Complete Genome Sequence of the Novel *Escherichia coli* Phage phAPEC8

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Bacteriophage phAPEC8 is an *Escherichia coli*-infecting myovirus, isolated on an avian pathogenic *Escherichia coli* (APEC) strain. APEC strains cause colibacillosis in poultry, resulting in high mortality levels and important economic losses. Genomic analysis of the 147,737-bp double-stranded DNA phAPEC8 genome revealed that 53% of the 269 encoded proteins are unique to this phage. Its closest relatives include the *Salmonella* phage PVP-SE1 and the coliphage rv5, with 19% and 18% similar proteins, respectively. As such, phAPEC8 represents a novel, phylogenetically distinct clade within the *Myoviridae*, with molecular properties suitable for phage therapy applications.

Phage vB_EcoM_phAPEC8 was isolated in Belgium in 2010, from a water sample in the vicinity of a poultry farm, on the avian pathogenic *Escherichia coli* (APEC) strain CH2 (16). The virulent nature of the phage was demonstrated *in vitro* by the absence of PFU in an overnight culture of phAPEC8-insensitive CH2 mutants. PhAPEC8 lyses 11 out of 31 tested APEC isolates and various CH2 mutants that are resistant against 21 other APEC phages (J. Tsonos, unpublished data). The phage was cultured by the standard soft agar layer technique and purified on a CsCl gradient (13). Transmission electron microscopy revealed phAPEC8 as a myovirus, similar to PVP-SE1, featuring an isometric head (85 nm) and a contractile tail (110 nm).

The double-stranded DNA (dsDNA) phAPEC8 genome was sequenced using paired-end reads (Illumina Sequencing Technology). The *de novo* assembled genome has a G+C content of 39.1%, which is lower than that of its bacterial host (3, 4). In total, 269 open reading frames (ORFs), larger than 100 bp, were identified by GeneMarkS (2) and NCBI ORF-finder (<http://www.ncbi.nlm.nih.gov/projects/gorf/>), followed by a manual correction where needed. The gene products were screened for similar proteins using Batch BLASTP at the Greengene facility (http://greengene.umd.edu/programs/Local_Blast.html). Of the 269 presumed proteins, 143 are unique and did not show any similarity with the NCBI nonredundant database. The highest degree of similarity was observed with *Salmonella* phage PVP-SE1 (51 proteins) and with *Escherichia* phage rv5 (49 proteins), with 42 gene products shared by all three phages.

Eleven clustered tRNA genes were identified (9, 15). One of the regulatory motifs found, TTGACA(N16)TAANAT, is similar to the σ^{70} consensus *E. coli* promoter sequence and can therefore be considered a potential host-dependent promoter (1, 7). The motif RTAGGGCGGSAATRGTKCCS was always (10 times) present in intergenic regions, systematically upstream of proteins unique to phAPEC8 (10). Eleven rho-independent transcription terminators were identified with ARNold (11).

Of the 37 structural proteins in PVP-SE1, 24 are similar to proteins in phAPEC8, explaining the structural similarity between these phages (14). This morphogenesis cluster of phAPEC8 also encodes a predicted protein with 74% amino acid identity to the endo-*N*-acetylneuraminidase of the K1-dependent phages K1-dep(4) and K1-dep(1) (accession numbers GU196277.1 and

GU196278.1, respectively). It was already suggested that the sialidase activity of K1-dependent phages provides an increased fitness on capsule-bearing *E. coli* cells in serum and thus these phages might be more efficient in phage therapy (5). The polysaccharase activity of a predicted protein similar to the colanic acid-degrading protein of phage NST1 (accession number HM214492.1) could allow the phage to penetrate into biofilms (8). Bacterial capsular polysaccharides and colanic acid are both considered virulence factors of APEC (6, 12).

In conclusion, phAPEC8 can be considered phylogenetically unique within the *Myoviridae*. The combination of those characteristics, together with the broad host range of phAPEC8, makes this unique *Myoviridae* an interesting candidate for phage therapeutical applications.

Nucleotide sequence accession number. The genome sequence of phAPEC8 is available under GenBank accession number JX561091.

ACKNOWLEDGMENT

This research was supported by grant RT 09/8 APECON from the Federal Public Services of Health, Food Chain Safety and Environment (Belgium).

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Received 31 August 2012 Accepted 1 September 2012

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doi:10.1128/JVI.02374-12

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