

Complete Genome Sequence of the Novel Lytic Avian Pathogenic Coliphage NJ01

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Bacteriophages of the C3 morphotype, characterized by very long heads that exceed their width several times, are extremely rare among the *Podoviridae* family members and constitute only 0.5% of over 5,500 phages that have been examined by the electron microscope (H. W. Ackermann, *Arch. Virol.* 152:227–243, 2007; H. W. Ackermann, *Arch. Virol.* 146:843–857, 2001). To date, among those phages proven to be C3, only coliphage phiEco32, *Lactococcus* phage KSY1, *Vibrio* phage 71A-6, and *Salmonella enterica* phage 7-11, but no avian pathogenic *Escherichia coli* (APEC) bacteriophages, have been completely sequenced (A. Chopin, H. Deveau, S. D. Ehrlich, S. Moineau, and M. C. Chopin, *Virology* 365:1–9, 2007; S. A. Khan, et al., *Mol. Cell Probes* 15:61–69, 2001; A. M. Kropinski, E. J. Lingohr, H. W. Ackermann, *Arch. Virol.* 156:149–151, 2011; D. Savalia, et al., *J. Mol. Biol.* 377:774–789, 2008) and are available in public databases. We isolated a bacteriophage from a scale duck market in Nanjing, Jiangsu province, named NJ01, that infects APEC. Sequence and morphological analyses revealed that phage NJ01 is a C3-like bacteriophage and belongs to the *Podoviridae* family. Here, we announce the complete genome sequence of phage NJ01 and submit the results of our analysis.

Avian pathogenic *Escherichia coli* (APEC), an important etiologic infection among the most significant infectious diseases, causes colibacillosis, airsacculitis, and associated pericarditis, perihepatitis, and peritonitis, frequently, resulting in severe economic losses in the poultry industry worldwide (5). Bacteriophage therapy is considered to be an alternative method for the treatment of bacterial infection (3). To date, there is no report of a C3-like APEC phage genome sequence. We report here the full genome sequence and organization of NJ01, a special-shaped virulent bacteriophage.

Our genomic phage DNA was basically isolated by organic extraction, and the principle was similar to the previous description (8, 10). It was sequenced by using the GS-FLX DNA library preparation kit (Roche Applied Sciences, United States), amplified by emPCR, and sequenced on a GS-FLX (454 Life Sciences, United States). The 454 reads were assembled with Newbler (version 2.0) (Roche, United States) using default assembly parameters. Open reading frames (ORFs) were predicted with Glimmer3.0 (<http://www.ncbi.nlm.nih.gov/projects/gorf/>) software. BLASTX and BLASTP (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) were used to search for homologous proteins. tRNA was identified with the TransTerm program and the tRNA-scan-SE program (<http://lowelab.ucsc.edu/tRNAscan-SE/>).

Transmission electron microscopy (TEM) showed that the NJ01 has a short, noncontractile tail, an elongated head (150 by 48 nm), and an 18- by 10-nm tail. It is suggested that it belongs to the *Podoviridae* family by morphological features. After ultra-high-throughput GS FLX sequencing was employed, a 77,448-bp double-stranded DNA genome was identified with no gap after the fragment was assembled. After bioinformatics assay, the genome contained 136 open reading frames (ORF) and 1 tRNA, with a G+C content of 42.05%. Structural proteins included the major head protein, tail protein, tail fiber protein, tail tip fiber protein, portal protein, scaffolding protein, internal virion protein, and lipoprotein, as well as the functional proteins, like holin, lysis, and DNA injection protein, while nonstructural proteins included a putative DNA replication enzyme, DNA and RNA polymerase,

DNA topoisomerase I, DNA ligase, and putative DNA and GTP binding protein.

The genome sequence showed that the DNA shared the greatest base pair identity, up to 80%, with the phage phiEco32, which was a coliphage isolated from cattle in the United States and belonged to the C3-like group (9). This is the first report of the complete genome sequence of the C3-like phage infecting avian pathogenic APEC. Studies investigating the complete genome of phage NJ01 would provide new antibacterial therapy to isolate bacteriophages against avian pathogenic colibacillosis.

Nucleotide sequence accession number. The complete genome sequence of phage NJ01 is available in GenBank under accession number [JX867715](https://www.ncbi.nlm.nih.gov/nuccore/JX867715).

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REFERENCES

1. Ackermann HW. 2007. 5500 Phages examined in the electron microscope. *Arch. Virol.* 152:227–243.
2. Ackermann HW. 2001. Frequency of morphological phage descriptions in the year 2000. Brief review. *Arch. Virol.* 146:843–857.
3. Brussow H. 2005. Phage therapy: the *Escherichia coli* experience. *Microbiology* 151:2133–2140.
4. Chopin A, Deveau H, Ehrlich SD, Moineau S, Chopin MC. 2007. KSY1, a lactococcal phage with a T7-like transcription. *Virology* 365:1–9.
5. Dho-Moulin M, Fairbrother JM. 1999. Avian pathogenic *Escherichia coli* (APEC). *Vet. Res.* 30:299–316.
6. Khan SA, et al. 2001. DNA packaging and developmental intermediates

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- of a broad host range *Vibrio vulnificus* bacteriophage 71A-6. *Mol. Cell Probes* 15:61–69.
7. Kropinski AM, Lingohr EJ, Ackermann HW. 2011. The genome sequence of enterobacterial phage 7-11, which possesses an unusually elongated head. *Arch. Virol.* 156:149–151.
 8. Lehman SM, Kropinski AM, Castle AJ, Svircev AM. 2009. Complete genome of the broad-host-range *Erwinia amylovora* phage phiEa21-4 and its relationship to *Salmonella* phage felix O1. *Appl. Environ. Microbiol.* 75:2139–2147.
 9. Savalia D, et al. 2008. Genomic and proteomic analysis of phiEco32, a novel *Escherichia coli* bacteriophage. *J. Mol. Biol.* 377:774–789.
 10. Sillankorva S, Neubauer P, Azeredo J. 2008. Isolation and characterization of a T7-like lytic phage for *Pseudomonas fluorescens*. *BMC Biotechnol.* 8:80.