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Short communication

The first whole genome sequence of a Fowl adenovirus B strain enables interspecies comparisons within the genus *Aviadenovirus*

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ABSTRACT

Fowl adenoviruses (FAdVs) are grouped into five different species: *Fowl adenovirus A* through *Fowl adenovirus E* (FAdV-A to FAdV-E), and so far, complete nucleotide sequences are only available for the genomes of FAdV-A, FAdV-C, FAdV-D and FAdV-E members. The aim of this study was to sequence and analyze the complete genome of the reference strain representing FAdV-B (FAdV-5, strain 340). By applying Next Generation Sequencing, the genome was found to be 45,781 bp long with 56.5% G + C content, and being very similar to the other FAdV strains. Genome organization and phylogenetic analyses confirmed the present division of the genus *Aviadenovirus* into species and the closer genetic relationship between FAdV-D and FAdV-E. In the left end region of the genome, homologues to the first predicted genes (ORF0–ORF12) described for FAdV-C (strain KR5) were present in the genome of strain 340, but ORFs 14B and 14C were absent. The central part of the strain 340 genome (IVa2–pVIII) showed an organization identical to that of other adenoviruses (but lacking the gene of protein V, which occurs only in mastadenoviruses). Just one fiber gene was identified. The right end region of the genome showed more heterogeneity. The predicted gene content within this region varies among aviadenoviruses, while the gene order and orientation of shared ORFs are conserved between different aviadenoviruses. With the completion of full genomes from all fowl adenovirus species, additional insights into the evolution of genus *Aviadenovirus* were obtained.

1. Introduction

Adenoviruses (AdVs) were detected or even cause infectious diseases in a broad vertebrate host spectrum (Benkő, 2008). The family *Adenoviridae* is divided into five genera: *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus* and *Ichtadenovirus* (Harrach et al., 2011). AdVs share a similar genome organization in the middle of their genome. However, there are extensive and characteristic differences between representatives of the five genera at the genome ends, which have strikingly different lengths and gene layout (Davison et al., 2003). The phylogeny and evolution of the genus *Mastadenovirus*, whose members infect different mammalian hosts, have been extensively studied. However, our understanding of the phylogeny, evolution and coevolution of the aviadenoviruses, which infect different avian hosts, is currently based on a few

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whole genome sequences without covering all FAdV species.

Fowl adenoviruses (FAdVs) belong to the genus *Aviadenovirus*. FAdVs were grouped into five "groups" (now species *Fowl adenovirus A* through *Fowl adenovirus E* (FAdV-A to FAdV-E)) first on the base of restriction enzyme digest pattern (Zsák and Kisary, 1984). They were separated into 12 serotypes (FAdV-1 to 8a and -8b to 11) based on cross-neutralization test (Hess, 2000). Recently, at least 12 genotypes within the five species were revealed by sequence analysis of the hexon loop 1 (L1) gene region (Marek et al., 2010a). Several additional aviadenoviruses, isolated from or detected in hosts other than chicken, have been described, *e.g.*, from turkey, goose, duck, pigeon, from different falcon and psittacine species (Hess et al., 1998; Schrenzel et al., 2005; Smyth and McNulty, 2008).

Until now, the complete nucleotide sequences were available for the FAdV genomes of FAdV-1 (CELO virus, species FAdV-A; Chiocca et al., 1996), FAdV-4 (strains ON1 and KR5, FAdV-C; Griffin and Nagy, 2011; Marek et al., 2012), FAdV-9 (strain A-2A, FAdV-D; Ojkic and Nagy, 2000) and FAdV-8 (isolate HG, FAdV-E; Grgic et al., 2011), and partial nucleotide sequences at the left and right ends of the genomes were available for representatives of several serotypes (Corredor et al., 2006, 2008). Additionally, the whole genome sequence of two non-chicken aviadenoviruses, turkey adenovirus 1 (TAdV-1) and goose adenovirus 4 (GoAdV-4) were determined (Kaján et al., 2010, 2012). So far, approximately 10% of the genome sequence was available for FAdV-B strain 340 (Marek et al., 2010a, 2012; Günes et al., 2012).

Aviadenoviruses are reported worldwide and cause infectious diseases in several avian species (Harrach and Kaján, 2011). FAdVs are mainly responsible for naturally acquired outbreaks of inclusion body hepatitis (IBH), hepatitis-hydropericardium syndrome (HHS) and gizzard erosions (GE) in chickens (Adair and Fitzgerald, 2008; Smyth and McNulty, 2008). Most commonly, FAdVs isolated from IBH cases belong to FAdV-D and FAdV-E (Ojkic et al., 2008). FAdV strains related to HHS belong to FAdV-4 (FAdV-C) and they are highly pathogenic to chickens (Hess et al., 1999). Gizzard erosions associated with FAdV infection have been reported and FAdV-1 (FAdV-A) has been isolated from most cases (Ono et al., 2003; Marek et al., 2010b).

FAdV-B strains have been isolated from bantam chickens, which had died suddenly and from healthy mallard duck (McFerran et al., 1976). A FAdV-B strain has also been isolated from the Achilles tendon of a chicken (Kaján and Kecskeméti, 2011). Recently, FAdV-B strains have been found in chickens suffering from lameness, swelling of the tarsal joint or inclusion body hepatitis (Marek et al., 2010a). Although FAdV-B strains can be isolated from diseased chickens, their pathogenicity for chickens would have to be confirmed by experimental studies.

The main purpose of this study was to apply high-throughput sequencing technology to provide the complete genome sequence of strain 340, which is the reference strain of FAdV-5 belonging to FAdV-B. Completing full genome sequences of all FAdV species should provide an update of the genetic content, phylogeny and evolution of the genus *Aviadenovirus*, whose members infect different avian hosts.

2. Materials and methods

2.1. Virus isolate

Plaque purified reference strain 340 (FAdV-5, FAdV-B) was kindly provided by Brian McFerran and propagated on confluent monolayers of chicken embryo liver (CEL) cells as described earlier (Marek et al., 2010a).

2.2. DNA extraction

Cell culture supernatants were clarified by low speed centrifugation (10 min, 6000 rpm) then ultracentrifuged (3 h, 24,000 rpm) and the pelleted cell-free virions were used for DNA isolation (Marek et al., 2012). Presence of FAdV DNA in sample and its classification within FAdV-B were verified by PCR targeting the loop1 (L1) region of the hexon gene (data not shown).

2.3. Illumina sequencing

Sequencing of the whole genome was done using GAIIx Illumina system (Central Service Facility NGS Unit, Vienna, Austria). Paired-end libraries with 200 bp paired-end distance were generated and the barcodes were used for sequencing of multiple virus samples in one lane (Illumina, San Diego, CA) but the topic of the present paper is only strain 340. Reads corresponding to different strains were separated based on perfect match to the barcode sequence. Since we suspected contamination by *Gallus gallus* reads due to propagation of the virus isolates in chicken embryo liver cells, we initially mapped all reads against the available genome of *G. gallus* v. 3.0 and the *G. sonneratii* mtDNA (AP006746.1) and used only unmapped reads for assembly of the virus genomes (Marek et al., 2012).

2.4. De novo assembly of strain 340

CLC Genomics Workbench v. 3.0 (CLC bio, Aarhus, Denmark) was used for assembly of the genome sequence. Since excess coverage can result in a lower quality of the *de novo* assembly, we sub-sampled a number of reads equivalent to an expected coverage of approximately 50 to 200 reads/site that resulted in longer and fewer contigs (Text S1). Based on the genome sequence available for different aviadenovirus strains and the left and right end of some FAdV types, the resulting contigs were manually ordered and orientated (Marek et al., 2012).

2.5. Partial genome sequencing

Primers were designed based on the sequence of adjacent contig ends and PCR performed in order to close the gap by classic Sanger sequencing. Oligonucleotide primers for the amplification of the DNA at the right end of the genome were designed on the basis of the obtained sequence data and genome ends of FAdV-C, FAdV-D and