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IN*SILICO* STRUCTURE PREDICTION OF ORF61 GENE RESPONSIBLE FOR CHICKEN POX

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ABSTRACT

Bioinformatics can be used to develop and improve methods for storage, retrieval, organization and analysis of biological data. A major activity in bioinformatics is to develop software tools to generate useful biological knowledge. Techniques of image and signal processing allow extraction of useful result from large amount of raw data. In this study work has been done on ORF61 gene that is responsible for causing chicken pox which is a highly contagious disease caused by primary infection of Varicella Zoster Virus (VZV). It usually starts with vascicular skin rash mainly on the body and head rather than at the periphery and becomes itchy. The early symptoms in children and adults are nausea, loss of appetite, aching and headache. VZV genes ORF4, ORF61 and ORF62 modulate the expression of the VZV genes of different kinetic classes in human T lymphocytes cell lines. The complete sequential, functional and structural information of all the VZV genes were obtained using different biological databases. The blast result gave 11% similarity. As the structure was not available in PDB, molecular modeling with 4 templates were done. 10 target models were generated out of which target model no. 7 with best dope score (-32793.12109) for structure prediction.

Keywords: Molecular Modeling, Chicken Pox, ORF61, VZV genes, PDB.

INTRODUCTION

Chickenpox is an infectious disease caused by the varicella-zoster virus [1]. Mostly found in children under the age of 15, but the adults and other children can also get it. May causes complication in adults & pregnant women. Once a person is caught with chickenpox, the virus usually stays in the body. The person may not get chickenpox again, but the virus can cause shingles in adults. The major symptom of chickenpox is an uncomfortable, itchy rash. The rashes usually shown on the face, chest, and back and then spreads to the rest of the body. It is easily passed between members of families and school classmates through airborne particles, droplets in exhaled air and fluid from the blisters or sores. It can also be transmitted indirectly by contact with articles, clothing and other items exposed to fresh drainage from open sores. Patients are contagious up to five days (more commonly one or two days) before and five days after the rash appears. When the sores are crusted over, the person is usually no longer contagious. Chicken pox may develop into a serious complication with Pediatric Liver Transplantation (PLT) [2]. After spreading the rash turns into fluid-filled blisters and eventually into scabs. Other symptoms include fever, headache, tiredness, loss of appetite. At the beginning, chicken pox is mild and it remains from 5 to 10 days. To get relief from itching Calamine lotions and oatmeal baths can be used. Acetaminophen can treat the fever. Use of aspirin during chickenpox can cause Reye syndrome.

Varicella-zoster virus (VZV), a neurotropic alphaherpes virus, is the etiologic agent of chicken pox and shingles (zoster) in human [3]. Varicella-zoster virus (VZV) infection of differentiated cells within the host and establishment of latency, likely requires evasion of innate immunity and limits secretion of antiviral cytokines [4]. Varicella-zoster virus (VZV) open reading frame 61 (ORF61) encodes a protein that transactivates viral and cellular promoters in transient-transfection assays and is the ortholog of herpes simplex virus ICP0 [5]. Varicella-zoster virus (VZV) open reading frame (ORF) 62 potentially encodes a protein with considerable amino acid homology to the herpes simplex virus (HSV) immediate-early regulatory polypeptide ICP4 (or IE3) [6].

Using an in vitro transient expression assay, it has been evaluated that they have ability of the putative immediate early VZV genes, ORF4, ORF61, and ORF62, to modulate the expression of VZV genes of different putative kinetic classes in a human T lymphocyte cell line. These cells are of the type in which VZV can be readily detected in the viremic phase of human infection. The gene product of ORF62 (IE62) is a major regulatory protein in VZV and is

capable of activating VZV genes of all putative kinetic classes. The gene product of ORF61 may play an accessory regulatory role in synergizing the activation of VZV genes induced by the gene product of ORF62 in human T lymphocytes [7].

Using the M13-dideoxynucleotide technology the entire DNA sequence of varicella-zoster virus (VZV) was determined. It was observed that the genome is variable in size, but the sequence which was obtained comprises 124884 bp. Analysis of the sequence indicated that the genome contains 70 genes distributed about equally between the two DNA strands [8]. A randomized double-blind, placebo-controlled, multicenter investigation assessed the usefulness of acyclovir in the treatment of immunosuppressed children with chickenpox [9].

In this work chicken pox has been chosen as a target disease which is caused by Varicella-zoster virus (VZV). The common VZV genes are ORF4, ORF61, and ORF62 which are responsible to modulate the expression of VZV genes of different putative kinetic classes in a human T lymphocyte cell line. First of all the complete sequential, functional and structural information of all the VZV genes was obtained using various biological databases. To find the similarity between homologous superfamilies, BLAST was done through which it has been examined that the structure of all the four proteins (ORF4, ORF61, ORF62 and IE62) was not available in PDB database as the similarity was below 95%. So, instead of working on all the responsible genes we have worked only on ORF61. Then the structure of ORF61 was generated using Modeller 9.10. The validation of modeled structure was done by using Procheck program present in SAVES server. The loop modeling process was done through modloop server to make our protein structure more stable.

METHODOLOGY

Sequence alignment and similarity search: The FASTA sequence of query protein (ORF61) was retrieved from NCBI Entrez sequence search (http://www.ncbi.nlm.nih.gov). For sequence similarity check of the protein blastp was carried out where ORF61 protein showed 11% query coverage and 44% identity (Table. 1). Protein 3D structure was then downloaded from PDB (http://www.rcsb.org/pdb/home/home.do) and we obtained our 4 templates (**2BCN, 2CKL, 3RPG and 2HOD)** for the proteins out of which the template with highest similarity was chosen for further modeling.

Structure prediction: The 3D-structure of query protein was predicted by automated homology modeling program, Modeller9.10 [10]. For Modeller, the template and query

sequences were carefully aligned to remove potential alignment errors. The default modeling process did end up with a loop. On the basis of DOPE score best model for structure was selected (Table. 2).

Loop modeling for protein 0RF61: Loop correction of the protein model is carried out by loop modelling through modloop (Fig. 1). After loop modelling stable structure of the protein ORF61 was obtained (Fig. 2). By verification of each model using Ramachandran Plot through SAVES-NIH by Procheck14 [11] the best model was selected.

Validation of Predicted Model: The model obtained was further submitted to modbase server (http://modbase.compbio.ucsf.edu/modloop/server) to rebuild the loop into its secondary structure. Validation of the model was done by Ramachandran plot analysis. Structural models were visualized by SPDBV 3.7 (Fig. 3).

RESULT AND DISCUSSION

In this study a query coverage of 11% and max identity of 44% was obtained for Protein ORF61. For homology modeling, obtained templates 2BCN, 2CKL, 3RPG and 2HOD for protein ORF61. After Modeling for the Protein ORF61 according to DOPE score the best model obtained was target.B99990007 with 75% amino acids in core region. For the validation and stability of the predicted structure loop modeling was done until all the residues were not shifted into core region or allowed region in Ramachandran Plot analysis. The protein 14 shows zero amino acids in both generous allowed and disallowed regions Loop Modeling for the protein ORF61 is carried out and result is verified through SAVES using Procheck by Ramachandran plot analysis. Finally our resultant protein ORF61 is PROTEIN 14 which may be used for drug designing.

Table. 1 Similarity Search Result for the protein ORF61

Protein	Accession no.	Query Cover	E-value	Max identity	PDB Accession no.
ORF61	AEL30876.1	11%	2e-11	44%	1CHC A

Table 2. DOPE SCORE obtained after Modeling

Filename	molpdf	DOPE score
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target.B99990001.pdb	3854.45581	-31229.99609
target.B99990002.pdb	4446.18457	-32058.93359
target.B99990003.pdb	3824.36279	-31444.33398
target.B99990004.pdb	3568.97046	-31366.44727
target.B99990005.pdb	3623.86572	-32308.99414
target.B99990006.pdb	4192.55078	-31973.37695
target.B99990007.pdb	3820.74609	-32793.12109
target.B99990008.pdb	4042.08984	-31145.32227
target.B99990009.pdb	3850.85449	-31518.92188
target.B99990010.pdb	3713.34668	-32407.91406

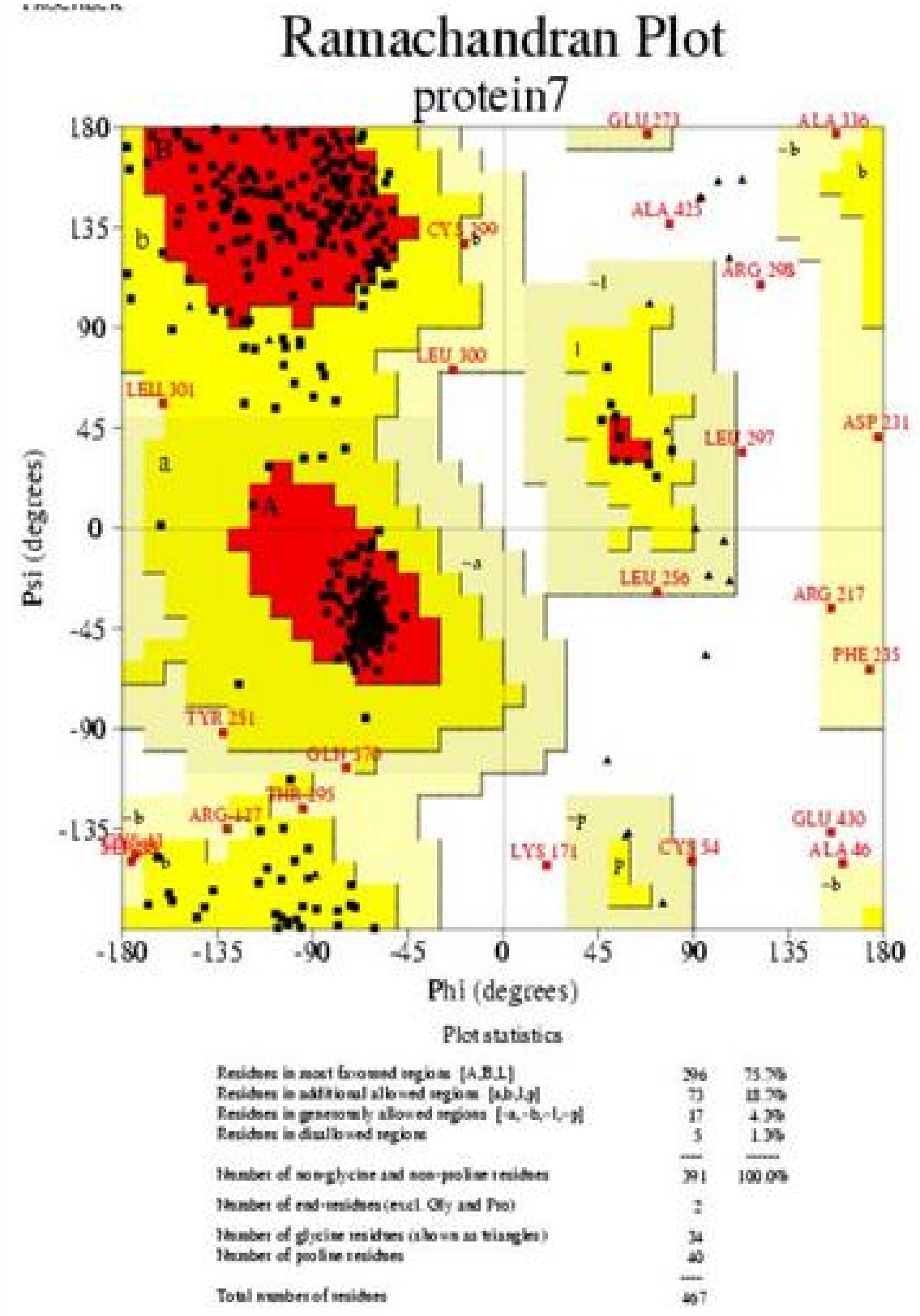

Fig. 1. Ramachandran Plot of the modeled structure (B99990007) with best dope score

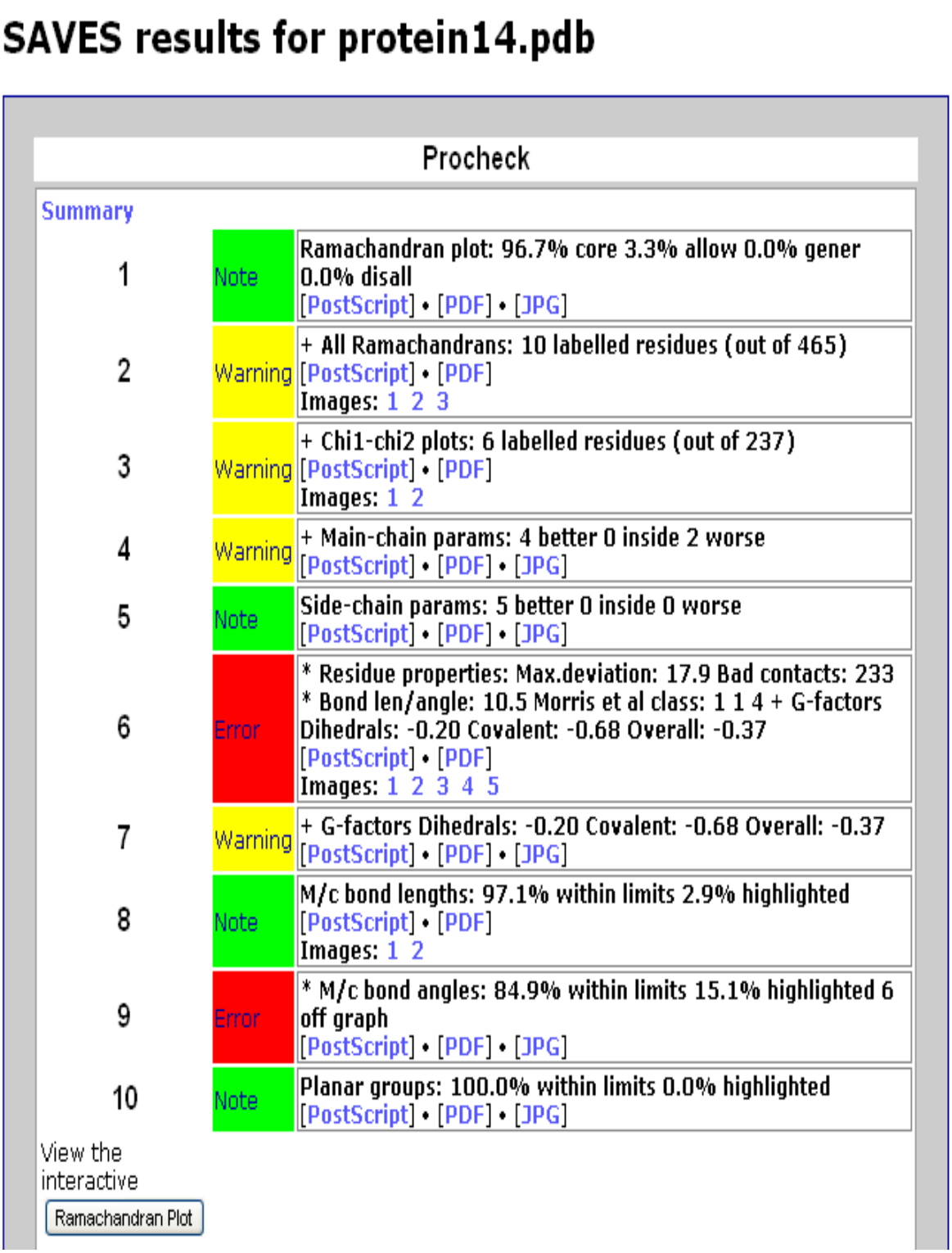

Fig 2. Ramachandran Plot of the final protein structure with all the amino acids in Core and allowed Region after loop modeling.

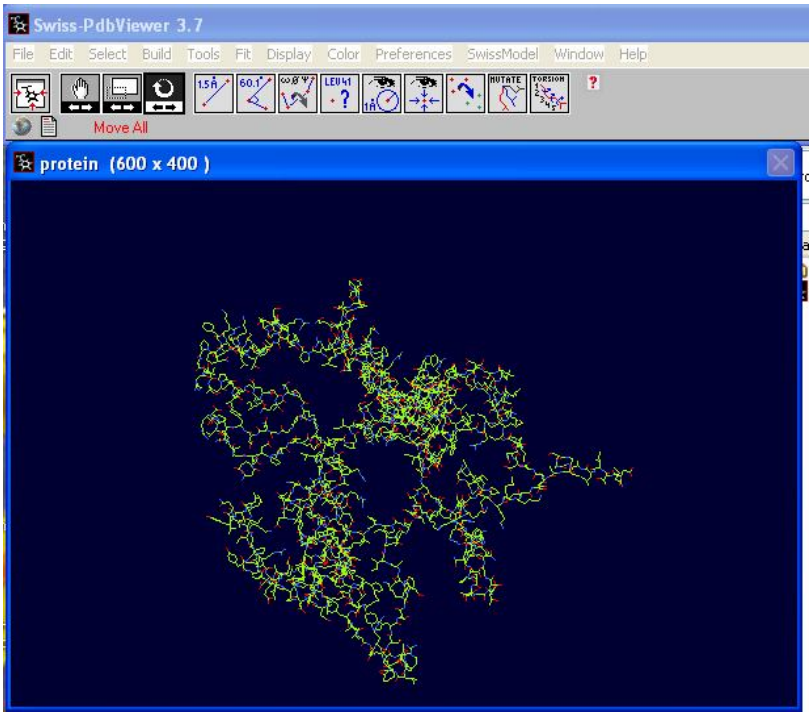

Fig 3. Modeled Structure of ORF61 Protein

CONCLUSION

The present work chicken pox has been chosen as a target disease which is caused by Varicella-zoster virus (VZV). The complete sequen, functional and structural information of all the VZV genes were obtained using various biological databases. The predicted structure can be further used for identifying the target sites for a potential drug.

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