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Research Article

Bioinformatics Analysis of the Duck Enteritis Virus UL54 Gene

[1, 3]Chaoyue Liu, [1, 2, 3]Anchun Cheng and [1, 2, 3]Mingshu Wang
[1]Institute of Preventive Veterinary Medicine,
[2]Key Laboratory of Animal Disease and Human Health of Sichuan Province, Sichuan Agricultural University, Wenjiang, Chengdu City, Sichuan, 611130, P.R. China
[3]Avian Disease Research Center, College of Veterinary Medicine of Sichuan Agricultural University, 46 Xinkang Road, Ya'an, Sichuan 625014, P.R. China

Abstract: In this study, we analyze the Duck Enteritis Virus (DEV) UL54 gene, which has been isolated and identified in our lab (GenBank accession NO EU071033), to help deeply research on DEV. DNA sequence analysis showed that the identified ORF which composed of 1377 bp nucleotides encoded 458 amino acids with a predicted Mr. of 51.75 kDa. Multiple sequence alignment suggested that the UL54 gene was highly conserved in Alphaherpesvirinae and was similar to the other herpesviral UL54 gene. Phylogenetic analysis of the DEV UL54 gene revealed that DEV had a close evolutionary relationship with Gallid, Herpesvirus 2 (GaHV-2), Gallid Herpesvirus 3 (GaHV-3), Meleagrid Herpesvirus1 (MeHV-1) and should belong to a single cluster within the Alphaherpesvirinae subfamily.

Keywords: DEV, molecular characterization, sequence analysis, UL54

INTRODUCTION

Duck Viral Enteritis (DVE), also called duck plague, is an acute, contagious and lethal disease of ducks, geese and other water flows. Since it was reported in Holland ducks in 1923 for the first time, more outbreaks were reported all over the world and produced significant economic losses in both domestic and wild waterfowl due to mortality, elimination and decreased egg production. DVE caused some typical pathologic features, vascular damage, tissue hemorrhage, digestive mucosal eruptions, lesions of lymphoid organs and degenerative changes in parenchymatous organs (Sandhu and Leibovitz, 2008). The pathogens of DEV is Duck Enteritis Virus (DEV), which has been classified as belonging to Alphaherpesvirinae subfamily of Herpesviridae based on the report of the 9th International Committee on Taxonomy of Viruses (ICTV), but it has not been grouped into any genus (King *et al*., 2011).

DEV is composed of a linear, double-stranded DNA genome with 44.89% G+C content (Ying *et al*., 2012). To date, the DEV genomic library was successfully constructed and the vast majority sequences of DEV were published, including UL5, UL6, UL7, UL23 (TK), UL24, UL25, UL26 (vp24), UL26.5 (vp22a), UL27 (gB), UL28, UL29, UL30, UL31, UL32, UL33, UL34, UL35, UL44 (gC), UL45, UL46, UL47, UL51, UL55, gE, gK, dUTPase (Chanjuan *et al*., 2009; Ying *et al*., 2011; Liting *et al*., 2010; Lichan *et al*., 2008a; Hua *et al*., 2010, 2009; Shunchuan *et al*., 2010; Bei *et al*., 2010; Mingsheng *et al*., 2009; Renyong *et al*., 2009; Lichan *et al*., 2008b; Wei *et al*., 2010). The mainly structure and Multifunction in the regulation of gene expression and DNA replication of some herpes virus UL54 gene has been identified (Meili *et al*., 2011; Corbin-Lickfett *et al*., 2009, 2010; Hernandez and Sandri-Goldin, 2010). However, researches on DEV UL54 protein still lacked owing to high homology between the amino acid sequences encoded by UL54 gene of PRV and DEV, we predicted DEV UL54 product has the similar functions. So, in the study, we identified the isolated DEV UL54 gene (GenBank accession No EU071033) in our laboratory by bioinformatics analysis software (Table 1). The characterization of the DEV UL54 gene aids in understanding of gene expression and DNA replication profoundly.

MATERIALS AND METHODS

The DEV UL54 gene GenBank accession No EU071033) was identified in our lab (Anchun *et al*., 2006). We analyze the Duck Enteritis Virus UL54 Gene and its encoding protein by software or websites listed in Table 1.

Corresponding Author: Chaoyue Liu, Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Wenjiang, Chengdu City, Sichuan, 611130, P.R. China

Table 1: Bioinformatics analysis software

Program	Software or website
Sequences of amino acids	http://web.expasy.org/cgi-bin/protparam/protparam
ORF	NCBI ORF finder
Signal peptide	SignalP V4.0
Trans-membrane region	http://genome.cbs.dtu.dk/services/TMHMM/
Hydrophobicity	http://www.expasy.org/cgi-bin/protscale.pl
B-epitope	BepiPred 1.0 server, IEDB
T-epitope	BJTEpitope
Motif	PredictProtein
Secondary structure	PredictProtein
Comparison and analysis	BLAST, ClustalX
Phylogenetic tree	ClustalX, Treeview
NES prodiction	NetNES (http://www.cbs.dtu.dk)
NLS prodiction	ProdictNLS
Location	PSORT

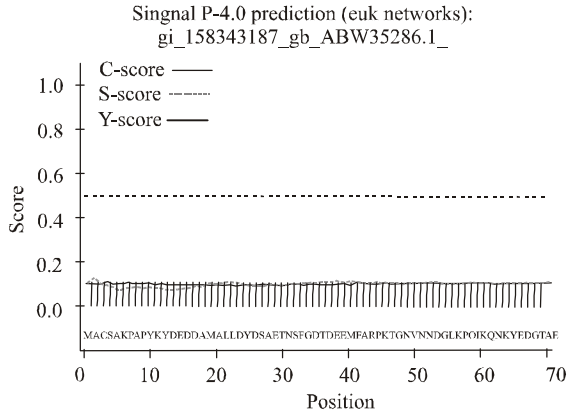

Fig. 1: The signal peptide analysis of DPV UL54 by SignalP V4.0. The protein contained no signal peptide

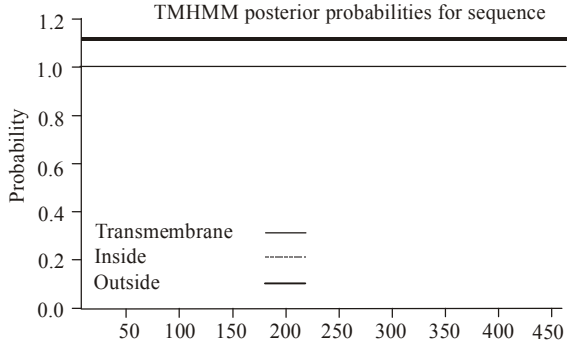

Fig. 2: The transmembrane region of DPV UL54 was analyzed by http://genome.cbs.dtu.dk/services/TMHMM/. And there is no transmembrane region in the protein

RESULTS

Analysis of DEV UL54 gene nucleotides sequences: The UL54 gene owns a 1377 bp long nucleotides sequence and contained a single ORF, which composed of 415 adenine (30.1%), 307 cytosine (22.3%), 329 guanine (23.9%), 326 thymine (23.7%) and a GC content 46.19%. The NC value of the nucleotides sequence of DEV UL54 gene was 57.04, less than the average number, indicated that the condones usage in the gene exist some differences.

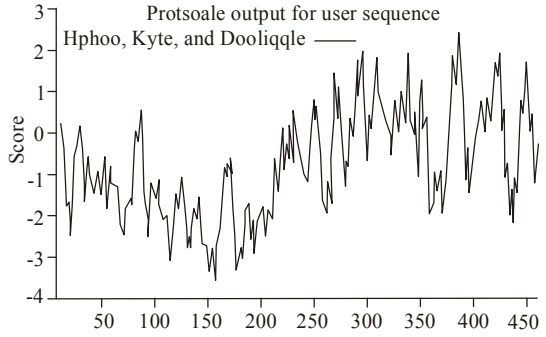

Fig. 3: The hydrophobicity analysis of DPV UL54 by the online program of EXPASY. The defining hydrophobicity regions located 373-384 amino acids

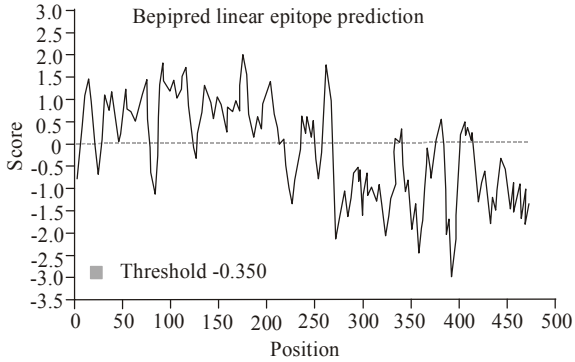

Fig. 4: The epitopes analysis of DPV UL54 by IEDB. The B-epitopes were located mainly at amino acids regions 3-17, 25-73, 82-117, 122-210, 225-240, 249-259, 323-329, 363-371, 389-400

Analysis of UL54 amino acids sequences: This DEV UL54 gene was predicted to encode a polypeptide which consisted of 458 amino acids. The polypeptide with a putative molecular mass of 51.75 kDa and a theoretical Isoelectric Point (PI) of 7.87, contains 65 acidic amino acids (55.1%), 51 basic amino acids (40%), 125 hydrophobic amino acids (27.3%), 67 hydrophilic amino acids (14.6%).

There were no signal peptide (Fig. 1) and transmembrane region in the protein (Fig. 2). The Defining hydrophobicity regions located 373-384 aa (Fig. 3). The predicted B-epitopes were pitched mainly at amino acids regions 3-17, 25-73, 82-117, 122-210, 225-240, 249-259, 323-329, 363-371, 389-400.

Figure 4 Expect for the B-epitopes, the protein owns 9 nonnumeric epitopes located at 11, 14, 20, 42, 236, 293, 302, 321 and 335, respectively.

The protein contained 7 Protein kinase C phosphorylation sites, 15 Casein kinase IIPhosphorylation sites, 9 N-Nutmeg acylation sites, 1 Amidation site, 1RGD and 6cAMP (or/and cGMP)

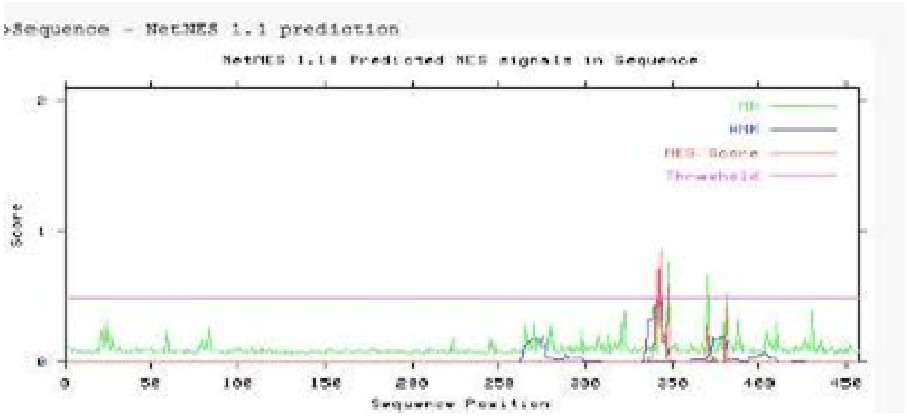

Fig. 5: The NES prediction of DPV UL54 by NetNES (http://www.cbs.dtu.dk). A leu-rich NES was located at 339-348 aa in the protein

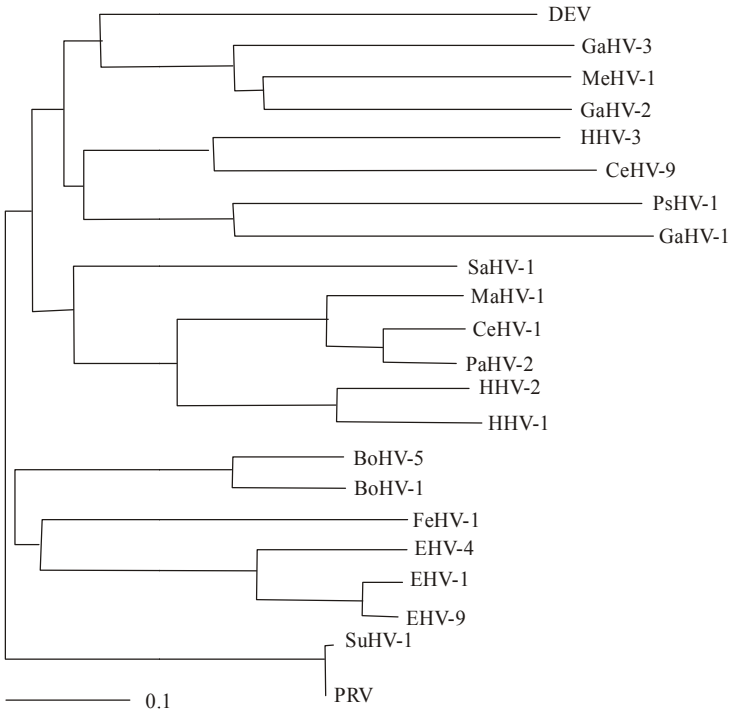

Fig. 6: Phylogenetic analysis of UL54 in 22 herpesviruses. Phylogenetic tree of the protein in the herpesvirusess was generated by Clustal X and Treeview

dependence Protein kinase phosphorylation sites. Forecasting the secondary structure revealed alpha helix 22.05%, extended strand 5.46% and loop 72.49% in the protein.

The putative sub-cellular localization showed that the protein was located mainly into nuclear (98%) and the rest was located in mitochondrial matrix space and lysosome (lumen) 1.0%, respectively.

Whether existed in endoplasmic reticulum (membrane) or not is still unknown. The analysis result shows no classic NLS and a Leu-rich NES (Fig. 5) located at 339-348 aa in the protein.

A phylogenetic tree (Fig. 6) was constructed by Clustal X and Treeview according to the amino acid sequences of the UL54 gene in DEV and other 21 herpesviruses. The result is in accordance with the subtype classification of DEV proposed previously and the DEV UL54 gene was closed to the Gallid Herpesvirus 2, Gallid Herpesvirus3, Meleagrid Herpesvirus1, although DEV was branched independently to others.

DISCUSSION

Up to now, the specific characteristics and functions of the UL54 protein in DEV are still unknown. In this study, we analyzed DEV UL54 gene by bioinformatics methods. UL 54 gene was selected

from the completed DEV gene libraries and identified by comparing and analyzing the nucleotide and amino acids sequences. The gene was analyzed by NCBI ORF Finder programs and sequence results revealed a single ORF of 1377 nucleotides encoded a protein of 458 amino acids with predicted Mr of 51.75 kDa.

Previously studies indicated that UL54 protein was the unique alpha-protein which can find its homology in all Herpesviridaes (Judith *et al*., 1995). Consequently, the UL54 protein was a conserved protein, but the genus has not been determined yet (Andrew and Davison, 2010). To help know clearly the classification of DEV, phylogenetic tree was constructed on the putative proteins of UL54 (Fig. 6). The result showed that the DEV CHv strain was more similar to alpha-herpervirus than beta-herpervirus or gamma-herpervirus. The DEV CHv formed an independent branch and was closed to GaHV-2, GaHV-3 and MeHV-1.

The HSV-1 UL54 protein, known as the ICP27, was identified as a multi-function protein. Due to the high similarity among the three alphaherpesviruses, HSV-1, PRV and DEV, we anticipated that DEV UL54 product has the same role as HSV-1 or PRV. The function of a protein was fundamentally rested with its spatial structure, so it was important to explore the structural characteristics of UL54 protein.

The analysis of signal peptide and transmembrane region suggested that the UL54 protein was not a membrane protein, which provided some information for its expression. The contact may play a key role in regulating the DEV gene expression. From the secondary structure and hydrophobicity analysis, we can see that the protein contained alpha helix 22.05%, extended strand 5.46%, loop 72.49% and the domain located 373-384 aa was the highest hydrophobicity regions.

Many function sites were found in the UL54 protein of DEV-CHv, including phosphorylation sites, Nutmeg acylation sites, Amidation site and RGD. These potential sites may provide more chances to interact with other proteins and regulate the functions. The antigenic determinants analysis results can improve knowledge of the antigenic and structural properties of DEV UL54 protein, as well as revealed the immunologic mechanism and yield methods for developing new vaccine. The protein was located mainly into nuclear without a classic NLS indicated that a new type of NLS should be identified. The NES also help the protein shuttle between the nuclear and cytoplasm (Yaju *et al*., 2005).

In conclusion, we complete the characterization analysis of DEV UL54 gene in the present study. The results of bioinformatics analysis, coupled with the previously published data strongly suggested that the DEV UL54 protein also served as a multi-function protein, but more experiments should be needed to elucidate its specific function and mechanism in the future study.

REFERENCES

Anchun, C., W. Mingshu and W. Ming, 2006. Construction of duck enteritis virus gene libraries and discovery: Cloning and identification of viral nucleocapsid protein gene. High Technol. Lett., 16: 948-953.

Andrew, J. and Davison, 2010. Herpesvirus systematic. Vet. Microbiol., 143: 52-69.

Bei, L., X. Chao and C. Anchun, 2010. Identification and characterization of duck plague virus glycoprotein C gene and gene product. Virol. J., 7: 349.

Chanjuan, S., C. Anchun and W. Mingshu, 2009. Identification and characterization of the duck, enteritis virus UL51 gene. Arch Virol., 154: 1061-1069.

Corbin-Lickfett, K.A., S.K. Souki and M.J. Cocco, 2010. Three Arginine residues within the RGG Box is crucial for ICP27 binding to Herpes Simplex Virus 1 GC-rich sequences and for efficient viral RNA export. Virol. J., 84: 6367-6376.

Corbin-Lickfett, K.A., I.H. Chen, M.J. Cocco and R.M. Sandri-Goldin, 2009. The HSV-1 ICP27 RGG box specifically binds flexible, GC-rich sequences but not G-quartet structures. Nucl. Acids Res., 37(21): 7290-7301.

Hernandez, F.P. and R.M. Sandri-Goldin, 2010. Herpes Simplex Virus 1 regulatory protein ICP27 undergoes a head-to-tail intramolecular interaction. Virol. J., 84: 4124-4135.

Hua, C., C. Anchun and W. Mingshu, 2009. Complete nucleotide sequence of the duck plague, virus gE gene. Arch Virol., 154: 163-165.

Hua, C., C. Anchun and W. Mingshu, 2010. Cloning, expression and characterization of gE protein of Duck plague virus. Virol. J., 7: 120.

Judith, B., G.K. Barbara and C.M. Thomas, 1995. Pseudorabies virus and equine herpesvirus 1 share a nonessential gene which is absent in other herpesviruses and located adjacent to a highly conserved gene cluster. Virol. J., 69: 5560-5567.

King, A., E. Lefkowitz and M.J. Adams, 2011. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego.

Lichan, Z., C. Anchun and W. Mingshu, 2008a. Characterization of codon usage bias in the dUTPase gene of duck enteritis virus. Prog. Nat. Sci., 18: 1069-1076.

Lichan, Z., C. Anchun and W. Mingshu, 2008b. Identification and characterizeation of duck enteritis virus dUTPase gene. Avian Dis., 52: 324-331.

Liting, L., C. Anchun and W. Mingshu, 2010. RPoly clonal antibody against the DPV UL46M: Protein can be a diagnostic candidate. Virol. J., 7: 83.

Meili, L., W. Shuai and C. Mingsheng, 2011. Identification of nuclear and nucleolar localization signals of Pseudo Rabies Virus (PRV) early protein UL54 reveals that its nuclear targeting is required for efficient production of PRV. Virol. J., 85: 10239-10251.

Mingsheng, C., C. Anchun and W. Mingshu, 2009. Characterization of synonymous codon usage bias in the duck plague virus UL35Gene. Intervirology, 52: 266-278.

Renyong, J., C. Anchun and W. Mingshu, 2009. Analysis of synonymous codon usage in the UL24 gene of duck enteritis virus. Virus Gen., 38: 96-103.

Sandhu, T.S. and L. Leibovitz, 2008. Duck virus enteritis (Duck Plauge). Dis. Poultry, pp: 384-393.

Shunchuan, Z., M. Guangpeng and X. Jun, 2010. Expressing gK gene of duck enteritis virus guided by bioinformatics and its applied prospect in diagnosis. Virol. J., 7: 168.

Wei, X., C. Anchun and W. Mingshu, 2010. Molecular cloning and characterization of the UL31 gene rom Duck enteritis virus. Mol. Biol. Rep., 37: 1495-1503.

Yaju, H., C. Mawsheng and W. Chingying, 2005. Mapping of functional regions conferring: Nuclear localization and RNA-binding activity of pseudorabies virus early protein UL54. J. Virol. Methods, 130: 102-107.

Ying, W., C. Anchun and W. Mingshu, 2011. Characterization of the duck enteritis virus UL55 protein. Virol. J., 8: 256.

Ying, W., C. Anchun and W. Mingshu, 2012. Complete genomic sequence of Chinese virulent: Duck enteritis virus CHv strain. Virol. J., 10: 5965.