of clones with increased virulence, resistance to environmental factors and antibiotics. One of the new molecular methods is INDEL-typing which is based on the search for spontaneous inserts/deletions of several nucleotides that differ in length in different clones.

Vibrio parahaemolyticus is a common and important pathogen that causes human gastroenteritis worldwide. We have developed a method for typing *V. parahaemolyticus* strains based on the analysis of six INDEL-locus (S.O. Vodop'yanov et al., 2016). The INDEL analyses of the *V. parahaemolyticus* collection revealed that strains of different INDEL-genotypes circulate in environment. The discriminating power of INDEL-typing for environmental strains was 0.95. However, to date, there is no information about the INDEL-genotypes of clinical strains.

The aim of this work was to study INDEL-markers of *V. parahaemolyticus* strains isolated during two foodborne disease outbreaks in the Russian Federation.

It was investigated 29 clinical strains of *V. parahaemolyticus* isolated in July-October 2012 in the Primorsky region of the Russian Federation. The study was performed on INDEL loci Vp967, Vp08, Vp619, Vp2256, VpA472, Vp506. The result showed that all 29 studied *V. parahaemolyticus* strains had identical INDEL genotype with the formula Vp967 — 112, Vp08 — 89, Vp619 — 114, Vp2256 — 111, VpA472 — 95 and Vp506 — 79 base pairs. Thus, both outbreaks were caused by one clone of the pathogen. At the same time, strains with other INDEL genotypes circulated in the environment.

In our opinion, the INDEL-typing method of *V. parahaemolyticus* strains can be useful in carrying out epidemiological investigation of outbreaks of food gastroenteritis.

2.4 doi: 10.15789/2220-7619-2018-4-2.4

EXPRESSION OF RECOMBINANT NS1 PROTEINS OF WEST NILE, DENGUE AND ZIKA FEVER VIRUSES IN *NICOTIANA TABACUM* FOR FUTURE USE IN DIAGNOSTICS

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In connection with the increasing frequency of infectious diseases outbreaks caused by arboviruses, the monitoring of the epidemiological situation in the Russian Federation requires development of immunological diagnostic kits for differential diagnosis. These kits could be developed using individual recombinant antigen proteins of selected viruses. Standard eukaryotic systems, for example insect cells, have a number of limitations in terms of productivity and costs. In our work, we used plants for the production of flavivirus antigens which are an ideal biofabric system because of their ability to generate large amounts of proteins with low cost and to produce an appropriate post-translational modification of recombinant proteins. Protein targets for expression were NS1 non-structural proteins of flaviviruses which were described in the literature as reliable serological markers.

The sequences of the NS1 proteins of Zika virus (ZIKV), West Nile virus (WNV) virus and the two serotypes of Dengue virus (DNV1 and DNV3), have been optimized for expression of the target proteins in the *Nicotiana tabacum*. The resulting DNA sequences were submitted in the GenBank database under accession numbers: MH134590, MH134591, MH134592, MH134593 for ZIKV, DNV1, DV3 and WNV respectively. Sequences were synthesized *de novo* using oligonucleotides by the enzymatic "Two step PCR" method. Expression cassettes containing 35S CaMV promoter and tNOS terminator for strong constitutive expression of the target were constructed on the base of the pBI121 plasmid. Four binary vector systems for the expression of NS1 proteins in plants were developed. Tobacco leaf discs were transformed using *Agrobacterium tumefaciens* Ti-plasmids of strain AGL0 and further regeneration of tobacco plants was carried out. For each expression structure, 10 independent transgenic lines were obtained and were transferred to rooting media for further transfer to conditions of closed soil, which would enable the collection of the necessary amount of biomass to isolate antigen proteins, for their further use in the creation of diagnostic systems. The target gene insertions in each line were confirmed by PCR.

Thus, the plant expression system of West Nile, Dengue and Zika virus antigens was developed and our future studies would include purification of target antigens and their verification as serological markers in diagnostic systems (ELISA, immunochip).

This study was supported by the RSF grant #17-75-10093.

2.5 doi: 10.15789/2220-7619-2018-4-2.5

WHOLE-GENOME SEQUENCING AS A TOOL FOR COMPREHENSIVE ASSESSMENT OF THE PATHOGENIC POTENTIAL OF ANCIENT ARCTIC MICROBIOMES

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Arctic permafrost is a natural reservoir of ancient prokaryotic mobile genetic elements (MGE) associated with pathogenicity or resistance to antimicrobials. It has been shown that ancient MGE have possibility to integration and effective expression in the genomes of modern bacteria. For example, an ancient *Psychrobacter* sp. pKLH80 plasmid from strain isolated in the Pleistocenic permafrost, contains blaRTG-6 β-lactamase gene, able to be mobilized in the modern epidemic *Acinetobacter baumannii* (Petrova M. et al., 2014). Horizontal genetical transfer of virulence and antibiotic resistance determinants from ancient microorganisms can lead to the appearance of genotypes with high epidemic potential. Thus the process of removal of paleomicroorganisms or their genetic material by degradation of permafrost due the global climatic changes is associated with the risk of emergence of new pathogenes or activation of forgotten infectious diseases. An effective monitoring of the pathogenic potential of the polar microbiota should be implemented.

In our opinion, one of the most promising approaches to the study of the pathogenic characteristics of bacteria found in permafrost is the whole genome sequencing. As a result of our team's studies several bacterial genomes isolated from Pleistocenic mammoth fauna were annotated. In particular, the ancient genomes of *Enterococcus* sp. (GenBank Acc. No. LGAN000000000000, NZ_LGAO00000000), *Arthrobacter* sp. ((Acc. No. QDAE00000000), *Clostridium perfringens* (Bac. No. QDAE00000000, QDAF00000000), *Serratia* spp. (Acc. No. MQRH00000000, MQML00000000), *Acinetobacter lwoffii* (Acc. No. LZDF00000000) were described.

The presence of of the modern epidemic clones markers in the genomes of Arctic paleobacteria was found. For example, IS16 genetic element characteristic for modern vancomycin-resistant enterococci in the ancient *E. faecium*