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GENETIC DIVERSITY OF LACTOFERRIN GENE IN-SILICO ON SELECTED MAMMALIAN SPECIES

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Abstract: A total of 17 lactoferrin gene sequences belonging to 6 species: cattle (3), buffalo (3), sheep (3), goat (3), horse (2) and camel (3), were retrieved from Genbank (www.ncbi.nlm.nih.gov). Sequences alignment, translation and comparison were done with ClustalW of the MEGA 6.0. The present study therefore aimed at examining the genetic diversity of Lf gene *in-silico* on selected mammalian species. The Dxy inferred using p-distance revealed a maximum value of 0.50 between buffalo, sheep and goat whereas a minimum value of 0.01 was realized between sheep and goat. The maximum Dxy value of 0.15 were observed between horse and camel whereas no minimum value was recorded during the investigation. The Neighbour Joining tree from the phylogenetic analysis showed trans-species evolution however, UPGMA tree topology was species-wise. This genetic tree obtained advance some form of proximity and differentiation in Lactoferrin gene sequences within and among the mammalian species studied which provide basis for selection of livestock in terms of genetic relationship.

Keywords: Diversity, In-silico, Lactoferrin, Mammalian, Sequence, Phylogenetic

Introduction

Lactoferrin (LF) is a single-chain, iron-binding glycoprotein of 80 kDa that belongs to the serum transferrin gene family called the red protein. LF is present in milk but also in other exocrine secretions such as tears, semen, saliva, and cervical mucus (*Wakabayashi et al., 2006*). The protein is synthesized by granulocytes and mammary epithelial cells in response to infections such as mastitis (*Kaminski et al., 2006*). Lactoferrin is able to sequester 2 molecules of iron making them unavailable to pathogenic organisms. In addition to this bacteriostatic activity, LF is endowed with antifungal and bactericidal effects (*Wakabayashi et al., 2006*).

Indeed, LF can interact with the lipid A of LPS contained in bacterial membrane. Lactoferrin can affect the destabilization of gram-negative bacterial membranes by preventing LPS from interacting with the main actors of LPS signaling such as CD14 (*Baveye et al., 1999*). Further, LF modulates the inflammatory process, immune system response, and cell growth. This multifunctional protein plays a key role in the health of mammary gland. Thus, it could be considered as a potential candidate gene in dairy mastitis resistance selection (*Seyfert et al., 1996; Wojdak-Maksymiec et al., 2006).*

Protein molecule of lactoferrin contains two lobes, both built of two globular domains (*Moore et al. 1997)*. There is a galore of lactoferrin biological functions and among them a special attention is being paid to its antibacterial (*Małaczewska and Rotkiewicz, 2007; Gonzalez-Chavez et al., 2009*), antiviral (*Rotkiewicz, 2007; Gonzalez-Chavez et al., 2009*), antitumor (*Małaczewska and Rotkiewicz, 2007; Gonzalez-Chavez et al., 2009*) and immunomodulatory properties (*Wakabayashi et al., 2006; Małaczewska and Rotkiewicz, 2007; Gonzalez-Chavez et al. 2009*).

A direct antimicrobial activity of lactoferrin affecting the bacterial cell wall, occurs due to two antimicrobial peptides of an N-terminal part of amino acid chain of this protein, called lactoferricin and lactoferrampin. These peptides are released from native protein by pepsin-mediated digestion (*Kraan et al., 2004; Exposito and Recio, 2006*). Lactoferrampin derived from bovine lactoferrin is bactericidal, where as this human peptide is probably inactive under normal conditions (*Haney et al., 2009*).

In human milk, there is 1 to 5 mg of lactoferrin /ml (*Teng, 2002*), contrary to bovine milk, where this protein concentration reaches maximum level of 0.1 mg/ml (*Schanbacher et al., 1993; Molenaar et al.,1996*). A dramatic increase in lactoferrin has been noticed in colostrum, mammary gland secretion during involution (*Schanbacher et al., 1993*) and in milk obtained from females suffering from a mammary gland inflammation (*Hagiwara et al., 2003; Malinowski et al. 2008*). Milk from quarters, in which *mastitis* pathogens are observed, contains more lactoferrin than that obtainable from uninfected ones, and protein concentration is to some extent pathogen-specific (*Chaneton et al., 2008*).

A lactoferrin gene has developed during evolutional mutations in a transferrin gene. There is 60-65% identity of nucleotide sequences between these two genes (*Baker and Baker, 2005*).The present study therefore aimed at examining the genetic diversity of Lf gene *in-silico* on selected mammalian species.

Materials and methods

Sequence sources

A total of seventeen (17) Lf sequences from six species: Cattle (3), Buffalo (3), Sheep (3), Goat (3), Horse (2) and Camel (3) were retrieved from the GenBank (www.ncbi.nlm.nih.gov). The GenBank accession number of these cattle, buffalo, sheep, goat, horse and camel, sequences were: AH010864.2, AB046664.1, L19981.1 (Cattle), EU669579.1, KC415279.1, HG515533.1 (Buffalo), AF091651.1, NM_001009769.1, LQ223516.1 (Sheep), DI012102.1, GQ149766.1, FM8875929.1 (Goat), AJ010930.1, NM_001163974.1 (Horse), KF915308.1, NM_001303567.1, AJ131674.1 (Camel).

Sequence alignment, translation and comparison

Sequence alignments, translations and comparisons were done using ClustalW as described by (*Larkin et al.,* 2007).

Phylogenetic analysis

Neighbor-Joining trees were constructed each using P-distance model and pair wise deletion gap/missing data treatment. The construction was on the basis of genetic distances, depicting phylogenetic relationships among the lactoferrin nucleotide sequences of the investigated species. The reliability of the trees was calculated by bootstrap confidence values (*Felsenstein, 1985*), with 1000 bootstrap iterations using MEGA 6.0 software (*Tamura et al., 2013*).

UPGMA tree construction

Unweighted pair group method using arithmetic average (UPGMA) trees for each gene was constructed with consensus sequences (a sequence from each species based on similarity was selected for the UPGMA); using same model as that of the tree. All sequences were trimmed to equal length corresponding to same region before generating the tree.

Results

Table 1. **Lactoferrin sequence variation within and among selected mammalian species**

Species	Number of sequences	Sequence length variation (bp)
Cattle	3	1784, 2127, 2357
Buffalo	3	281, 822, 1505
Sheep	3	299, 468, 2127
Goat	3	2326, 2327, 2356
Horse	2	2231, 2270
Camel	3	2250, 2304, 2337

bp means base pair

The length of the Lf gene varied from 281- 2357 within and across species. Cattle and Sheep have similar coding region of 2127 base pair as presented in table 1.

Table 2. Evolutionary divergence between species (Dxy) per site

	Cattle	Buffalo	Sheep	Goat	Horse	Camel
Cattle		0.01	0.00	0.00	0.01	0.01
Buffalo	0.49		0.01	0.01	0.01	0.01
Sheep	0.06	0.50		0.00	0.01	0.01
Goat	0.05	0.50	0.01		0.01	0.01
Horse	0.21	0.54	0.20	0.20		0.01
Camel	0.18	0.54	0.17	0.17	0.15	

Standard error estimate is presented at the upper diagonal while average genetic distances between species is presented at the lower diagonal.

Estimates of Evolutionary Divergence (Dxy) between Sequences is presented in table 2. The number of base differences per site from between sequences are shown. The analysis involved 6 nucleotide sequences.

Estimated distance matrix for lactoferrin gene between consensus sequences of 6 mammalian species selected is as shown. In ruminants, a maximum Dxy of 0.50 between buffalo, sheep and goat and minimum Dxy value of 0.01 was realized between sheep and goat. Amongst non-ruminants (pseudo- ruminant), maximum Dxy value of 0.15 was seen between horse and camel respectively. Generally a maximum value of 0.54 Dxy was realized between buffalo, horse and camel respectively.

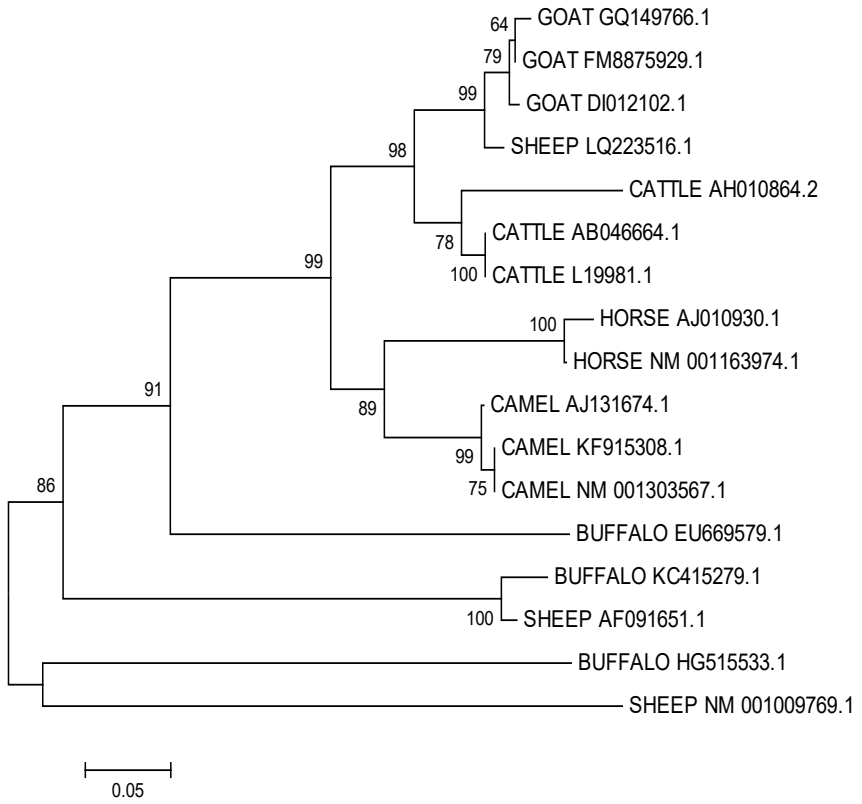

Fig 1.0 Phylogenetic tree construction from the p-distance option of neighbour joining tree.

Neigbhour Joining tree, showed that the sequence of ruminants and non-ruminants were separated from each other due to their respective genetic distance (Dxy) value. The larger the Dxy value the more the distance across the mammalian species selected.

This variation of the genetic distance across species could clearly be explained based on the UPGMA tree deduced from the consensus sequence of the neighbour Joining tree.

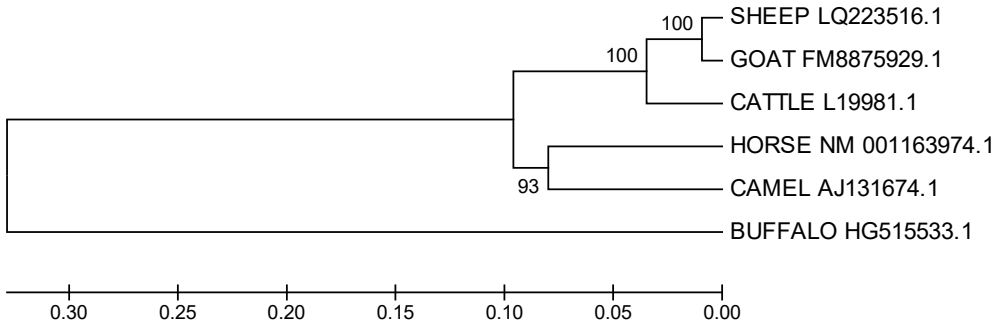

Fig 2. Consensus sequence of the UPGMA tree derived from the neighbour joining tree.

Evolutionary history inferred using UPGMA, revealed that all the goat sequences (FM8875929.1) were closer to sheep (LQ223516.1) sequences compared to those of cattle sequence (L19981.1) (Figure 2). Horse sequences (NM001163974.1) were also closer to those of camel sequence (AJ131674.1). Also this current result revealed that the sequence of buffalo (HG515533.1) tends to be closer to sequence of camel and horse respectively than the ruminant especially the cattle, this variation could be explained based on their genetic distance value from each other from evolution.

Discussion

Dxy is the index of DNA divergence between or among the sequences. The larger the Dxy is, the smaller the genetic distance is (*Kang et al., 2008*).

Lactoferrin is an iron-binding glycoprotein and is considered a major part of the non-specific disease resistance complex in the mammary gland (*Hiss et al., 2008*). The length variation of the LF gene within and among species might result from evolution and differentiation. Many length variations caused by insertions and deletions resulting in amino acid variation within species have been found by comparison with known sequences. This kind of mutation may be related to antibacterial activity or other functions, and needs to be investigated further (*Kang et al., 2008*).

The neighbour-joining tree clearly revealed that clustering was largely species-wise. The presence of numerous alleles at a particular Lf locus is evidence of the long-term evolutionary persistence of the locus (*Yakubu et al., 2013*). This is suggested by the fact that the alleles in one species are often more closely related to the alleles in closely related species than to the other alleles in the same species. The species wise clustering might be due to species specific residues (*Takahashi and Nei, 2000*) and such patterns of the sequences may be explained by gene conversion and balancing selection.

This study may also provide a useful marker for selection of highly expressing animals. By identifying more efficient lactoferrin promoters, a potential exists to identify better sires faster, thereby accelerating the improvement of livestock through breeding (*Daly et al., 2006*).

The genetic relationships of the Lf gene in sheep and goat showed by the phylogenetic tree were in accordance with the speciation of these species in the evolution history as reported in similar work by (*Yakubu et al., 2014*). The same is applicable to the association between horse and camel. The close relationship between horse and camel was also in accordance with the results of *Yakubu et al. (2014),* Yang *et al.* (2004) and Tang *et al.* (2006), which showed that the comparability of cDNA sequences was highest between the pig and the camel by alignment of the full-length sequences of gene caBD21 cDNA of camel, pig, cattle, and sheep. The analysis of molecular evolution could help us to understand Lf antibacterial mechanism from the view of evolution pressure.

Conclusion

There was a great genetic variation in the aligned sequences of Lf gene within and across species whereas the genetic tree obtained advance some form of proximity and differentiation in Lactoferrin gene sequences within and among the mammalian species studied.

Genetička različitost gena laktoferina in-silico na izabranim vrstama sisara

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Rezime

Ukupno 17 laktoferin genetskih sekvenci koje su pripadale 6 vrsta: goveda (3), bivoli (3), ovce (3), koze (3), konji (2) i kamile (3), su preuzete iz banke gena (www.ncbi.nlm.nih.gov).Ređanje sekvenci, translacija i upoređivanje sekvenci je urađeno korišćenjem ClustalW - MEGA 6.0. Stoga, ovo istraživanje je usmereno na ispitivanje genetičke raznovrsnosti Lf gena *in-silico* na odabranim vrstama sisara. Dxy zaključen korišćenjem p-distance pokazao je maksimalnu vrednost od 0,50 kod bivola, ovaca i koza, dok je između ovaca i koza ostvarena minimalna vrednost od 0,01. Maksimalna Dxy vrednost 0,15 je primećena između konja i kamile, dok tokom istraživanja nije zabeležena minimalna vrijednost. Međutim, filogenetsko stablo u okviru filogenetske analize je pokazalo evoluciju između vrsta međutim, UPGMA topologija stabla bila je specifična za vrstu. Ovo genetsko stablo unapređuje neki oblik blizine i diferencijacije u sekvencama gena lactoferina unutar i između proučavanih vrsta sisara koje pružaju osnovu za selekciju stoke u smislu genetskog odnosa.

Ključne reči: Različitost, in-silico, lactoferin, sisar, sekvence, filogenetski

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