

Mechanism-based dual reporter screening system assists in identification of translational inhibitors

P-02.1-17

D. Lukianov^I, I. Lisevich^I, V. Marina^{II}, M. Wieland^{III}, T. Maviza^I, E. Rasumova^{II}, A. Ferberg^{II}, M. Tochilkina^{IV}, O. Ibitoye^I, V. Tashlitsky^{II}, V. Polshakov^{II}, S. Iarovenko^{IV}, K. Komarova^{IV}, D. Wilson^{III}, P. Sergiev^{II}, O. Dontsova^{III,V}, I. Osterman^{I,II}

^ISkolkovo Institute of Science and Technology (Skoltech), Moscow, Russia, ^{II}Lomonosov State University, Chemistry Department, Moscow, Russia, ^{III}Institute for Biochemistry and Molecular Biology, University of Hamburg, Hamburg, Germany, ^{IV}Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia, ^VShemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

In the last decade, many bacteria with outstanding resistance have been discovered and the already known pathogenic bacteria gained resistance genes. It caused a gradual decrease in the efficiency of the known antibiotics. According to a population-level modelling analysis in the European Union in 2015 it was estimated 33000 deaths caused by antimicrobial resistance. In our laboratory method, convenient for high throughput screening was invented. A broad-specificity system for detecting translation inhibitors is built upon the tryptophan attenuator trpL and Katushka2S fluorescent protein. DNA-damaging antibiotics can be detected by the expression of Red Fluorescent Protein(RFP) cloned under sulA promoter. Using this approach 50000 individual chemical compounds and 4000 cultural broth were tested. Among tested substances we have found chemicals that induce translational reporter system. Molecule «Y020-7126» from ChemDiv compound library inhibits translation in the non-cellular system and in vivo incorporation of C¹⁴-valine in cells proteins, stops translation on the stage of elongation according to the toe-printing assay. Also, two cultural broth which contains inhibitors of translation were found—the compound auroplanin from a cultural broth of *Actinoplanes sp. VKM Ac-2862*. It has the empirical formula C₂₃H₂₅NO₄ and, according to the structure solved by Nuclear Magnetic Resonance, is a novel molecule. *E.coli* strains resistant to this active compound have mutations in 560 helices of 16S rRNA. These mutations were not described in the literature before. Another interesting broth sample of bacterium *Amycoloplasia sp.* This culture produces aromatic polyketide antibiotic tetracenomycin X(TcmX), which is a potent inhibitor of protein synthesis and does not induce DNA damage as previously thought. TcmX binds to a novel site within the polypeptide exit tunnel on the large subunit. The reported study was funded by RFBR according to the research project №20-34-90048.