

POSTERS

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the molecular evolution of the bacterial nicotine pathways and we are using the well characterized pAO1 encoded *nic*-genes from *Paenarthrobacter nicotinovorans* (GenBank GI: AJ507836) as a model for comparative genomics. A rather similar *nic*-gene arrangement was identified in the draft genome of *Arthrobacter* AK-YN10, a bacterial strain isolated for its ability to degrade atrazine. In the current approach, we aimed to investigate the location of these *nic*-genes and to establish whether these genes are functional or not. For this, total and plasmidial DNA was extracted from *Arthrobacter* AK-YN10 and the presence of the nicotine catabolism genes was assessed by PCR using specific primers. The nicotine consumption during the growth of pAO1 and AK-YN10 strains on citrate medium was monitored by HPLC. We showed that the AK-YN10 strain contains at least one plasmid and that the 6-hydroxy-L-nicotine oxidase gene involved in the nicotine metabolism on pAO1 is placed on one of these plasmids. The AK-YN10 strain is both resistant to and can grow on nicotine containing media, but it is not able to degrade this alkaloid.

P.28-009-Wed

The ethnic characteristics of distribution of EDNRA H323H polymorphic genetic marker of cardiovascular diseases

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The Endothelin receptor type A (ETA) is a G protein-coupled receptor that binds endothelin-1 and mediates its vasoconstrictor effect. Many studies suggest that genetic variations in the *EDNRA* gene coding for the ETA receptor are associated with different cardiovascular diseases, however, others could not find this association, probably due to ethnic characteristics of studied populations. In our previous study of the *EDNRA* H323H (T>C) polymorphism (rs5333) we found a significant difference in allelic and genotypic frequencies between Arab Syrians and Russian populations. In the present study, we investigated for the first time the distribution of this polymorphism among 36 Lebanese with the mean age of 25.37 ± 7.57 years. The genotypes were determined using PCR with restriction fragment length polymorphism. The frequency of C allele of *EDNRA* gene was 19%. The genotype frequencies were in Hardy-Weinberg equilibrium ($P > 0.05$). The TT, TC and CC genotype frequencies were 63.89%, 33.3%, and 2.81%, respectively. A significant difference was revealed in genotype distribution between Lebanese and Russians from Central Russia ($P = 0.00096$), as well as in allelic distribution ($\chi^2 = 4.281$, $P = 0.03852$), whereas genotypic frequencies tended to be different between Arab Syrians and Lebanese ($P = 0.05708$). The results suggest that the analyzed polymorphism seems to have an ethnic distribution; therefore, the allelic and genotypic frequencies of the H323H *EDNRA* polymorphism should be investigated in larger samples and compared with clinical implications through additional research within studied populations. The publication was prepared with the support of the "RUDN University Program 5-100".

P.28-010-Mon

A novel synthetic flavonoid with potent antibacterial properties: in vitro activity and proposed mode of action

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The emergence of pathogenic multidrug-resistant bacteria demands new approaches in finding effective antibacterial agents. Synthetic flavonoids could be a reliable solution due to their important antimicrobial activity. We report here the potent *in vitro* antibacterial activity of CICI-flav – a novel synthetic tricyclic flavonoid. The antimicrobial effects were tested using the minimum inhibitory concentration (MIC), time kill and biofilm formation assays. Fluorescence microscopy and scanning electron microscopy were employed to study the mechanism of action. MTT test was used to assess the cytotoxicity of CICI-flav. Our results showed that Gram positive bacteria were more sensitive (MIC = 0.24 µg/ml) to CICI-flav compared to the Gram negative ones (MIC = 3.9 µg/ml). We found that our compound showed significantly enhanced antibacterial activities, 32 to 72-folds more active than other synthetic flavonoids. CICI-flav showed a bactericidal activity at concentrations ranging from 0.48 to 15.62 µg/ml. At twice the MIC, all *Escherichia coli* and *Klebsiella pneumoniae* cells were killed within 1 h. Also CICI-flav presented a good anti-biofilm activity. The mechanism of action is related to the impairment of the cell membrane integrity. No or very low cytotoxicity was evidenced at effective concentrations against Vero cells. Based on the strong antibacterial activity and cytotoxicity assessment, CICI-flav has a good potential for the design of new antimicrobial agents.

P.28-011-Tue

Low molecular weight metabolites secreted through an ABC-type efflux pump MacAB protect *Serratia marcescens* against hydrogen peroxide

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The emergence of bacterial multi-drug resistance is a growing problem of public health worldwide. Bacterial drug efflux systems are membrane protein complexes that function to expulse drugs from the cell. In addition to well established role of multidrug efflux pumps in antibiotic resistance, efflux pumps also play important additional roles in biology of bacteria that are independent of their role in drug efflux. Macrolide-specific ABC-type drug efflux pump MacAB first identified in *E. coli* has been linked to virulence of *Salmonella enterica* serotype Typhimurium in mice. Here we show that MacAB is essential for survival of *Serratia marcescens* SM6 in the presence of hydrogen peroxide. We further show that the growth of *S. marcescens* Δ MacAB mutant cells in the peroxide-containing media could be restored by co-culture with wild type cells. This protection is mediated by heat- and proteinase K-sensitive metabolites present in the media used for growth of wild type *S. marcescens* SM6 cells but not in the media used for growth of its isogenic Δ MacAB mutant cells. Moreover, the synthesis of these metabolites does not require active ribosomes. Fractionation of the conditioned media showed

that protective antioxidant molecules are present in the fraction containing low molecular weight metabolites (under 10 kDa). Additional HPLC analysis resulted in the identification of five fractions with anti H₂O₂ protective properties which are currently evaluated for their metabolite composition. This work was supported by the Russian Science Foundation project 16-14-10200 and performed in accordance with the Russian Government Program of Competitive Growth of Kazan Federal University.

P.28-012-Wed Identification of fucoidan sulfatases using bioinformatics and functional screening approaches

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Sulfatases play a key role in the catabolism of various sulfated polysaccharides (ulvans, carrageenans, agarans, fucoidans, etc.) of marine organisms. The variety of sulfated polysaccharide structures implies a large amount of sulfatases with different substrate specificity. Despite advances in processing and annotating the genomic and metagenomic data of marine microorganisms, the correct annotation of carbohydrate sulfatases is still difficult. Due to the fact that sulfatases acting on many marine polysaccharides have been poorly characterized, and some have not yet been discovered. To date, only a few carrageenan sulfatases and agaran sulfatases have been biochemically characterized. There are only fragmentary data about fucoidan sulfatases. Amino acid sequences, specificity, mode and mechanism of action of fucoidan sulfatase are still unknown. We analyzed the genome of the fucoidan degrading marine bacterium *Wenyngzhuangia fucanilytica* CZ1127 and identified 80 hypothetical sulfatases genes. Six hypothetical sulfatases genes, named by us as swf1-6 were located in close proximity to genes of fucoidanases (107 family of glycoside hydrolases CAZy). We assumed that the presence of sulfatases coding genes in the same locus with fucoidanases indicates their participation in the catabolism of fucoidans. To confirm their function, genes of sulfatases were cloned and proteins were produced in *Escherichia coli* cells. Functional screening among hypothetical sulfatases using sulfated fucooligosaccharides and fucoidans resulted to identification of two fucoidan sulfatases SWF1 and SWF4. Specificity and some catalytic features of sulfatases were determined using various sulfated fucooligosaccharides. Based on the substrate specificity, the enzymes are classified as fucoidan exo-2O-sulfatase (SWF1) and fucoidan exo-3O-sulfatase (SWF4). This work was supported by the Russian Science Foundation (Project No. 18-04-00905).

P.28-013-Mon ATP-synthase inhibition by semi-synthetic oligomycin A derivatives

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Macrolide antibiotic oligomycin A (OlgA) is a high-active ATPase inhibitor, which is widely used for biochemical studies of mitochondrial F₀F₁ ATP synthase. In micromolar concentrations, OlgA binds to F₀ c-subunit and blocks proton translocation, resulting in disruption of bioenergetic metabolism. It has

been proposed that hydroxyl group at C33 position of the OlgA side chain might have an influence on its binding with the target. Also, recently we have found that modifications of OlgA macrolactone core led to significant changes in its biological properties. In order to investigate mitochondrial ATP-synthase inhibition by oligomycins more closely, a series of semi synthetic OlgA derivatives with site-selective modifications were synthesized and their inhibitory activity on the F₀F₁ ATP synthase were determined on inverted membrane vesicles obtained from cells of *Streptomyces fradiae* ATCC 19609 (strain, supersensitive to OlgA). It has been found that nitron-oligomycin, modified at positions C7 and C3 into intramolecular heterocycle and 2,3,16,17,18,19-hexahydrooligomycin A with reduced double C-C bonds didn't inhibit ATP-synthesis in the vesicles, probably due to significant change of macrolactone geometry and, consequently, decreasing the affinity to the target. These data were in agreement with results of molecular modelling of binding of OlgA and its derivatives to the intracellular target. In striking contrast, 33-azido-33-deoxy-oligomycin and 33-O-mesyl-oligomycin inhibited ATP synthesis more potently than parent antibiotic. Finally, 33-dehydrooligomycin and 33-deoxy-33-thiocyanato-oligomycin were slightly less active than OlgA. Thus, we can conclude that structural changes in macrolactone cycle can be critical for binding affinity of OlgA to ATP synthase and functional groups at C33 position indeed play the important role in ATP-synthase inhibition by OlgA. This work was supported in part by Russian Science Foundation (agreement № 15-15-00141).

P.28-014-Tue Relationship between iFGF23, iPTH and phosphorus in renal transplant patients

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iFGF23, a bone-produced hormone, plays a key role in phosphorus metabolism. The aim of this study is to investigate the relationship between iFGF23, iPTH and Phosphorus in renal transplant patients. 40 males (age 39.28 ± 11.01) and 20 female (age 40.05 ± 12.09) renal transplant patients who are followed in the transplantation polyclinic of Gazi University Medical Faculty Hospital were included in the study. The control group consisted of 18 males (age 41.22 ± 6.34) and 16 healthy females (age 41.88 ± 8.24) were included in the study. The mean transplant year was 6.95 ± 5.44. The median (min-max) values of serum iFGF23 in renal transplant patients were found to be 219.67 (93.7–652.83) pg/ml respectively, while serum iPTH levels were 83.6 (16.52–278.3) pg/ml, and serum Phosphorus was found to be 3.195 (1.6–5.7) mg/dl. Serum iFGF23 levels were found to be 119.67 (52.39–361.74) pg/ml in the control group, while serum iPTH levels were 38.05 (3.41–109.1) pg/ml serum Phosphorus 3.52 (2.54–4.62) mg/dl respectively. Serum iFGF23 and iPTH levels were statistically significantly higher in the patient group than the control group (*P* < 0.05). Whereas serum Phosphorus levels were statistically significantly lower in the patient group than the control group (*P* < 0.05). Correlation study of transplant patients showed a strong positive correlation between serum iFGF23 and serum iPTH (*P* < 0.01) but no correlation between iFGF23 and Phosphorus (*P* > 0.05). There was no correlation between iFGF23 and iPTH and Phosphorus in the correlation study performed in the control group (*P* > 0.05).