Structural and Functional analysis of Interferon Gamma From *Bos taurus* by Bioinformatic Tools

Anila Hoda*, Lorena Hysi, Vilson Bozgo, Lumturi Sena
Department of Animal Science
Agricultural University of Tirana,
Kodër Kamëz – Tirana, Albania
E-mail: ahoda@ubt.edu.al*, lhysi@ubt.edu.al, vbozgo@ubt.edu.al, lsena@ubt.edu.al,

Citation: Hoda, A., Hysi, L., Bozgo, V., & Sena, L. (2020). Structural and Functional Analysis of Interferon Gamma From *Bos taurus* by Bioinformatic Tools. *Zhivotnovadni Nauki*, *57*(4), 25-37 (Bg).

Abstract

Interferon gamma is a dimerized soluble cytokine that is the only member of the type II class of interferons. Interferon, IFN gamma plays an important role in generating immunity against viruses and intracellular infections. A total of 17 full-length protein sequences from different farm animals were retrieved from NCBI database. Sequence analysis such as multiple sequence alignment (MSA), conserved motif identification, computation of amino acid composition, and phylogenetic tree construction were performed on these primary sequences. Phylogenetic tree indicated three subclusters comprising ruminant species, animals from equidae genius and swine individuals respectively. In silico analysis of interferon gamma from *Bos taurus* revealed that it is a thermostable, alkaline protein having molecular weight of about 20 kDa, having mainly two predicted conserved domains that belong to the Interferon gamma superfamily and to PKc_like superfamily. Prediction of motifs, patterns, disulphide bridges and secondary structure were performed for functional characterization. The 3D structure and protein model were developed by the use of Swiss Model, iTAsser, Phyre2 and RaptorX, and were validated using protein structure checking tools PROCHECK, ERRAT and QMEAN, which indicated that the model generated by Swiss Model was more acceptable

***Key words**:* in silico analysis, protein model, structure validation, phylogeny

Introduction

Native bovine interferon gamma is a 143 amino acid cytokine with potent activating, antiviral and antiproliferative properties. It is produced as a pro-peptide with an additional 23 amino acid N-terminal signal peptide sequence having a molecular weight of ~ 20 kDa. IFNgamma has antiviral activity and immunoregulatory functions. It is produced by T lymphocytes activated by specific antigens or mitogens. Properties include macrophage activation, upregulation of MHC class I and II molecules on various cells and induction of cytotoxicity.

IFN gamma plays an important role in generating immunity against viruses and intracellular infections (Gray and Goeddel, 1982) and is associated with resistance against tick (Maryam et al., 2012). IFN-γ is a master checkpoint regulator for many cytokines which are protein mediators that are known to be involved in many biological processes, including cell growth, survival, inflammation, and development (Zha et al., 2017). Bovine interferon gamma has been investigated for its therapeutic potential and could be a new product used in the control of bovine diseases (Jalali et al., 2010).

(Ealick et al., 1991) have determined the x-ray crystal structure of recombinant human interferon-'gamma with the use of multiple-isomorphous-replacement techniques and have shown that the protein is dimeric with six helices in each subunit. The 3D structure prediction of protein require X-ray crystallography and NMR spectroscopy which is very time consuming, tedious method and generate a large amount of data creating a gap between available sequences and solved structure, the gap that is reduced by *in silico* method of predicting 3D structure (Bhalla and Lal, 2014). Nacheva et al., 2012 have investigated the potential conformational changes in the structure of the human mutant proteins employing molecular dynamics simulations. The prediction of protein structure from amino acid sequences is a fundamental scientific problem and is regarded as a grand challenge in computational biology and chemistry (Bibinu et al., 2019). Analysis of a protein, which includes characteristics and structure determination, can be done *in silico* by the use of bioinformatics tools and various online databases. The online available bioinformatics tools for protein research enable the possibility to reveal characteristics of the protein of interest by only starting from the predicted or known amino acid sequence without fully depending on experimental approaches (Yavuz and Ozturk, 2017).

In the present study we describe an *in silico* comparative structural and functional analysis of interferon gamma from *Bos taurus*, by the use of biocomputational tools. We intend to study the physicochemical properties, predict secondary structure, modeling the 3D protein, evaluate and analyze the interferon gamma.

Material and methods

Sequence retrieval

Sequences of Interferon Gamma from different farm animals were retrieved in FASTA format from the GenBank (https://www.ncbi.nlm.nih.gov/protein). Table 1 shows the list of protein sequences considered in this study.

Phylogenetic comparison

Phylogenetic analyses were performed by employing a maximum likelihood (ML) method, by the use of MEGA X software (Kumar et al., 2018). The bootstrap method with 500 replica set, in order to test the robustness of phylogeny, amino acid substitutions, Poisson model and complete deletion were selected.

Primary structure analysis

Amino acid composition, pI or isoelectric point (pH at which net charge is zero), extinction coefficient (quantitative study of protein–protein and protein–ligand interactions), instability index (stability of proteins), aliphatic index (relative volume of protein occupied by aliphatic side chains), GRAVY or Grand Average Hydropathy (sum of all hydropathy values of all amino acids divided by number of residues in a sequence) were analyzed using the Expasy'sProtParam server (Gasteiger et al., 2005) (*http://us.expasy.org/tools/protparam.html*). The Hum-mPLoc v2.0 server (Shen and Chou 2009) was used to predict the subcellular localization of interferon gamma protein. The TMPred server (Hofmann, 1993) was used to analyze the presence of the transmembrane domains within the interferon gamma protein.

Secondary structure prediction

Secondary structure and the percentage of secondary elements i.e. helix, turn and sheet were predicted using PSIPRED and CFSSP: Chou and Fasman Secondary Structure Prediction server (http://www.biogem.org/cgi-bin/chofas.pl) (McGuffin et al., 2000), (Kumar, 2013).

Homology Modeling and model evaluation

The query sequence was interferon gamma precursor of Bos taurus (NP_776511.1). The tertiary structure of query sequence was predicted through four online homology modeling programs: Expasy SWISS-MODEL (ProMod Version 3.70), I-TASSER (Yang et al., 2015), (http://zhanglab.ccmb.med.umich.edu/I-TASSER/,), Phyre2 (Kelley et al., 2015) (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) and RaptorX structure prediction server, (http://

raptorx.uchicago.edu/StructurePrediction/predict/).

The built models were evaluated and verified from QMEAN and SAVES server (http://services.mbi.ucla.edu/SAVES/). Ramachandran plot was constructed by using the pdb file in SAVES server (http://services.mbi.ucla.edu/SAVES/Ramachandran/) and the highest overall quality factor was generated by ERRAT (http://services.mbi.ucla.edu/ERRAT/).

Functional analysis

For functional analysis CYS_REC tool (http://www.softberry.com/berry.phtml) was used to identify position of cysteine and compute most probable SS bond pattern of pairs in protein (Hooda, 2011). The set of conserved amino acid residues were analyzed using NCBI database (http://www.ncbi.nlm.nih.gov/cdd/) and Motif search tool (http://www.genome.jp/tools/motif/) (Singh et al., 2012). SOSUI server that was able to predict transmembrane helices from amino acid sequences with high precision and accuracy (Suwa et al., 2011).

Prediction of functionally interacting partners of the protein was performed by STRING (http://string-db.org/) analysis (Szklarczyk et al., 2014).

The active site residues of the modeled protein were assessed by CASTp serve (Dundas et al., 2006). The calculation was carried out using a solvent probe of radius 1.4 A°.

Results

Sequence retrieval

A total of 17 sequences, with a similarity are least 75%, were retrieved for different farm animals, in FASTA format from GenBank. The details about the selected protein sequences are shown in Table 1.

Multiple sequence alignment (MSA) of selected sequences was performed by Clustal Omega. The results of alignment are shown in Fig. 1.

Phylogenetic tree

A bootsrap Maximum likelihood tree for 17 selected protein sequences was generated by the use of MEGA X software (Fig. 2). In the tree are shown three major groups: individuals from ruminant species belong to one group, individuals from Equidae genius belong to another group and the last group is composed with swine individuals. the bootstrap consensus tree with node statistics.

Primary sequence analysis

The physicochemical properties for each sequence are shown in Table 1. The isoelectric point (pI) ranged from 9.36 to 9.70, indicating that the proteins are alkaline in nature. The instability indices for all sequences ranged from 34.92 to 42.99. The percentage of different amino acids of interferon gamma of different farm animals is shown in Fig. 3. The prediction of transmembrane segments within the protein was done by ProtScale tool. In Fig. 4 is shown the hydrophobicity profile obtained by ProtScale using the Kyte & Doolittle Scale (Kyte and Doolittle, 1982). The recommended threshold level is 1.6. Similarly transmembrane region prediction was performed using TMPred server (Hofmann and Stoffel) which showed two transmembrane helices one intrinsic and one extrinsic in nature. Similar patterns were observed with those provided by ProtScale hydrophobicity profile. The peaks indicated two potential transmembrane regions present within the protein. Results provided by Hum-mPLoc v2.0 server (Shen and Chou, 2009) showed that interferon gamma is an extracellular protein.

Secondary structure prediction

Secondary structure prediction of interferon gamma precursor from different farm animals by the use of SOPMA (Table 2) showed that the proteins are mostly comprised of alpha helix and random coil. In Fig. 5 and 6 are shown the secondary structure map of interferon gamma and the graphical representation of secondary structures. There was no disordered protein binding site present (Fig. 5). In this secondary structure analysis alpha-helical conformation was higher which indicates the thermostable nature of the protein.

Table 1. Parameters for the protein encoded by AtNHX1 gene using the ProtParam program: molecular weight (MW) (g/mol); isoelectric point (pI); extinction coefficient (EC) (M-1 cm-1); instability index (Ii); aliphatic index (Ai); grand average hydropathy(GRAVY); number of negative residues (-R); number of positive residues (+R)

Accession No	Sp	Length	M weight	pI	-R	+R	EC (Cys residues not reduced)	EC (Cys residues reduced)	II	Comment	AI	GRAVY
NP_776511.1	Bos taurus	166	19363.39	9.63	19	28	12950	12950	40.67	unstable	86.93	-0.497
Q8SPW9.1	Bos taurus	166	19434.47	9.63	19	28	12950	12950	36.23	stable	86.93	-0.515
BAE75855.1	Bubalus bubalis	166	19464.56	9.63	19	28	12950	12950	36.23	stable	86.93	-0.499
NP_001277834.1	Bubalus bubalis	166	19513.63	9.60	19	28	14440	14440	36.23	stable	86.93	-0.486
ADR71665.1	Ovis aries	166	19327.42	9.65	19	28	11460	11460	42.99	unstable	86.93	-0.483
NP_001272611.1	Capra hircus	166	19325.43	9.70	19	28	11460	11460	40.52	unstable	88.67	0.472
AAT72315.1	Capra hircus	166	19312.43	9.70	19	28	11460	11460	40.52	unstable	88.67	-0.455
ABS28998.1	Equus caballus	166	19342.37	9.50	19	28	14565	14440	34.92	stable	85.18	-0.462
ABS28999.1	Equus asinus somalicus	166	19216.21	9.41	19	27	14565	14440	35.91	stable	85.18	-0.421
ADD13972.1	Sus scrofa	166	19384.50	9.54	16	25	13075	12950	39.69	stable	82.23	-0.332
BAA05876.1	Equus caballus	166	19352.41	9.45	19	27	13075	12950	36.88	stable	87.53	-0.407
ABV04318.1	Sus scrofa	166	19518.64	9.54	17	26	13075	12950	42.33	unstable	79.88	-0.363
NP_999113.1	Sus scrofa	166	19418.52	9.54	16	25	13075	12950	40.20	unstable	79.88	-0.338
NP_001075418.1	Equus caballus	166	19338.34	9.37	19	26	13075	12950	36.88	stable	87.53	-0.405
ABH11657.1	Sus scrofa	166	19446.53	9.56	16	25	13075	12950	41.11	unstable	79.88	-0.342
ABI85319.2	Sus scrofa	166	19419.46	9.36	17	24	13075	12950	40.71	unstable	79.88	-0.336
ABI73977.1	Sus scrofa	166	19347.40	9.47	16	24	13075	12950	41.05	unstable	79.28	-0.319

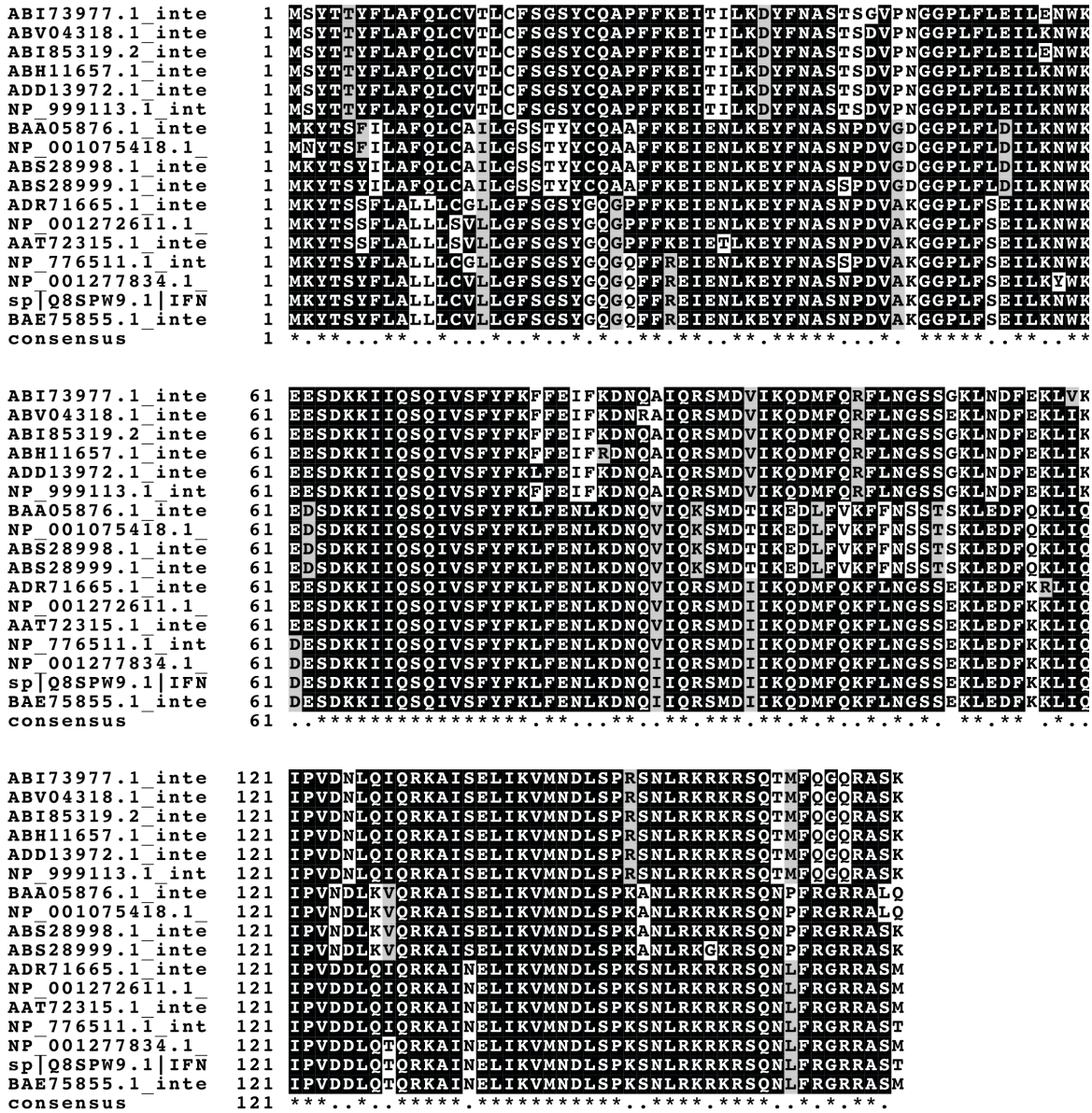


Fig. 1. Multiple Sequence Alignment of interfernon gamma precursor sequences from different farm animal species

Homology modeling and model evaluation

The 3D models of interferon gamma from *Bos taurus* (NP_776511.1) were gained by four protein structure homology model building programs Swiss Model, Phyre2, ITasser and RaptorX. The results of validation of each model by QMEAN4 and SAVES server are summarized in Table 3.

The G-Factor provides a measure of how unusual a property is. A value below -0.5 is considered unusual and a value below -1.0 is considered highly unusual. The results of Table 3 show that all models were not unusual, but the more reliable model is Swiss Model.

Functional analysis

Functional analysis of these proteins includes prediction of transmembrane region, disulfide bond and identification of important motifs. The SOSUI server shows that NP_776511.1 is

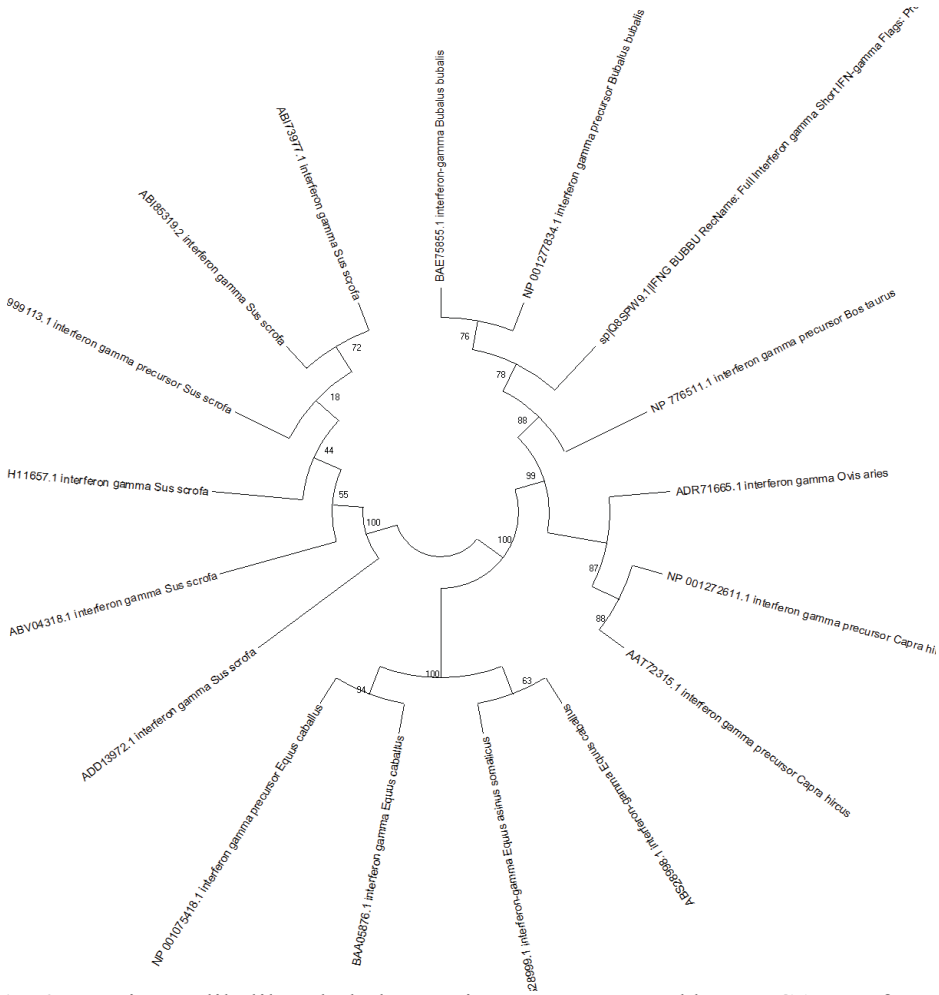


Fig. 2. Maximum likelihood phylogenetic tree constructed by MEGA X software

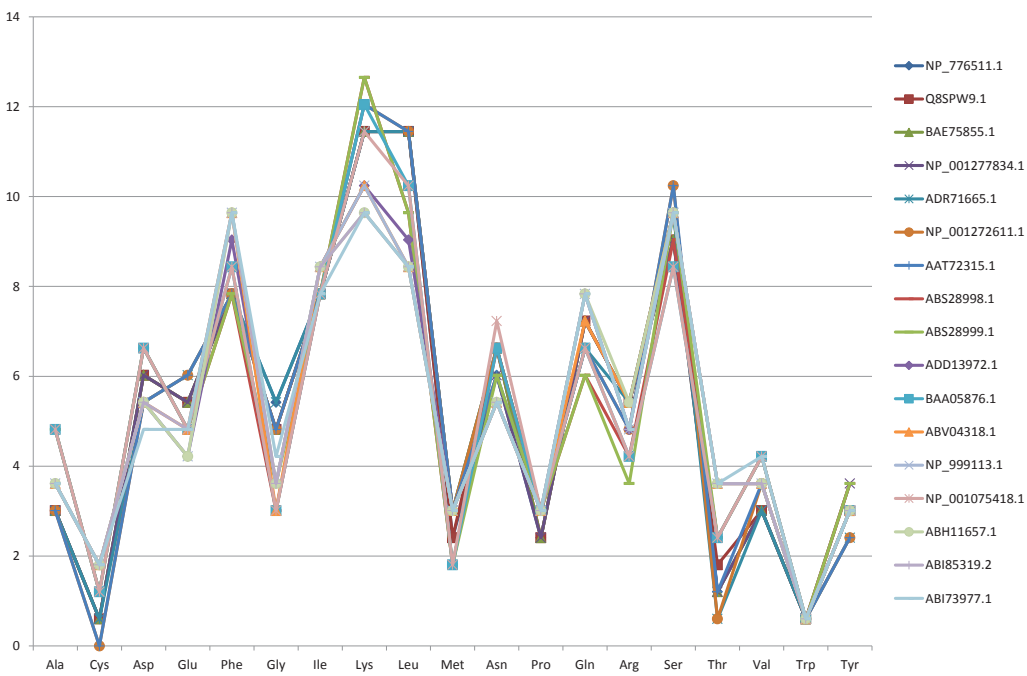


Fig. 3. Graphical representation of amino acid composition of selected sequences

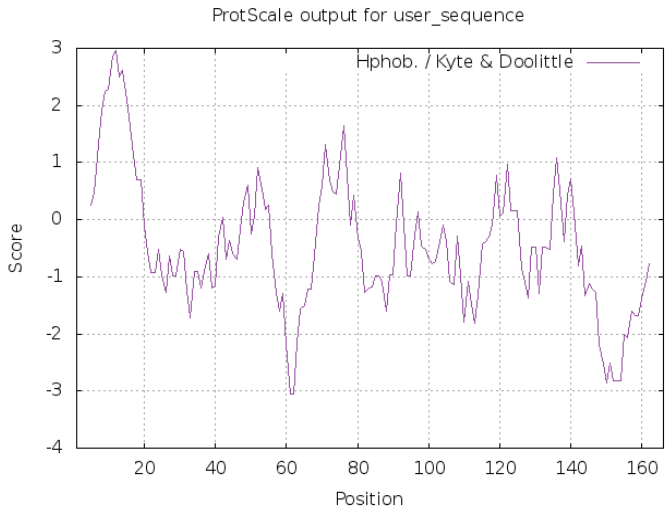


Fig. 4. ProtScale output of interferon gamma (Bos taurus)

Table 2. Predicted secondary structure content and disulphide bridges using NPS@ SOPMA and CYS-REC tools

Accession No	Sp	Alpha Helix	Extended Sheet	Beta Turn	Random coil	Disulfide bridge prediction (CYSREC) (%)
NP_776511.1	Bos taurus	62.65	3.01	3.61	30.72	None
Q8SPW9.1	Bos taurus	59.04	5.42	3.61	31.93	None
BAE75855.1	Bubalus bubalis	59.64	5.42	3.01	31.93	None
NP_001277834.1	Bubalus bubalis	59.64	.02	4.82	29.52	None
ADR71665.1	Ovis aries	62.65	3.01	4.22	30.12	None
NP_001272611.1	Capra hircus	60.84	3.01	3.61	32.53	None
AAT72315.1	Capra hircus	63.25	2.41	3.61	30.726	None
ABS28998.1	Equus caballus	1.45	4.82	2.41	31.33	None
ABS28999.1	Equus asinus somalicus	62.65	4.22	1.81	31.33	None
ADD13972.1	Sus scrofa	57.23	6.63	2.41	33.73	The most probable pattern of pairs: 13-23,
BAA05876.1	Equus caballus	63.86	3.01	3.01	30.12	None
ABV04318.1	Sus scrofa	61.45	6.02	1.81	30.72	The most probable pattern of pairs: 13-23
NP_999113.1	Sus scrofa	61.45	6.63	3.01	28.92	The most probable pattern of pairs: 13-23
NP_001075418.1	Equus caballus	60.24	6.02	1.81	31.93	None
ABH11657.1	Sus scrofa	59.64	6.63	1.81	31.93	The most probable pattern of pairs: 13-23
ABI85319.2	Sus scrofa	61.45	6.63	3.01	28.92	The most probable pattern of pairs: 13-23
ABI73977.1	Sus scrofa	62.05	0.02	2.41	29.52	None

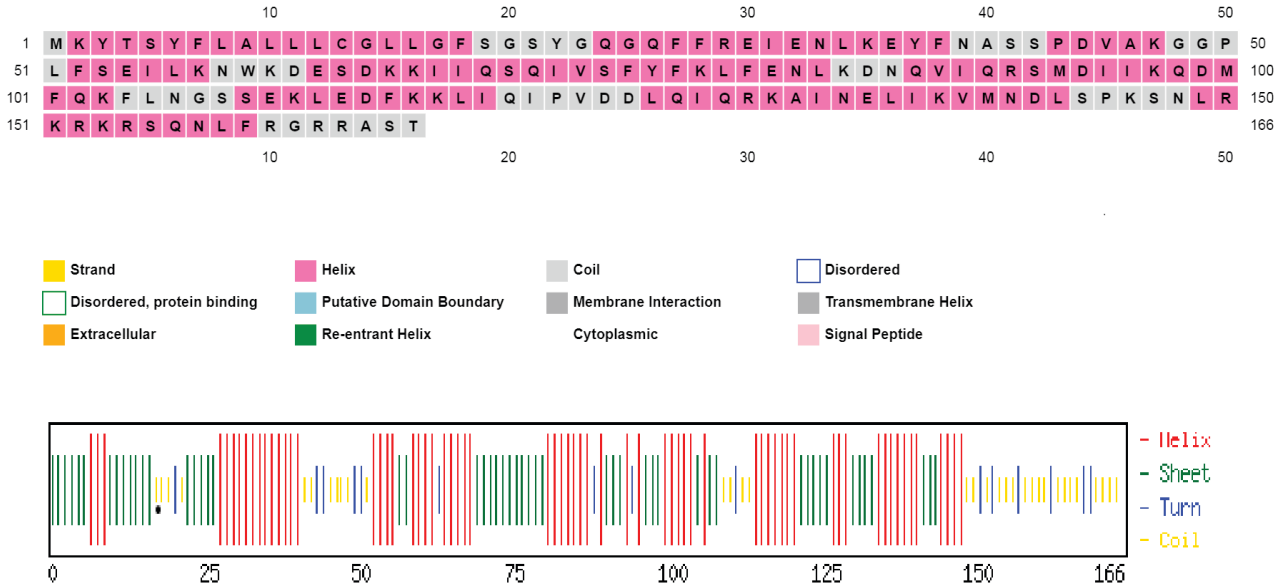


Fig. 5. Secondary structure map of interferon gamma precursor attained from PSIPRED

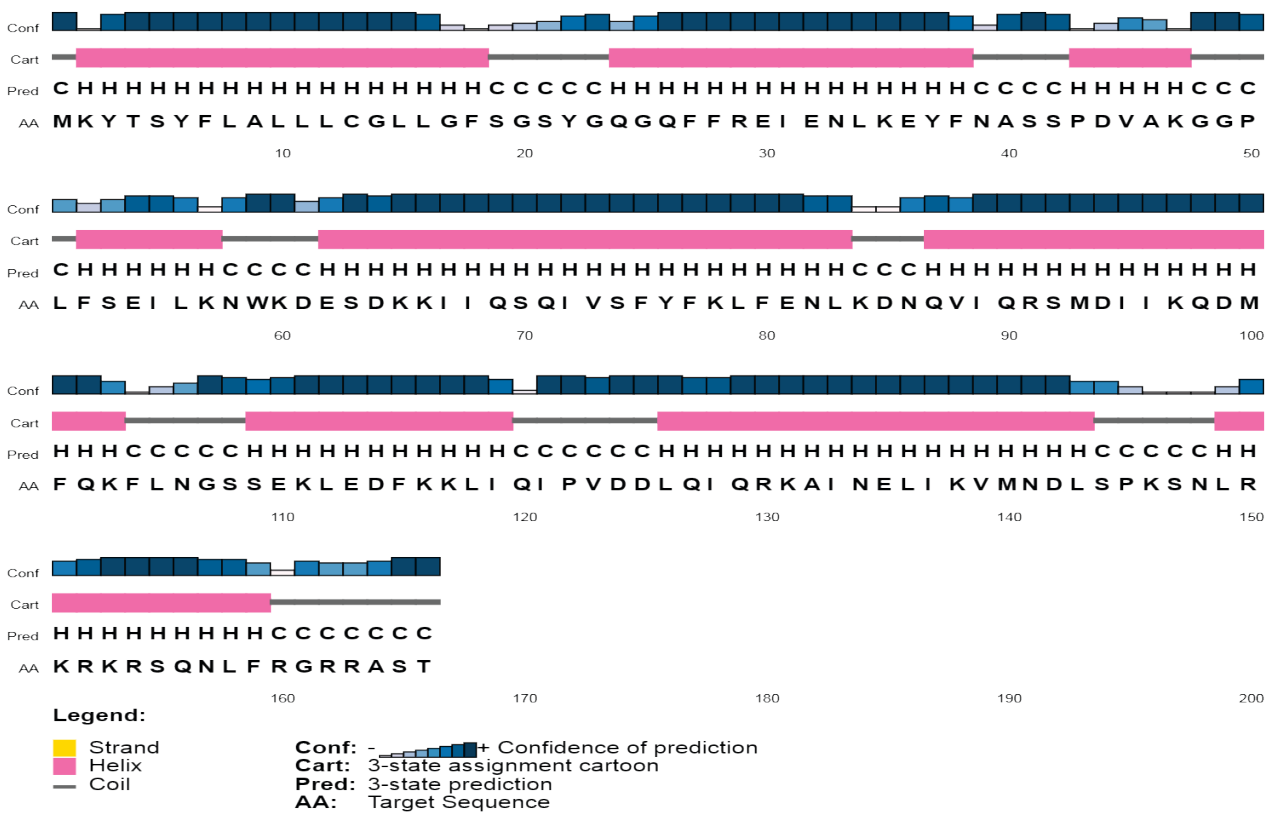


Fig. 6. Graphical representation of the predicted secondary structures present within the target protein interferon gamma precursor

Table 3. Comparative values of PROCHECK, G-Factor, QMEAN scores between all predicted models

Validation		Swiss model	Phyre	Tasser	RaptorX
PROCHECK Ramachandran Plot	Most favored regions	95.9	91.4	82.8	93.4
	Additional allowed regions	4.1	6.0	12.6	6.6
	Generously allowed regions	0.0	0.7	2.6	0.0
	Disallowed regions	0.0	2.0	2.0	0.0
G-Factor overall average		0.04	-0.00	-0.38	0.02
QMEAN	QMEAN4	0.93	-2.01	-4.09	-1.14
	Global Score	0.77 ± 0.05	0.63 ± 0.07	0.65 ± 0.07	0.59 ± 0.07
ERRAT Overall Quality Factor		99.0476	84.8101	98.1013	86.3309

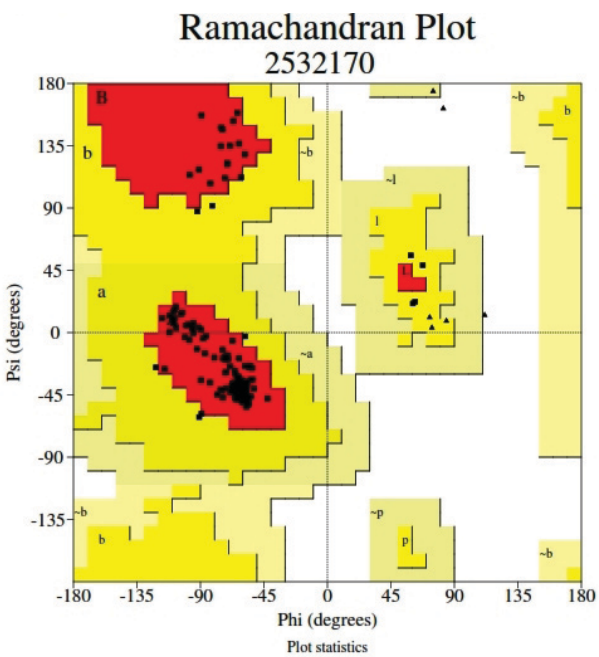


Fig. 7. Functionally interacting partners with the selected protein

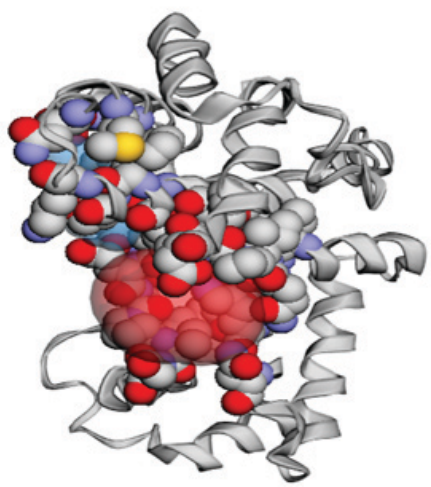


Fig. 8. Active site prediction of the predicted 3D structure of interferon gamma precursor of Bos taurus by CASTp server

a membrane protein with a transmembrane region (MKYTSYFLALLLCGLLGFS), of 19 aa length. The transmembrane regions are rich in hydrophobic amino acids. The presence of disulphide bonds in all sequences, obtained by CYS_REC are shown in Table 2. Fig. 7 shows the protein interaction network resolved by STRING analysis.

There are 41 predicted structural pockets, but only the pockets with volume > 50 Å were reported (red and green) (Fig. 8). In Table 4, are shown details about the area and volume, according to solvent accessible surface (SA, Richards' Surface) and the molecular surface (MS, Connolly's surface) for each cavity.

Table 4. Details of the active sites obtained by CASTp

POC_ID	Area_sa[1]	Area_ms[2]	Vol_sa[3]	Vol_ms[4]	Lenth[5]
1	252.863	519.352	212.673	725.258	248.132
2	113.372	204.546	50.070	270.781	114.613
3	63.921	126.167	28.380	152.769	54.725
4	40.332	76.301	20.329	100.334	45.372
5	29.600	48.399	14.822	67.188	22.531

[1]Area as pert Richards'surface; [2]Area as per Connolly' surface; [3]Volume as per Richards surface; [4]Volume as per Connolly's surface; [5]Length of the cavity

Discussion

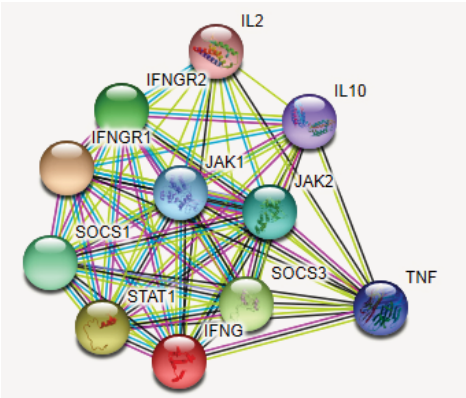
Interferon gamma is a master checkpoint regulator for many cytokines (Zha et al., 2017). One important role of IFN-γ is to regulate immunity and bridge the innate and specific immune response pathways (Payne, 2017). In the present study the interferon gamma from *Bos taurus* was analyzed by bioinformatic tools. Similar *in silico* studies are also previously carried out (Bixheku et al., 2019, Bozgo et al., 2014, Bozgo et al., 2017). The MSA results displayed protein blocks that do not contained gap-free regions. Thus, it was concluded that the selected protein is highly conserved amongst the farm animals. The phylogenetic tree, where the node statistics were greater than 50 (except one node with 45), indicated that the clades formed during divergence were strongly supported.

All proteins with instability indices lower than 40 are stable, meanwhile the proteins having instability index higher than 40 were unstable. This is also according to (Pramanik et al., 2017, Verma et al., 2016). All proteins showed high aliphatic index suggesting that proteins are thermostable (Ikai, 1980). The Grand Average Hydropathy (GRAVY) was negative for all proteins, indicating that proteins are hydrophilic.

The highest the quality G-factor, the better is the quality of the protein structure (Colovos and Yeates, 1993). The resulting GMQE score is expressed as a number between 0 and 1. Swiss model has the highest value of 0.77, indicating higher reliability. Swiss model has QMEAN z-score around zero indicating a good agreement between the model structure and experimental structures of similar size. iTasser model display low quality since have a low score (-4.09). PROCHECK Ramachandran Plot supported Swiss model as it had the highest number of residues in the most favored regions (95.9%) (Fig. 9) According to (Yadav et al., 2013) > 90% of the residues residing in favored region implies the characteristics of a good quality model. Therefore the more reliable model is Swiss model (Fig. 10). The built model through SWISS-MODEL was deposited to Protein Model Database (PMDB) and obtained the accession no.: PM0082612.

There are ten potential interacting partners. The closest interacting protein having the shortest node was found IFNGR1, which is *Bos taurus* interferon gamma receptor while the distant interacting protein was IL2 - Interleukin-2 which is produced by T cells in response to antigenic or mitogenic stimulation

Protein interaction network resolved by STRING analysis showed two that type of conserved domain were found, one of which belong to the Interferon gamma superfamily and the other to PKc_like superfamily, which is mainly composed of the catalytic domains of serine/threonine-specific and tyrosine-specific protein kinases. It also includes RIO kinases, which are atypical serine protein kinases, aminoglycoside phosphotransferases, and choline kinases. These proteins catalyze the transfer of the gamma-phosphoryl group from ATP to hydroxyl groups



		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
IFNG	*Interferon gamma; Produced by lymphocytes activated by specific antigens or mitogens. IFN-gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons; Belongs to the type II (or gamma) interferon family (166 aa)*									
Predicted Functional Partners:										
IFNGR1	*Bos taurus interferon gamma receptor 1 (IFNGR1), mRNA (466 aa)*					●	●	●		0.995
STAT1	*Bos taurus signal transducer and activator of transcription 1, 91kDa (STAT1), mRNA (1162 aa)*					●	●	●		0.987
SOCS3	*Suppressor of cytokine signaling 3; SOCS family proteins form part of a classical negative feedback system that regulates cyto...*						●	●		0.961
IFNGR2	*Interferon gamma receptor 2; Uncharacterized protein (307 aa)*					●	●	●		0.955
SOCS1	*Suppressor of cytokine signaling 1; Suppressor of cytokine signaling 1-like; Uncharacterized protein (223 aa)*						●	●		0.950
JAK2	*Janus kinase 2 (1133 aa)*						●	●		0.948
JAK1	*Bos taurus Janus kinase 1 (JAK1), mRNA (1158 aa)*						●	●		0.947
TNF	*Tumor necrosis factor; Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages...*					●		●		0.947
IL10	*Interleukin-10; Inhibits the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by a...*							●		0.939
IL2	*Interleukin-2; Produced by T-cells in response to antigenic or mitogenic stimulation, this protein is required for T-cell proliferatio...*							●		0.929

Fig. 9. Graphical representation of Ramachandran Plot, for the Swiss model by SAVES

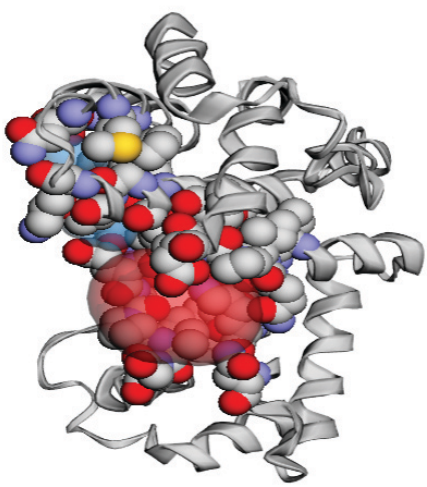


Fig. 10. Predicted 3D model interferon gamma (NP_776511.1) generated by Swiss model viewed in PyMol

in specific substrates such as serine, threonine, or tyrosine residues of proteins.

According to (Kelley and Sternberg, 2009), structural elements in the model will be reliable if a template is detected with > 30% sequence identity to the query, therefore findings from this study were reliable as all the query sequences of various species were detected with more than 75% identity.

Conclusions

Standard bioinformatic tools are useful to provide physicochemical characteristics, structural properties including 3D model, model quality analysis, phylogenetic assessment and functional analysis of interferon gamma. The results obtained here will help researchers to get an idea about the predicted protein structure and for detection and identification of such type of proteins *in vivo* or *in silico*.

References

Bhalla, A. S., & Lal, R. (2014). In Silico Sequence Analysis, Structure Prediction and Evaluation of Fatty Acid Synthase Protein (FAS) of Gallus gallus (Chicken) by Homology Modelling. *IJSR*, *4*(9), 843-847.

Bibinu, B. S., Gwaza, D. S., & Dim, N. I. (2019). Molecular analysis of proteins of BMP15 gene in ruminants and non-ruminants. *MOJ Proteomics Bioinform*, *8*(1), 22-25.

Bixheku, X., Hoda, A., Hysi, L., & Bozgo, V. (2019). In silico analysis of osteocalcin gene in Cyprinidae. *Albanian Journal of Agric. Sci*, *18*(Special edition), 197-203.

Bozgo, V., Hysi, L., & Hoda, A. (2017). Characterization of human CRB gene product by the use of bioinformatic tools. *Albanian Journal of Agricultural Sciences*, *16*(Special Edition), 347-351.

Bozgo, V., Hysi, L., Hoda, A., & Sena, L. (2014). In silico analysis of myostatin gene in some farm animals. *Albanian J. Agric Sci.*, *13*(Special editidon), 441-446.

Colovos, C., & Yeates, T. O. (1993). Verification of protein structures: patterns of nonbonded atomic interactions. *Protein science*, *2*(9), 1511-1519.

Dundas, J., Ouyang, Z., Tseng, J., Binkowski, A., Turpaz, Y., & Liang, J. (2006). CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic acids research*, *34*(suppl-2), W116-W118.

Ealick, S. E., Cook, W. J., Vijay-Kumar, S., Carson, M., Nagabhushan, T. L., Trotta, P. P., & Bugg, C. E. (1991). Three-dimensional structure of recombinant human interferon-gamma. *Science*, *252*(5006), 698-702.

Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. In *The proteomics protocols handbook* (pp. 571-607). Humana press.

Gray, P. W., & Goeddel, D. V. (1982). Structure of the human immune interferon gene. *Nature*, *298*(5877), 859-863.

Hofmann, K. A. W. S. (1993). TMbase-A database of membrane spanning proteins segments. *Biol. Chem. Hoppe-Seyler*, *374*, 166.

Hooda, V. (2011). Physicochemical, Functional and structural characterization of wheat germin using in silico methods. *Current Research Journal of Biological Sciences*, *3*(1), 35-41.

Ikai, A. (1980). Thermostability and aliphatic index of globular proteins. *The Journal of Biochemistry*, *88*(6), 1895-1898.

Jalali, S. A. H., Nikbakht, B. G. R., Tajbakhsh, H., Kouhi, M., Gholkar, M., & Rabani, M. (2010). Cloning and high level expression of bovine interferon gamma gene in eukaryotic cells (COS-7).

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature protocols*, *10*(6), 845-858.

Kelley, L. A., & Sternberg, M. J. (2009). Protein structure prediction on the Web: a case study using the Phyre server. *Nature protocols*, *4*(3), 363.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, *35*(6), 1547-1549.

Kumar, T. A. (2013). CFSSP: Chou and Fasman secondary structure prediction server. *Wide Spectrum*, *1*(9), 15-19.

Kyte, J., & Doolittle, R. F. (1982). A simple method for displaying the hydropathic character of a protein. *Journal of molecular biology*, *157*(1), 105-132.

Maryam, J., Babar, M. E., Nadeem, A., & Hussain, T. (2012). Genetic variants in interferon gamma (IFN-γ) gene are associated with resistance against ticks in Bos taurus and Bos indicus. *Molecular biology reports*, *39*(4), 4565-4570.

McGuffin, L. J., Bryson, K., & Jones, D. T. (2000). The PSIPRED protein structure prediction server. *Bioinformatics*, *16*(4), 404-405.

Nacheva, G., Lilkova, E., Petkov, P., Petkov, P., Ilieva, N., Ivanov, I., & Litov, L. (2012). In silico studies on the stability of human interferon-gamma mutants. *Biotechnology & Biotechnological Equipment*, *26*(sup1), 200-204.

Payne, S. (2017). *Immunity and Resistance to Viruses in Viruses: from understanding to investigation*. Academic Press.

Pramanik, K., Soren, T., Mitra, S., & Maiti, T. K. (2017). In silico structural and functional analysis of Mesorhizobium ACC deaminase. *Computational Biology and Chemistry*, *68*, 12-21.

Shen, H. B., & Chou, K. C. (2009). A top-down approach to enhance the power of predicting human protein subcellular localization: Hum-mPLoc 2.0. *Analytical biochemistry*, *394*(2), 269-274.

Singh, S., Singh, G., Sagar, N., Yadav, P. K., Jain, P. A., Gautam, B., & Wadhwa, G. (2012). Insight into Trichomonas vaginalis genome evolution through metabolic pathways comparison. *Bioinformation*, *8*(4), 189.

Suwa, M., Sugihara, M., & Ono, Y. (2011). Functional and structural overview of G-protein-coupled receptors comprehensively obtained from genome sequences. *Pharmaceuticals*, *4*(4), 652-664.

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., ... & Kuhn,

M. (2015). STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic acids research*, *43*(D1), D447-D452.

Verma, A., Singh, V. K., & Gaur, S. (2016). Computational based functional analysis of Bacillus phytases. *Computational biology and chemistry*, *60*, 53-58.

Yadav, P. K., Singh, G., Gautam, B., Singh, S., Yadav, M., Srivastav, U., & Singh, B. (2013). Molecular modeling, dynamics studies and virtual screening of Fructose 1, 6 biphosphate aldolase-II in community acquired-methicillin resistant Staphylococcus aureus (CA-MRSA). *Bioinformation*, *9*(3), 158.

Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., & Zhang, Y. (2015). The I-TASSER Suite: protein structure and function prediction. *Nature methods*, *12*(1), 7-8.

Yavuz, C., & Öztürk, Z. N. (2017). Working with Proteins in silico: A Review of Online Available Tools for Basic Identification of Proteins. *Turkish Journal of Agriculture-Food Science and Technology*, *5*(1), 65-70.

Zha, Z., Bucher, F., Nejatfard, A., Zheng, T., Zhang, H., Yea, K., & Lerner, R. A. (2017). Interferon-γ is a master checkpoint regulator of cytokine-induced differentiation. *Proceedings of the National Academy of Sciences*, *114*(33), E6867-E6874.