Short communication

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Genomic characterization of *Pseudomonas* bacteriophage AN14

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ABSTRACT. Siphoviruses with a prolate capsid infecting *Pseudomonas* sp. were isolated from environmental sources through the world, and were recently defined as a separate taxonomic genus *Yuavirus*. Besides a distinguishable morphology, the hallmarks of the genus are heavily modified genomic DNA and a lytic infection cycle while putative lysogeny genes are proposed in the genomes. Bacteriophage AN14 was isolated from Lake Baikal ecosystem and was assigned as a member of *Yuavirus*. We report the biological and morphological features if this phage, as well as the comprehensive re-annotation of its genome. Modern bioinformatics analysis resulted in the refinement of the taxonomic attributation of the YuA-like phages and highlighting the specific genomic and proteomic features typical for *Yuavirus* phages including AN14.

Keywords: Bacteriophage, *Pseudomonas*, genomics, phylogeny, taxonomy

Introduction

Bacteriophage AN14 infecting *Pseudomonas* was discovered in the aquatic ecosystem of Lake Baikal in 2010. According to the current nomenclature justified by the International Committee for Virus (ICTV) in 2020, phage AN14 belongs to the genus *Yuavirus* of family *Siphoviridae*. ICTV database lists six previously reported phages as members of *Yuavirus*— *Alphaproteobacteria* virus φJL001, and *Pseudomonas* viruses LKO4, M6, MP1412, PAE1 and YuA. Moreover, the NCBI database and taxonomic browser contains information on another 12 phages presumably belonging to the genus *Yuavirus*. Extreme diversity of the bacterial hosts of this phage group comprising alpha-, beta- and gamma-proteobacteria raises questions on the correction and possible revision of the current taxonomic classification.

The presented work reports the parameters of the AN14 infection cycle and morphology. We have re-annotated the AN14 genome taking into account the recent data on structures and functions of phage proteins. Genomic and phylogenetic research have refined the taxonomic position of phages belonging to genus *Yuavirus*.

Results and discussion

The genome of AN14 contains 86 putative ORFs, putative functions of 58 proteins can be predicted, and 28 ORFs are assigned as hypothetical proteins. There are no tRNA genes found in the genome. All genes are oriented in the same direction. G+C content of the AN14 genome is 64.5%, evenly distributed through the genome. *Yuavirus* phages infecting *Pseudomonas* have the G+C value most close to the average G+C (65%) of the bacterial host.

The genes of AN14 genome are clustered in three blocks – the first one encodes structural and lysis proteins, the second one is responsible for nucleotide metabolism and modification proteins, and the third block encodes replication, transcription and other proteins. The genes of structural proteins comprise about one third of the genome and are well conserved in the representatives of the genus. The functions of most of these genes can be predicted using BLAST and HMM-HMM pipelines.

The block for terminal lysis of the host cell in *Pseudomonas Yuavirus* phages contains genes encoding peptidoglycan-lysing enzyme, which has been proposed to function as murein transglycosylase F, and the holin located immediately downstream. One of the noticeable features of AN14 predicted proteome is a significant number of proteins involved in nucleotide modification and repair. At least 8 genes for such proteins can be found, and their homologs are present in other *Yuavirus* genomes. A large set of potential nucleotide modification proteins correlates with the experimentally found high content of modified bases in the genomes of phages YuA (Ceyssens et al., 2008). Another interesting feature of

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AN14 genome is the presence an anti-restriction system (Spoerel et al., 1979), including antirestriction protein (gp49) and predicted exonuclease inhibitor protein (gp03). The previous annotation of AN14 genome and the annotations of the genomes of YuA-like phages genomes have stated a presence of an integrase-like protein (gp27), but we propose that this protein is most likely a DNA primase.

In order to construct a consistent taxonomy and phylogenetic positioning of phage AN14, we performed a BLAST search using the terminase large subunit protein sequences and GenBank phage database, and constructed a list of phages belonging to the taxa, representatives of which were found with E -value $< 10^{-8}$. The maximum likelihood (ML) phylogeny of terminase large subunit protein sequence clearly showed the affiliation of AN14 with the *Yuavirus* clade. The tree groups 16 *Pseudomonas* phages into a distinct clade and points to *Vojvodinavirus* as the genus closest evolutionally. Contrary to the current ICTV classification, *Alphaprotebacteria* phage φJL001 (AY576273) (Lohr et al., 2005)469 bp with an overall G + C content of 62%. The genome has 91 predicted open reading frames (ORFs seems to belong to a group comparatively distant from *Yuavirus*, and *Bordetella* virus LK3 (KX961385) belongs to the genus *Vojvodinavirus*. These conclusions are in the agreement with proteome clustering, ANI calculations, genome sequence comparison among 10 AN14-related phage genomes, and ML phylogeny based on 39 core proteins concatenated sequences.

The terminase phylogeny points to *Pseudomonas* phages of the *Abijanvirus* genus as the next closest group to *Vojvodinavirus*. More distant relatives also represent the phages of gram-negative bacteria, mostly *Pseudomonas* spp. Phylogenetic analysis suggests that *Alphaprotebacteria* phage φJL001 terminase have diverged from the common clade comprising *Yuavirus* and related groups even earlier than the divergence to genera *Stenhofvirus*, *Pamexvirus*, *Abijanvirus* and *Vojvodinavirus* occured.

Previously, it was shown experimentally that *Pseudomonas* phage YuA and *Bordetella* phages, closely related to AN14, infect their hosts through a lytic cycle only. The main rationale to state a possible temperate lifestyle of *Yuavirus* phages is the presence of the putative conserved integrase gene (*g27* in case of AN14) and the repressor (*g26*) in the genome. However, the homologs of AN14 gp27, annotated in φJL001, YuA, M6 and other related phages as integrases, may perform a different function not related to lysogeny. Alternatively, it may be attributed as DNA primase/ polymerase belonging to AEP-primases. Undoubtedly, this hypothesis requires a future experimental proof. However, it gives a reasonable explanation for the long-disputed contradiction between the presence of predicted lysogeny genetic apparatus, and lysogeny never observed experimentally for *Yuavirus* phages.

Aknowlegements

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