemic reactions. Short peptides that mimic IgE epitopes (mimotopes) offer an opportunity for development of safer immunotherapeutics. Due to their small size, they do not possess the ability to cross-link IgEs on effector cells. Moreover, in combination with a suitable immunogenic carrier, they are able to stimulate T cell immune response. Peptides were selected from phage-displayed random peptide libraries and used to computationally map IgE epitopes. Peptide mimotopes of identified epitopes were synthesized. Additionally, peptide mimotopes fused to bacteriophage coat protein pIII were isolated from *Escherichia coli* periplasm and tested for IgE binding with sera of bee venom-allergic patients in the immunodot assay. Basophil