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The *In-Silico* Study on Structural, Functional and Sub-Cellular Localization of Hypothetical Proteins in the Orf Virus

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Abstract

The function, sub-cellular localization and structure of hypothetical proteins in the Orf Virus were explained by this study. The Orf Virus (OFRV) having a total of 130 NCBI genes out of which 42 are predicted for hypothetical proteins and these hypotheticals proteins were unknown for function, sub-cellular localization and structure and which was screened for function, sub-cellular localization and structure. For the determination of functional annotations the bioinformatics online tools as CDD-BLAST, INTERPROSCAN and PFAM were used. Cello v2.5 was used for the prediction of the sub-cellular localization and PS2 Server-Protein Structure Prediction server was used for the prediction of templates for conserved domains. To determine the 3-D structures, E-value and aligned percentage of the hypothetical proteins the PS2 Server-Protein Structure Prediction server also used. The study on this Orf virus may be useful for understanding the functional and structural characteristics of hypothetical protein as well as understanding the genetics at the molecular level to control Orf virus infection through vaccination.

Keywords: *ORFV; Contagious Ecthyma; E-Value; Parapoxvirus; Pfam*

Introduction

The Orf virus (ORFV) is classified into the genus *Parapoxvirus,* subfamily *Chordopoxvirinae* and family *Poxviridae with* double-stranded DNA [2]. The Orf is a zoonotic disease and reportable disease transmitted from animals to humans [12]. It is the causative agent of a severe exanthematic dermatitis and contagious ecthyma (CE) that involves domestic as well as wild small ruminants. In the members of the Cervidae family, camels and camelids and several other ruminants Orf has been reported. Orf virus can also be infected to squirrels, dogs and cats. After the evaluation of IgM antibodies in the extent of ruminant populations supported on late seroprevalence study, goats having a higher incidence of infections than sheep [9]. The Orf virus (ORFV) can be infected to Sheep at various times, although with briefer times to recovery with less easily noticeable pathological changes as compared to a primary infection [8]. The endemic disease distributed worldwide in many countries wheresoever sheep and goats embossed. The zoonotic potential of this disease affects the people who are in contact with infected animals directly like veterinarians, farmers, and butchers. Also infected are in indirect contact with infected animals especially during drenching, shearing, slaughtering, and docking [21]. During the study, the transmission of the disease from humans to humans has not been reported yet. But the cause of reinfection has seen [15].

It is reported that the transmission of infection by indirect or direct contact with infected materials to individuals as well as from animals to humans (adults) who are involved in slaughtering of farm animals, livestock fairs and children visiting the zoos [10]. Contagious ecthyma reported as a highly contagious disease that primarily affects goats, sheep, and wild ruminants and having qualities of the formation of nodules, papules, or vesicles that develop into midst crusts or dense scabs on the lips, tongue and gingiva. Zoonotic ORFV infectious Patients observed with the development of papillomatous and nodular lesions mainly found on the mouth, face, and hands [11].

The period of incubation is mostly 3 - 7 days. The lesions tardily converted to healed skin with little or no scarring from the shallow annular ulcer, papule, scab and vesicle. Each stage is just about last for one week for all the six stages of orf virus infection listed as a target, maculopapular, regenerative, papillomatous, acute and regressive [23]. lesions can also be observed occasionally within the esophagus or the Abomasum and in the buccal cavity [16]. first appearance of disease as erythematous macules that develop into papules with an appearance for 7 to 14 days. Subsequently, the lesions converted to vesicular and nodular which undergoes to ulcerate in 2 - 3 weeks. The lesions are generally asymptomatic types, until now secondary infections can leash to pain and discomfort [7]. The Orf virus disease normally lasts for 3 - 4 weeks which resolves impromptu in 1 - 2 months. Even though the morbidity rate of this disease is high up to 100% and the uncomplicated cases exceed 1% in mortality rate [17]. Contagious ecthyma outbreaks progress more ofttimes during the menses of extreme temperature. erythema in the initial stage which later progresses into papules and these papules develop into scabs [5].

The orf virus is the member of the genus parapoxvirus and it has four members as bovine popular stomatitis virus (BPSV), pseudocowpox virus (PCPV), orf virus (ORFV) and *parapoxvirus* of red deer in New Zealand (PVNZ). Morphologically comparison of *parapoxviruses* with other members of genera poxvirus with their crisscross pattern on the particle surface, ovoid shape, and relatively small size. The Orf virus particles are about 160 nm in width and about 260 nm in length which are ovoid [13]. A total of five genes namely ORFO20, ORF127, ORF117, ORF109 and B2L identified during the study of sequencing analysis of the Orf virus from the virus outbreaks in Argentina. Furthermore [18]. The Orf virus (ORFV) genome having a linear double-stranded DNA with a length of 138 kb which encodes a total of 132 putative gene products. The genome consists of the B2L gene (ORF 011) at terminal regions which implicated in virulence and host range. The ORFV B2L gene consists of 1137 bp in which about 42 kDa of highly and major immunogenic envelope protein present. The gene B2L is highly conserved amid ORFV isolates which used for detection, phylogenetic analysis and molecular characterization of ORFV in different outbreaks [6]. As a treatment for *the Orf virus*, systemic and topical antibiotics have been recommended during opportunistic infections pursual the disease could come forth. To control the Orf virus infection effectively remains as vaccination [22].

Methodology

Sequence retrieval

For the retrieval of the Orf virus whole genome sequences, the KEGG database was used (http://www.genome.jp/kegg/).

Functional annotation and categorization

The Screening, analysis, and prediction of the 3-D structures and conserved functional domains of the hypothetical proteins from the Orf virus were done by using the bioinformatics web tools. The CDD-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) [1,14,19,20], INTERPROSCAN (http://www.abi.ac.uk/interpro) [24], Pfam (http://www.pfam.sanger.ac.uk/) [4] and Cello (http://cello.life.nctu.edu.tw/) were used as bioinformatics online tools. The information for the presence of functional characteristics and conserved domains in the sequences of hypothetical proteins is available in databases CDD-Blast, Interproscan, and Pfam were used for the present study. To determine the cellular localization of the enzyme or identified protein within the cell the database Cello server v 2.5 was used.

Protein structure prediction

For the prediction of the 3-D structure of hypothetical proteins, PS2 server-Protein Structure Prediction Server is used. (http://www.ps2.life.nctu.edu.tw/) [3,19]. The 3D structures prediction of the proteins generated after running the FASTA format of hypothetical protein sequence by using PS2 server-Protein Structure Prediction Server. The 3-D structural model of the hypothetical protein is predicted on the detection of functional annotations for the template.

Results and Discussion

For characterizing 42 hypothetical proteins from the complete genome sequences for Orf virus were carried out based on computational studies. For the predictions of functional and structural characteristics of the total of 42 hypothetical proteins available online web servers that are CDD- Blast, Interproscan, Pfam, Cello and PS2server were used. Sub-cellular localization of all the hypothetical proteins present in the Orf virus was characterized successfully. The representation of order to as Template ID, E-value and aligned percentage given in PS2 structure template column as a scoring template for 3-D structures (Table 1).

Sr. No.	NCBI Gene Id	CDD Blast	Interproscan	Pfam	Cello V2.5	PS2 Server		
						Template	E-Value	Aligned Percentage
1	2947608	Chordopoxvirus L2 protein; This family consists of several Chordopoxvirus L2 proteins.	NA	Chordopoxvirus L2 protein, ABC-type cobalt transport system, permease component	Inner Membrane 2.193	NA	NA	NA
2	2947609	Poxvirus L3/FP4 protein, DNA polymerase III subunits gamma and tau; Validated	NA	Poxvirus L3/FP4 protein	Cytoplasmic 2.512	NA	NA	NA
3	2947615	Viral late protein H2; All Members of this family show similarity to the vaccinia virus late protein H2. This protein is often referred to by its gene name of H2R. Members from this family all belong to the viral taxon Poxviridae.	Viral late protein H2	Viral late protein H2	Periplasmic 1.706	2j69A	3.5	62.62
4	2947619	NA	Poxvirus E2/O1	Poxviridae protein	Cytoplasmic 3.640	1u6gC	0.22	100
5	2947620	Poxviridae protein; This family of proteins is restricted to Poxviridae. It contains a number of differently named uncharacterized proteins.	Poxvirus E2/O1	Poxviridae protein	Cytoplasmic 2.904	2bptA	0.082	98.51
6	2947622	NA	Poxvirus I2	Poxvirus entry protein complex L1 and I2	Cytoplasmic 2.139	NA	NA	NA

7	2947625	NA	NA	Poxvirus I6-like family	Cytoplasmic 2.461	NA	NA	NA
8	2947634	NA	Pox virus E6 protein	Pox virus E6 protein	Cytoplasmic 4.008	2qnaA	0.76	99.47
9	2947642	NA	NA	Chordopoxvirus A15 protein	Cytoplasmic 1.796	NA	NA	NA
10	2947646	NA	NA	KICSTOR complex C12orf66 like	Cytoplasmic 3.795	NA	NA	NA
11	2947651	NA	Poxvirus G5	Poxvirus G5 protein	Cytoplasmic 3.793	1iv8A	4	98.89
12	2947653	Permuted papain-like amidase enzyme, YaeF/YiiX, C92 family. Amidase_YiiX is a family of permuted papain-like amidases. It has amidase specificity for the amide bond between a lipid and an amino acid (or peptide). From the structure, a tetramer, each monomer is made up of a layered alpha-beta fold with a central, 6-stranded, antiparallel beta-sheet that is protected by helices on either side. The catalytic Cys154 in UniProtKB:Q74NK7, Structure 3kw0, is located on the N-terminus of helix alphaF. The two additional helices located above Cys154 contribute to the formation of the active site, where the lysine ligand is bound.	Permuted papain-like amidase enzyme, YaeF/YiiX, C92 family	Permuted papain-like amidase enzyme, YaeF/YiiX, C92 family	Cytoplasmic 2.136	2if6A	0.2	78.92
13	2947656	Late protein H7; Family of poxvirus late H7 proteins.	Late protein H7, poxvirus	Late protein H7	Cytoplasmic 3.341	NA	NA	NA

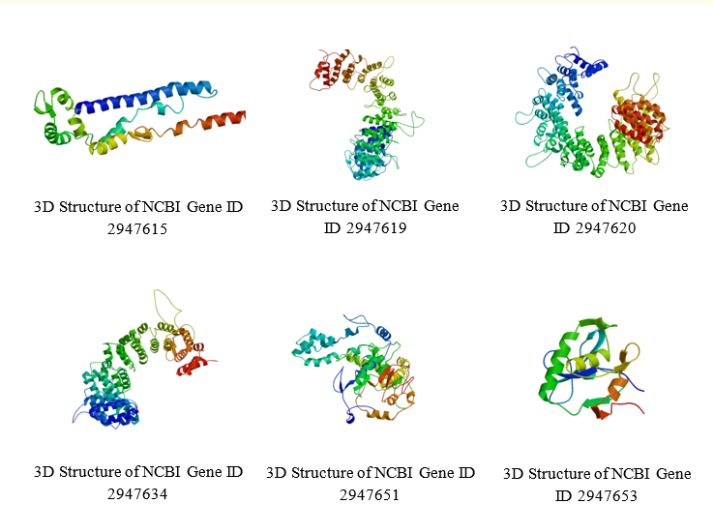
14	2947659	NA	NA	NA	Periplasmic 2.408	NA	NA	NA
15	2947660	NA	NA	NA	Periplasmic 2.408	NA	NA	NA
16	2947661	NA	NA	Rifin, Golgi-body localisation protein domain, RAM signalling pathway protein, Protein BYPASS1-related, AJAP1/PANP C-terminus	Extracellular 2.290	NA	NA	NA
17	2947662	NA	NA	NA	Extracellular 1.335	NA	NA	NA
18	2947663	NA	NA	Poxvirus A51 protein	Cytoplasmic 4.297	NA	NA	NA
19	2947666	Chordopoxvirus G3 protein; This family consists of several Chordopoxvirus specific G3 proteins. The function of this family is unknown.	Poxvirus G3	Chordopoxvirus G3 protein	Periplasmic 1.629	NA	NA	NA
20	2947668	NA	Poxvirus F16	Poxvirus F16 protein	Cytoplasmic 3.887	NA	NA	NA
21	2947671	NA	Poxvirus E2/O1	Poxviridae protein, Triabin	Cytoplasmic 4.035	NA	NA	NA
22	2947673	NA	NA	NA	Cytoplasmic 2.210	NA	NA	NA
23	2947678	NA	NA	NA	Cytoplasmic 1.709	NA	NA	NA

24	2947679	NA	NA	NA	Cyto-plasmic 3.416	NA	NA	NA
25	2947686	Poxvirus A28 family; Family of conserved Poxvirus A28 family proteins. Conserved region spans entire protein in the majority of family members.	Poxvirus A28	Poxvirus A28 family	Peri-plasmic 2.227	2pb9A	5.1	79.29
26	2947687	NA	NA	NA	Cyto-plasmic 1.666	NA	NA	NA
27	2947688	NA	NA	NA	Peri-plasmic 1.666	NA	NA	NA
28	2947689	Orthopoxvirus C10L protein; This family consists of several Orthopoxvirus C10L proteins. C10L viral protein can play an important role in vaccinia virus evasion of the host immune system. It may consist in the blockade of IL-1 receptors by the C10L protein, a homolog of the IL-1 Ra.	Orthopoxvirus C10L	Orthopoxvirus C10L protein	Extra-cellular 2.419	2pneA	0.49	86.08
29	2947696	Chordopoxvirus A35R protein; This family consists of several Chordopoxvirus sequences homologous to the Vaccinia virus A35R protein. The function of this family is unknown.	Chordopoxvi-rus A35R	Chordopoxvirus A35R protein	Inner Mem-brane 2.281	NA	NA	NA
30	2947698	NA	NA	Photosystem II reaction centre N protein (psbN)	Extra-cellular 1.639	1dmhA	2.7	96.5

31	2947699	Protein of unknown function (DUF1235); This family contains a number of viral proteins of unknown function, UV excision repair protein Rad23; All proteins in this family for which functions are known are components of a multiprotein complex used for targeting nucleotide excision repair to specific parts of the genome. In humans, Rad23 complexes with the XPC protein. This family is based on the phylogenomic analysis of JA Eisen (1999, Ph.D. Thesis, Stanford University). [DNA metabolism, DNA replication, recombination, and repair]	Vaccinia virus, A37	DUF1235 Family	Cytoplasmic 2.369	NA	NA	NA
32	2947700	NA	NA	NA	Extracellular 2.194	NA	NA	NA
33	2947701	NA	NA	NA	Extracellular 2.022	1zvoC	4E-09	99.03
34	2947702	NA	NA	Beta-lactamase2 Domain	Cytoplasmic 3.226	NA	NA	NA
35	2947703	NA	NA	NA	Cytoplasmic 2.273	NA	NA	NA
36	2947705	NA	NA	NA	Cytoplasmic 4.604	1ldjA	0.26	94.06

37	2947707	Poxvirus A11 Protein; Family of conserved Chordopoxvirinae A11 family proteins. Conserved region spans entire protein in the majority of family members.	Poxvirus A11	Pox_A11	Cyto-plasmic 4.487	NA	NA	NA
38	2947711	Protein of unknown function (DUF678); This family contains several poxvirus proteins of unknown function.	Poxvirus A19	DUF678 Family	Peri-plasmic 1.781	NA	NA	NA
39	2947712	NA	NA	Pox A21 Family	Cyto-plasmic 2.036	2qh0A	7.1	67.59
40	2947720	NA	NA	NA	Cyto-plasmic 1.666	NA	NA	NA
41	2947721	Poxvirus A6 protein	Poxvirus A6	Poxvirus A6 protein	Cyto-plasmic 2.858	NA	NA	NA
42	2947731	NA	NA	Poxvirus F11 protein	Cyto-plasmic 3.872	2ch7A	1.9	41.81

Table 1: *Predicted structures, functions and sub-cellular localizations of the hypothetical proteins in the Orf virus.*



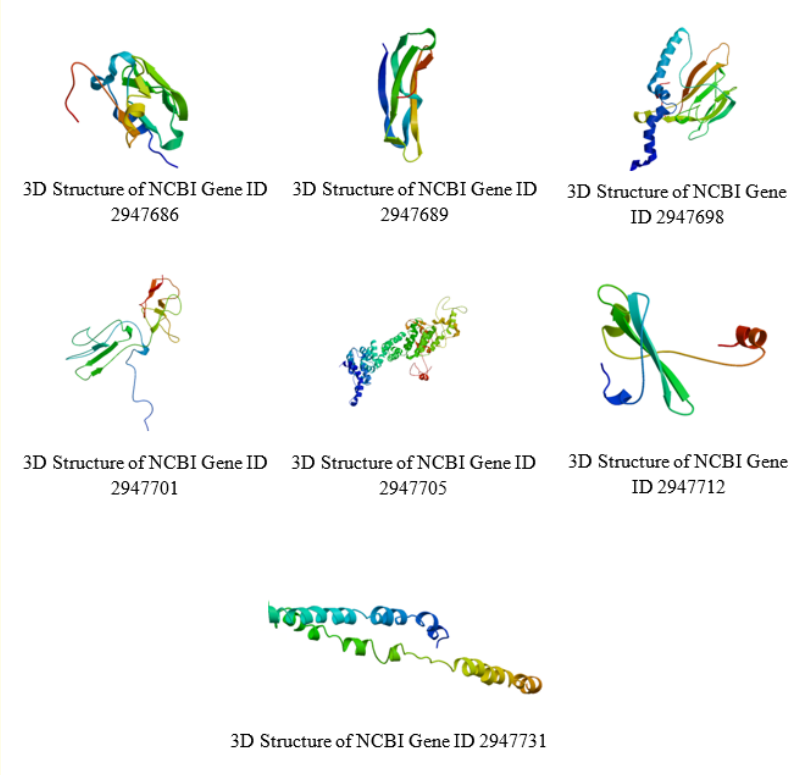


Figure 1: *Predicted 3D Structures of the hypothetical proteins from the Orf virus.*

During this study, we successfully found 14, 18 and 29 characterized probable functions of hypothetical proteins by using CDD-Blast, Interproscan, and Pfam respectively. A total of 13 three dimensional structure prediction templates out of 42 screened hypothetical proteins were also successfully characterized. The NCBI gene ID 2947615 of hypothetical protein having Viral late protein H2 which shows similarity to the vaccinia virus late protein H2. This protein is often referred to by its gene name of H2R. Members of this family all belong to the viral taxon Poxviridae. Chordopoxvirus and Poxvirus proteins include in several hypothetical proteins.

The NCBI gene ID 2947653 consists of Permuted papain-like amidase enzyme (YaeF/YiiX) from the C92 family. Amidase_YiiX is a family of permuted papain-like amidases. It has amidase specificity for the amide bond between lipid and amino acid (or peptide). From the structure, a tetramer, each monomer is made up of a layered alpha-beta fold with a central, 6-stranded, antiparallel beta-sheet that is protected by helices on either side. The catalytic Cys154 in UniProtKB: Q74NK7 with Structure 3kw0 is located on the N-terminus of helix alpha F. The two additional helices located above Cys154 contribute to the formation of the active site, where the lysine ligand is bound. The NCBI gene ID 2947689 shows the presence of Orthopoxvirus C10L protein. C10L viral protein can play an important role in vaccinia virus evasion of the host immune system. It may consist of the blockade of IL-1 receptors by the C10L protein with a homolog of the IL-1 Ra.

The gene ID 2947699 contains several viral proteins of unknown function and UV excision repair protein Rad23 and having components of a multiprotein complex used for targeting nucleotide excision repair to specific parts of the genome. In humans, Rad23 complexes with the XPC protein which are based on the phylogenomic analysis of JA Eisen (1999, Ph.D. Thesis, Stanford University).

Conclusion

In present studies, a total of 42 structurally and functionally important hypothetical proteins from Orf Virus have grouped. According to the results in the Orf virus, many probable functional proteins are available. By using CDD- Blast, Interproscan, Pfam, Cello and PS2 server a total of 130 NCBI genes were screened proteins out which 42 hypothetical proteins which are successfully characterized by functionally as well as structurally the characterized predicted three-dimensional structures and functions in the Orf virus can be assisting in establishing their infection criteria. The structural and functional characterization of hypothetical proteins in the Orf virus was from the present *in-silico* study revealed the functional and structural characterization of hypothetical proteins in the Orf virus. All the data screened from hypothetical proteins in the Orf virus may be useful for understanding the genetics at the molecular level for the infection criteria as well as the development of the vaccine.

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