Bioinformatics Protein Sequence Analysis of Contagious Bovine Pleuropneumonia

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ABSTRACT

A total of Twenty (20) contagious bovine pleuropneumonia (CBPP) proteins were retrieved from the GenBank (www.ncbi.nlm.nih.gov). The Genbank accession numbers of the sequences and sequence variations of the proteins were used to investigate the molecular identity of various CBPP proteins. The physico-chemical properties of CBPP protein were performed using protparam tool. Isoelectric point (pI), molecular weight (MW), extinction coefficient (EC); instability index (II), aliphatic index (AI) and grand average of hydropath city (GRAVY) were computed. The study also revealed that some of the pI of CBPP protein were acidic while some were basic in nature. The EC and II of CBPP proteins indicate better stability which is an indication of resistant to mutation. AI for some of CBPP protein have AI>100. This indicates thermo stability of the protein. The GRAVY of CBPP proteins revealed some are positive while some are negative. The positive value indicates solubility (hydrophilic) in water while negative is not soluble (hydrophobic) in water. The amino acid composition of CBPP proteins indicates that they are rich in isoleucine, leucine and lysine. The three dimensional structures (3D) of the CBPP proteins were determine using Phyre2 server. The amino acid sequences of CBPP proteins were subjected to secondary structure prediction using ExPASy's SOPMA tool. The proteins are more of alpha helix structure. The genetic information revealed in this study will help in carrying out research in mutagenesis and pharmacogenetic so as to increase cattle production and also to eliminating transboundary trade bearier in developing countries.

Keywords: *Bioinformatics, Protein, Contagious bovine and Sequence,*

INTRODUCTION

Contagious bovine pleuropneumonia (CBPP) is a disease of cattle that affects production through mortality and reduced productivity. It also retards genetic improvement and limits the ability of cattle to work. CBPP is an infectious disease of cattle caused by the small-colony type of *mycoplasma mycoides* subspecies *mycoides* (Masiga and Domenech, 1995). The Pan African Program for the Control of Epizootics (PACE) (this program is implemented by the African Union Inter african Bureau for Animal Resources [AU-IBAR] in 32 African countries and is funded principally by the European Commission with the support of the participating African countries) has identified CBPP as the second most important trans boundary disease in Africa after rinderpest (Tambi *et al*., 2006). Transmission occurs from direct and repeated contact between sick and healthy animals. The first incidence of the disease in Nigeria was recorded in 1924 when reliable records were first available (Foluso, 2004). As at today the disease is endemic in West, Central, East and parts of South Africa (Tambi *et al*., 2006). It is a major threat for cattle health, production and also the most significant epidemic disease of cattle in Africa where it was reported from seventeen countries in 2001 (OIE, 2002) and twenty seven countries in 2002 (Tambi *et al*., 2006). The study was to investigate protein sequence analysis of contagious bovine pleuropneumonia by in silico approach

MATERIALS AND METHODS

A total of twenty (20) CBPP proteins of cattle were retrieved from the GenBank (www.ncbi.nlm.nih.gov). The Genbank accession numbers o f the sequences and sequence variations are shown in Table 1. ProtParam Tool was used for the computation of various physical and chemical properties of the CBPP proteins using amino acid sequences. The commutated parameters were molecular weight,

theoretical pI (isoelectric point), amino acid composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) (Gasteiger, 2005). The amino acid sequences of CBPP proteins were subjected to secondary structure prediction using ExPASy's SOPMA tool. SOPMA is an improved SOPM method. It predicts 69.5% of amino acids for a 3 state description of the secondary structure (a helix, b sheets and coil). The Phyre2 server was used to predict the 3D structure of CBPP proteins.

These servers predict the three-dimensional structure of a protein sequence using the principles and techniques of homology modeling (Kelley and Sternberg, 2009). Currently, the most powerful and accurate methods for detecting and aligning remotely related sequences rely on profiles or Hidden Markov Models (HMMs). 3DligandSite was used to predict the binding site of the 3D structure of the CBPP proteins. Phyre2 was coupled to the 3DligandSite server for protein binding site prediction (Wass *et al*., 2010).

Table1._Amino acid names, accessions and numbers of bovine CBPP protein_

S/N	NAME OF PROTEIN	ACCESSION No	AMINO ACID No
1	Lipoprotein B	AAU26106	622
2	Proline- tRNA ligase	Q6MTR9	474
3	GTpase obg	Q6MTG9	433
4	Phosphoglycerate kinase	P62415	404
5	Transposase	NP975936-IS 1634BQ	557
6	Transposase-is4 family	ADK70040	557
7	Transposase-isMmy 1	CAL91969	470
8	Prolipoprotein A	CAE76667	532
9	Prolipoprotein	CAE76666	548
10	DNA-directed RNA polymerase subunit beta	Q6MRX5	1255
11	Amino acid permease	NP975877	512
12	Ribose/galactoseABC transport	CAE76664	550
13	UDP-glucose-4 epimerase	NP975938	334
14	Lipoprotein B precursor	AAUI4997	622
15	ATP synthases subunit alpha	Q6MS92	525
16	Phosphonate ABC transporter permease	NP975087	911
17	Methionine- tRNA ligase	CAE76665	549
18	ABC transporter-ATP binding protein	YP00781134	406
19	DNA topoisomerase 1	NP975898	643
20	Protein tanslocace subunit set	Q6MUE3	944

RESULTS

Physico-chemical characteristics of CBPP protein were predicted by protparam. The computed isoelectric points (pI) of CBPP of protein are shown in table 2. The result revealed that GT pase obg, Phosphogly cerate kinase, ATP syntheses subunit alpha, Methionine-tRNA ligase and Protein translocase subunit set are acidic (pI<7) while the rest of the CBPP proteins have (pI>7) which indicates they are basic in nature. The net charge of CBPP is shown in table 2. The result revealed that Lipoprotein B, Proline- tRNA ligase, Transposes, Transposase-is4 family, Transposase-isMmy 1, Prolipoprotein, DNA-directed RNA polymerase subunit beta, Amino acid permease, Ribose/galactoseABC transport, Lipoprotein B precursor, Phosphonate ABC transporter permease, ABC transporter-ATP binding protein, DNA topoisomerase 1 and Protein translocase subunit set have positive net charges. However, Phosphoglycerate kinases,

Prolipoprotein A, UDP-glucose-4 epimerase are neutral (no charge) while GTpase obg, ATP synthases subunit alpha and Methionine- tRNA ligase are negatively charged. The extinction coefficient (EC) values for CBPP proteins at 280 nm are shown in table 2. The protein with the highest EC value is Phosphonate ABC transporter permease (120560) and the least is Prolipoprotein (25330). The EC values depend on the concentration of Tyrosine and Tryptophan. The extinction coefficient of a protein at 280 nm depends almost exclusively on the number of aromatic residues, particularly tryptophan (Gill and Von-hippel, 1989). The Half life of all the proteins selected for the study is presented in table 2. Half life is the time it takes for half of the amount of protein in a cell to disappear after its synthesis in the cell of the proteins. The result showed 30 hours for all the CBPP protein. The instability index (II) of CBPP protein is presented in Table 2. The instability index provides an estimate of the stability of your protein in a test tube. A protein

whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable (Guruprasad *et al.,* 1990). The result from this study shown that all the CBPP proteins instability index value is <40. The result of the Aliphatic Index (AI) of CBPP is shown in Table 8. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). Amino acid permease, ATP synthases subunit alpha and ABC transporter-ATP binding proteins have AI>100 while the rest of the protein CBPP protein have AI<100. The result of Grand Average Hydropathicity (GRAVY) of CBPP

protein is shown in table 2. The grand average hydropathicity (GRAVY) in this study showed that only amino acid permease and phosphonate ABC transporter permerase have positive value while the rest of the CBPP protein have negative value. Table 3 showed the amino acid composition in percentages of (CBPP) protein. All the CBPP proteins used for this study have similar amino acid composition. All the proteins have higher percentage values in isoleucine, leucine and lysine amino acids composition. Isoleucine and leucine are aliphatic amino acid and lysine is polar amino acid. All the CBPP proteins have zero percentage of selenocystein and pyrrolysine amino acids.

Table 2. *physic-chemical characteristic of protein of CBPP predicted by protparam*

Protein	AA No	Mol Wt	PI	Q	EC	Half Life	II	AI	GRAVY
Lipoprotein B	622	69808.3	8.81	+	64860	30hrs	30.63	79.98	-0.621
Proline- tRNA ligase	474	55432.5	7.47	+	63425	30hrs	34.66	85.53	-0.487
GTpase obg	433	48344.4	6.65	-	41495	30hrs	25.16	94.36	-0.377
Phosphoglycerate kinase	404	44414.1	6.94	Neu	31065	30hrs	24.65	98.71	-0.163
Transposase	557	65645.7	9.44	+	90900	30hrs	28.30	87.47	-0.566
Transposase-is4 family	557	65590.6	9.42	+	90900	30hrs	29.21	87.13	-0.570
Transposase-isMmy 1	470	55428.1	9.38	+	47010	30hrs	31.05	90.64	-0.460
Prolipoprotein A	532	61092.0	7.07	Neu	29800	30hrs	33.82	78.97	-0.758
Prolipoprotein	548	62619.1	9.21	+	25330	30hrs	29.05	80.75	-0.846
DNA-directed RNA polymerase subunit beta	1255	141359.0	8.37	+	99170	30hrs	33.86	97.38	-0.312
Amino acid permease	512	566633.2	9.54	+	55725	30hrs	28.26	117.70	0.677
Ribose/galactoseABC transport	550	60936.9	8.99	+	86290	30hrs	24.70	81.04	-0.457
UDP-glucose-4 epimerase	334	37848.0	7.04	Neu	34520	30hrs	32.34	90.21	-0.287
Lipoprotein B precursor	622	69823.4	8.88	+	64860	30hrs	29.88	80.29	-0.613
ATP synthases subunit alpha	525	58202.9	6.09	-	39310	30hrs	34.12	105.85	-0.146
Phosphonate ABC transporter permease	911	105787.5	9.66	+	120560	30hrs	28.03	111.17	0.034
Methionine- tRNA ligase	509	59588.3	6.13	-	81960	30hrs	36.42	97.68	-0.360
ABC transporter-ATP binding protein	406	47495.3	9.64	+	41830	30hrs	22.22	103.20	-0.459
DNA topoisomerase 1	643	74161.2	8.33	+	94910	30hrs	31.53	83.41	-0.625
Protein tanslocace subunit set	944	107796.5	5.77	+	45730	30hrs	36.25	97.66	-0.452

AA=amino acid; pI=isoelectric point; Q=net charge; II=instability index; AI=alphatic index; GRAVY= grand average of hydropathicity ; EC= extinction coefficient; Mol wt=molecular weight.

Table 3. *Composition (%) of CBPP proteins*

PROTEIN	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	O	U
Lipoprotein B	5.9	2.4	7.4	6.9	0.2	3.5	6.9	5.8	0.8	7.6	8.2	12.5	1.8	4.2	3.2	7.4	7.4	1.3	2.3	4.3	0.0	0.0
Proline- tRNA ligase	3.6	4.2	6.3	6.3	2.1	4.9	7.8	3.8	1.3	9.9	7.2	6.1	1.7	5.5	3.4	5.5	5.7	1.3	4.2	5.3	0.0	0.0
GTpase obg	6.0	2.1	7.6	6.7	0.5	3.5	7.4	9.9	1.4	8.5	10.2	11.8	1.4	4.8	2.3	3.2	3.5	0.9	3.0	5.3	0.0	0.0
Phosphoglycerate kinase	7.7	1.7	6.2	5.9	0.5	3.0	7.4	7.9	1.0	8.7	9.2	11.6	1.2	5.2	2.7	5.9	4.2	1.0	1.5	7.4	0.0	0.0
Transposase	3.2	4.5	6.8	5.9	0.9	3.4	6.5	4.3	1.3	9.9	7.7	12.9	1.3	4.8	2.3	5.7	5.6	1.3	6.3	5.4	0.0	0.0
Transposase-is4 family	3.4	4.3	6.6	6.1	0.9	3.4	6.5	4.3	1.3	9.9	7.7	13.1	1.3	4.8	2.3	5.7	5.6	1.3	6.3	5.2	0.0	0.0

Protein	A	R	N	D	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	U	O
Transposase-isMmy 1	3.0	5.1	8.1	5.1	1.1	1.7	7.7	3.6	1.5	10.6	9.1	11.9	2.3	6.2	1.7	7.2	4.9	0.4	5.1	3.6	0.0	0.0
Prolipoprotein A	2.6	1.5	8.8	7.9	0.2	3.6	7.0	3.0	1.3	8.3	8.6	13.3	0.8	5.8	4.9	9.2	5.8	0.0	3.8	3.6	0.0	0.0
Prolipoprotein	3.1	1.6	13.0	6.9	0.2	2.7	6.4	3.5	1.3	7.7	9.1	14.4	1.3	4.4	5.3	6.6	5.3	0.0	3.1	4.2	0.0	0.0
DNA-directed RNA polymerase subunit beta	5.9	4.9	5.3	5.7	0.7	4.1	7.9	6.3	1.4	8.8	8.9	9.2	2.2	3.4	3.6	5.4	5.3	0.7	2.6	7.0	0.0	0.0
Amino acid permease	7.2	2.5	4.9	3.1	1.2	3.3	1.8	8.2	1.6	12.3	10.9	5.7	3.1	9.0	2.0	8.2	4.1	1.2	2.9	6.8	0.0	0.0
Ribose/galactoseABC transport	7.3	1.8	7.3	5.8	0.2	3.1	5.8	7.3	1.5	7.3	6.9	11.5	2.0	3.3	1.1	9.8	6.2	1.8	3.8	6.4	0.0	0.0
UDP-glucose-4 epimerase	5.4	2.1	9.3	5.7	1.5	2.7	5.4	6.9	2.1	6.9	8.4	9.0	0.9	5.7	2.7	6.6	4.2	0.0	6.9	5.1	0.0	0.0
Lipoprotein B precursor	5.8	2.4	7.2	6.8	0.2	3.4	7.1	5.8	0.8	7.6	8.2	12.7	1.8	4.2	3.2	7.6	7.4	1.3	2.3	4.5	0.0	0.0
ATP synthases subunit alpha	7.0	3.8	5.3	4.8	0.2	5.9	7.0	7.2	1.3	10.5	9.3	7.2	1.7	2.7	3.6	6.1	4.8	0.4	3.6	7.4	0.0	0.0
Phosphonate ABC transporter permease	4.7	2.7	.6.1	3.8	0.5	3.8	4.9	3.5	0.7	10.9	12.2	11.9	0.8	7.2	1.8	6.1	6.6	1.1	4.8	5.7	0.0	0.0
Methionine- tRNA ligase	3.3	2.2	9.0	5.1	1.0	4.1	7.5	4.1	2.0	10.2	11.2	9.4	1.4	5.1	2.8	4.9	5.9	1.4	5.7	3.7	0.0	0.0
ABC transporter-ATP binding protein	2.5	1.0	10.8	5.7	0.2	5.4	4.4	3.7	2.0	9.9	12.3	15.5	1.0	5.7	2.0	5.7	2.5	0.7	4.2	4.9	0.0	0.0
DNA topoisomerase 1	4.7	3.3	8.1	5.4	0.8	3.3	8.4	4.8	1.2	8.7	8.2	11.2	1.2	3.9	3.0	6.8	6.1	1.2	5.3	4.4	0.0	0.0
Protein tanslocace subunit set	7.3	5.6	6.0	7.3	0.1	4.9	8.8	5.2	1.4	9.5	9.9	9.1	2.8	3.4	1.7	3.7	5.3	0.1	2.9	5.1	0.0	0.0

A=Alanine, Arginine=R, Asparagini=N, Aspartic acid=D, cysteine=C, Glutamic acid=E,Glutamin=Q, Glycine=G, Histidine=H, Isoleucine=I, Leucine=L, Lysine=K, Methionine=M, Phenylalanine=F,Proline=P, Serine=S, Theonine=T,Tryptophan=W, Tyrosine=Y, Valine=V,Selenocystein=U,Pyrrolysine=O

The prediction of secondary structures of CBPP proteins of cattle is shown in Table 4. Phosphonate ABC transporter permease showed highest alpha helix (54.67%) and lowest Prolipoprotein (24.09%). The beta turns prediction showed highest in GTpase obg (37.02%) and lowest in Lipoprotein B (17.04%). Prolipoprotein also is lower (5.84%) extended strand while Transposase (11.31%) showed highest. Prolipoprotein showed highest (42.52%) in random coil while Phosphonate ABC transporter permease showed lowest (18.88%)

Table 4.*Prediction of secondary structures of CBPP proteins*

Protein	Alpha helix (%)	ExtendedStrand(%)	Beta Turn (%)	Random coil (%)
Lipoprotein B	40.03	17.04	9.81	33.12
Proline- tRNA ligase	35.02	23.21	9.49	32.28
GTpase obg	30.95	37.02	10.85	31.18
Phosphoglycerate kinase	42.82	23.51	10.89	22.77
Transposase	39.32	21.18	11.31	28.19
Transposase-is4 family	40.57	20.47	10.77	28.19
Transposase-isMmy 1	46.17	19.77	8.51	25.53
Prolipoprotein A	28.38	26.32	7.52	37.78
Prolipoprotein	24.09	27.55	5.84	42.52
DNA-directed RNA polymerase subunit beta	41.04	20.32	10.36	28.29
Amino acid permease	35.74	29.69	11.13	23.44
Ribose/galactoseABC transport	38.00	20.81	11.09	30.73
UDP-glucose-4 epimerase	35.93	24.55	11.08	28.44
Lipoprotein B precursor	39.87	18.49	9.81	31.83
ATP synthases subunit alpha	41.90	20.95	9.52	27.62
Phosphonate ABC transporter permease	54.67	20.31	6.15	18.88
Methionine- tRNA ligase	49.31	17.88	9.23	23.58
ABC transporter-ATP binding	38.92	27.34	8.87	24.88

protein				
DNA topoisomerase 1	40.28	20.06	8.86	30.79
Protein tanslocace subunit set	53.92	17.90	7.94	20.23

Parameters:
Window width: 17
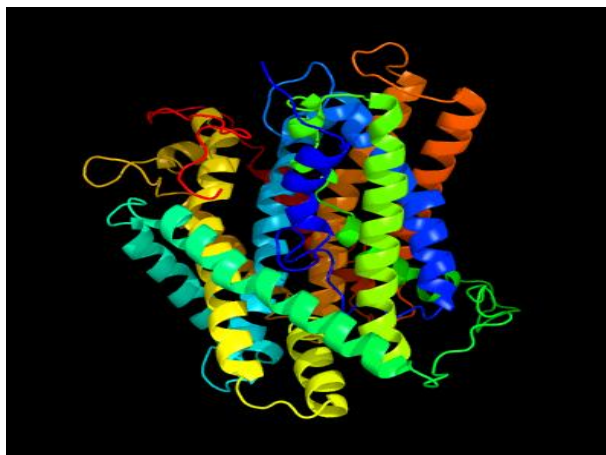*Similarity threshold: 8*
Number of states: 4

Figure1. *Schematic 3D structure of cattle amino acid permease-bovine protein Image colored by rainbow N → C terminus Model dimensions (Å): X: 68.312 Y:64.481 Z:61.432393 residues (97% of goat phosphoglycerate_kinase-caprine sequence) have been modeled with 100.0% confidence by the single highest scoring template.*

DISCUSSION

The isoelectric point (pI) will be useful for developing buffer system for purification by isoelectric focusing method. The isoelectric point is of significance in protein purification because it is the pH at which solubility is always minimal and at which mobility in an electro focusing system is zero and therefore the point at which the protein will accumulate (Fennema, 2008). This implies that the protein can accept electron or give out electrons depending on the charges. The net charge indicates the amino acid number of residues in a protein. The positively charged proteins are bind to the negatively charged proteins and vice versa. The neutral is not bound to either positively nor negatively charged proteins. This provides information about the location of the proteins. Intracellular proteins tend to have a higher fraction of negatively charged residues while extra cellular proteins tend to have fraction of positive charge residues (Munduganore *et al*., 2012). The positively charged protein may help to regulate gene expression or help to fold the DNA. The extinction coefficient of a protein at 280 nm depends almost exclusively on the number of aromatic residues, particularly tryptophan (Gill and Von-hippel, 1989). The extinction coefficient (EC) of CBPP protein has appreciable number of EC. This indicates that the higher the EC value of proteins, the higher the number of aromatic residues (Gasteiger

2003; Munduganore *et al*., 2012). Since EC depends on number of aromatic compound which have unusual stability as compared to other geometric or connective arrangements of the same set of atoms. As a result of their stability, it is very difficult to cause aromatic molecules to break apart and to react with other substances (Hofmann and Stoffel, 1993). This implies that they are resistance to mutation from generation to generation that is why drugs and vaccines targeted towards the control of this disease must be alter by post translation modification so that drugs and vaccine for control of this disease will have more effect on the disease causing organism. The Half life is the time it takes for half of the amount of protein in a cell to disappear after its synthesis in the cell of the proteins. The result showed 30 hours for all the CBPP protein**.** This indicates that the in this study is 30 hours. Half life plays an important role in determining its stability in vivo of the protein (Bachmair *et al*., 1986). The instability index provides an estimate of the stability of protein in a test tube. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable (Guruprasad *et al.,* 1990), the result from this study shown that all the CBPP proteins instability index value is <40. Which mean that there are stable and the reason for the stability is that, there are certain dipeptides, the occurrence of which is significantly difference in the unstable proteins

compared with those proteins that are stable? This implies that they do not react with other compounds and play a role in substrate/recognition (a point where mutation have less or no effect) which mean they are resistance to mutation. The aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). The AI>100 indicates thermal stability which mean the protein having AI>100 will withstand wide range of temperature while protein having AI<100 are not thermally stable which cannot withstand wide range of temperature (Munduganore *et al*., 2012). Amino acid permease, ATP synthases subunit alpha and ABC transporter-ATP binding proteins have AI>100 while the rest of the protein CBPP protein have AI<100. AI is regarded as a positive factor for the increase of thermal stability of globular proteins (Ikai, 1980). This may be the reason why this deadly disease of cattle prominent in tropical countries. The GRAVY of the protein in this study revealed that amino acid permease and phosphonate ABC transporter permerase proteins have positive value which mean that they are hydrophobic (not soluble in water) and have they surface rich with negatively charged amino acid like glutamate and aspartate amino acids. All the eight remaining proteins have negative value which mean they are hydrophilic (soluble in water) and have their surfaces rich with positively charged amino acids like lysine and arginine. The proteins that are bind to positively charge DNA they may regulate gene expression or to fold the DNA (Urry, 2004). In particular, hydrophobic amino acids can be involved in binding/recognition (point where mutation have few or no effect) of hydrophobic ligands (compounds) such as lipids (Betts *et al*., 2003). The CBPP proteins used for this study have similar amino acid composition and the higher percentages in leucine, isoleucine (are aliphatic amino acid) and lysine (basic amino acid). The aliphatic amino acids (isoleucine, and leucine), may be regarded as a positive factor for the increase of thermal stability of globular proteins (Ikai, 1980). All the CBPP proteins have zero selenocystein and pyrrolysine which is interpret as stop codons (protein cannot conclusively determine the identity of a residue) (Suchanek *et al*., 2005). This implies that because of the higher percentage composition of the aliphatic amino acid are thermally stable and the basic amino acid imply they can accept electron.

CONCLUSION

The physico-chemical properties, amino acid composition, and secondary structure of CBPP proteins indicated physical, chemical and thermal stability of the protein molecules. These indicated that the proteins are resistant to mutation and can withstand wide range of temperature. The genetic information revealed in this study will help in carrying out research in mutagenesis and pharmaco genetic so as to increase cattle production and also to eliminating trans boundary trade bearier in developing countries.

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