



## Expression of different isoforms of Cathelicidin-4 transcripts in river Buffalo mRNA

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### ABSTRACT

**Objective:** Mature cathelicidin peptides show inhibitory activity against Gram-positive and Gram-negative bacterial species, certain fungi, parasites or enveloped viruses. The function of cathelicidins is not limited to antimicrobial killing, but extends to other aspects of immunity and tissue repair. In the present study RNAs from different tissues of buffalo were extracted, reverse transcribed and amplified using specific pair of primer designed from published cathelicidin-4 cDNA sequences of *Bos taurus*.

**Methodology and results:** An amplified product of 247 bp was obtained in cDNA of lung whereas an amplified product of 349 bp was obtained in cDNA of mammary gland, ovary, colon, testis, intestine and trachea tissues. No reaction was found in tongue, muscle, liver and blood. The amplified fragments obtained from lung and trachea cDNA were chosen for sequencing. NCBI alignment revealed that an amplified product of buffalo lung does not have intron I sequence or other copy of the forward primer. The cDNA of buffalo trachea includes intron I sequence and possess a copy of the forward primer. The results indicate that native buffalo expressed different cathelicidin-4 transcripts (spliced and unspliced forms) which may be used to produce different functional proteins.

**Conclusion and application of findings:** Native buffalo could express cathelicidin-4 gene more efficiently than Egyptian native and Frisian crossbred cattle. Native; Frisian crossbred cattle and buffalo retained intron -1 (unspliced form) in their CATH4 mRNA and thus they could express cathelicidin-4 gene more efficiently than *Bos taurus* or *Bubalus bubalis* reared abroad. This cathelicidin-4 may be a good candidate antibiotic for preventing disease outbreaks in domestic livestock and a starting point for the development of novel synthetic antimicrobial agents for use on these animals.

**Key words:** antimicrobial peptides, Cathelicidin-4, buffalo, spliced and unspliced forms

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### INTRODUCTION

The antimicrobial peptides of innate immunity are a front-line, host-defence response to microbial invasion (Ganz, 2002). Mammalian antimicrobial peptides fall within one of two major groups: the cysteine-rich  $\alpha$  and  $\beta$  defensins (Martin *et al.*, 1995; White *et al.*, 1995), and the heterogeneous group of the cathelicidin-derived peptides (Zanetti *et al.*, 1995). Cathelicidin-4, also known as indolicidin,

was first discovered in the cytoplasmic granules of bovine neutrophils (Falla *et al.*, 1996).

Mature cathelicidin peptides generally exhibit broad-spectrum antimicrobial activity against a range of Gram-positive and Gram-negative bacterial species (Zanetti *et al.*, 2002). Cathelicidins also show inhibitory activity against certain fungi (e.g. *Candida albicans*), parasites (e.g. *Cryptosporidium parvum*) or enveloped viruses (e.g.

human immunodeficiency virus) (Nizeti & Gallo, 2003). Bacterial killing by cathelicidins is rapid and the mechanism of killing in most cases is thought to involve intercolation and assembly of the peptides within the bacterial membrane to disrupt membrane integrity (Gutsmann *et al.*, 2001). The function of cathelicidins is not limited to antimicrobial killing, but extends to other aspects of immunity and tissue repair (Gallo *et al.*, 2002). Their broad and rapid microbicidal effect on release may be critically important to prevent tissues from pathogen attacks and thus prevent the onset of infection (Dorschner *et al.*, 2001).

In mammals, these peptides are released by epithelial cells at the interface with the external environment. Cathelicidin is present in the mouth, tongue, esophagus, epithelia, and in the epithelial cells, submucosal glands of the airway, and lung surface fluid (Bals *et al.*, 1998). Cathelicidin is also expressed in the genitourinary tract (vagina, epididymis, and seminal plasma), where it can function as a host defense barrier (Malm *et al.*, 2000). In addition, cathelicidin is found in secretory granules of neutrophils and it is also expressed in skin keratinocytes during inflammatory disorders (Frohm *et al.*, 1997).

Thirty cathelicidin members have been found in mammals (Niyonsaba *et al.*, 2005). Human and mice each possess a single cathelicidin, whereas other species such as cattle and pigs express many different cathelicidins (Ramanathan *et al.*, 2002). Cathelicidin genes consist of 4 exons

#### MATERIAL AND METHODS

Different tissue samples were obtained from healthy native buffalo at the slaughter house located at El Moneib, Giza, Egypt. Total RNAs were isolated from the buffalo's frozen tissues (lung, mammary gland, ovary, colon, testis, tongue, muscle, intestine, trachea, liver and blood) using Trifast reagent (Peqlab, Biotechnologie GmbH) according to the manufacturer's instructions (Grubor *et al.*, 2004).

Cathelicidin-4 cDNAs were synthesized using the total RNAs obtained from the different tissues with the Ready-To-Go You Prime First- strand Beads (Amersham Biosciences) according to the instructions of the manufacturer. The first-strand cDNA synthesis was done on 2 µg of total RNA using reverse transcriptase (FPLC pure) and the oligo (dT) primer, pd (T) 12-18 (Amersham Biosciences).

and 3 introns: the first 3 exons comprise N-terminal signal peptide, a highly conserved prosequence and cathelin prodomain, while the fourth exon encodes the processing site and variable C-terminal antimicrobial peptide (Zanetti *et al.*, 2000).

In higher eukaryotes, the majority of genes produce nascent mRNA transcripts that contain several introns. Although cells appear to have developed checkpoints to ensure that only fully spliced mRNAs are exported and expressed (Chang & Sharp, 1989; Legrain & Rosbash, 1989), export of incompletely spliced RNAs with retained introns would potentially result in translation of aberrant proteins. Hwang and Cohen (1997) revealed the possible correlation between over-expression and failure to remove the intron.

The distribution of bovine and ovine sequences in a phylogenetic tree indicates that most Cathelicidins sequences do not cluster monophyletically within Bovidae, i.e. these sequences are more closely related between than within species, suggesting the existence of common ancestor genes before divergence of bovids from other Cetartiodactyla (Tomasinsig & Zanetti, 2005). Knowledge of the multiple functions and structures of cathelicidin may not only result in an understanding of the mechanism of action of this antimicrobial peptide family, but also provide a new lead in the design of potent antimicrobial peptides with therapeutic application (Hsu *et al.*, 2005).

To amplify the cathelicidin cDNAs, each PCR was primed with two gene-specific primers designed from the bovine Cathelicidin-4 gene (gi|31341226|ref|NM\_174827.2|) published on database. The sequence of the primers was determined using the software Primer 3 at <http://www.genome.wi.mit.edu> (Marone *et al.*, 2001). PCR primers were selected on the basis that the 5' and 3' ends span the two exons, so that the amplification product obtained from the cDNA would be of different length from that obtained from any contaminant genomic DNA comprising intronic sequences. The primers were synthesized by Amersham Pharmacia Biotech.

The primers used in these amplifications were: a sense primer synthesized at the 5'-end of the gene (5'- GTGGTCGCTGTGGCTACTGCTGC-3') and an antisense primer synthesized at the 3'-end of the gene (5'- CTCCGCGGGCTGCTGAATCG -3'). Amplification



reactions (100 µl) contained 5 µl of first-strand buffalo cDNAs, 0.2 mM dNTPs, 10 mM Tris, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01% galatin (w/v), 1.25 units Taq polymerase and 1 µM upper and lower primers. Cycling parameters were set as follows: one cycle of preheating at 94°C for 3 min and 30 cycles of denaturation at 94 °C each for 1 min, annealing at 63°C for 2 min, and extension at 72°C for 2 min. A final post-extension cycle was performed at 72°C for 10 min (Babiker *et al.*, 2002). PCR products were resolved on a 1.5% agarose gel. No

PCR products were detected in the absence of reverse transcriptase, which indicated the lack of contaminating genomic DNA.

The PCR products were purified and sequenced at the Center of Genetic Engineering, Ain-Shams University, and Cairo, Egypt. Sequence analysis and alignment were carried out using the Expert Protein Analysis System (CLUSTAL-W) server of the Swiss Institute of Bioinformatics (Gasteiger, 2003) and NCBI-BLASTN 2.2.13 version (Altschul *et al.*, 1997).

## RESULTS

An amplified product of 247 bp was obtained in cDNA of lung whereas an amplified product of 349 bp was obtained in cDNA of mammary gland, ovary, colon, testis, intestine and trachea tissues. No reaction was found in tongue, muscle, liver and blood (Fig. 1). The amplified fragments obtained from lung (247 bp) and trachea (349 bp) cDNA were chosen for sequencing. A one way sequence of cathelicidin-4 amplified segments of lung and trachea cDNA buffalo are presented in Figures 2 and 3, respectively.

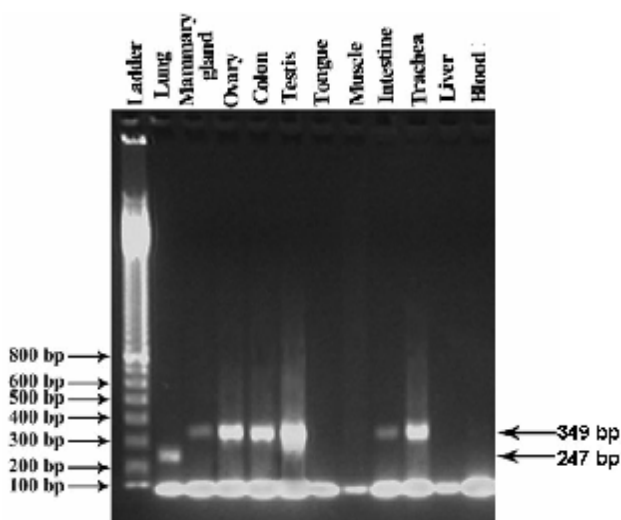


Figure 1: Stained gel of amplified PCR products of Cathelicidin 4 gene using cDNA constructed from mRNA of different tissues of buffalo. Arrows on the right indicate the amplified fragment sizes.

NCBI-Blast analysis of the amplified sequence (247 bp) of native buffalo Lung CATHL-4 cDNA (from 176 to 210 bp) showed 94% alignment with *Bos taurus* CATHL-4 mRNA (gi|31341226|ref|NM\_174827.2] and gi|462|emb|X67340.1|BTINDLCD] [from 83 to 49 bp]. Native buffalo lung CATHL-4 cDNA (from 148 to 203 bp) showed 85% alignment with *Bubalus bubalis* myeloid cathelicidin mRNA gi|111399450|gb|DQ832666.1| (from 66 to 11 bp).

When native buffalo Lung CATHL-4 cDNA (from 85 to 210 bp) was aligned with Egyptian native cattle trachea CATHL-4 cDNA (AB 294198, AB 294375, gi: 126149290) from 175 to 301 bp, Frisian crossbred cattle trachea CATHL-4 cDNA (AB 294209, gi: 126149291) from 190-316 bp and *Bos taurus* chromosome 22 (gi: 76649266) from 8390 to 8264 bp (exon I extend from 8425 to 8216bp), a 77% alignment was reported. Whereas the alignment of the amino acid translated sequence of buffalo lung CATHL-4 cDNA showed 61 - 67% identities with both amino acid translated sequence of *Bos taurus* CATHL-4 mRNA (gi: 31341226 ref |NM174827.2]; gi: 462| emb X67340.1] and 58%-65% identities with *Bubalus bubalis* gi: 51950347| emb AJ 812216.11] (data not shown).

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GAATTGTTTTTATAATGATGGCGCAGTAGCACACCGACTACAATTGTGATTCTCTTCTTGTAGGGGTGA
GAAAGATAGAGCGGAGAGATTAGTTTCTGTGTGACGTTTCATTGTTGTGATCCACTGCAGATGCACGAAC
TCCTTGTTGCTGATGTCCTGGTGGCTGGTGGAGGAGACCACTAGTTCAGCAGCAGTAGCCACACCGA
CCA
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Figure 2: Sequence of the downstream strand of cathelicidin-4 amplified fragment of native buffalo lung cDNA. The forward primer for CATH-4 are underlined and indicated in bold typeface.

On the other hand, NCBI Blast analysis of the second amplified sequence (349 bp) of native buffalo CATHL-4 trachea cDNA revealed no significant alignment with both published *Bos taurus* and *Bubalus bubalis* CATHL-4 mRNA. However CLUSTAL-W analysis revealed that the whole sequence of native buffalo trachea CATHL-4 cDNA had identities of 65% and 66% with published Egyptian native cattle trachea CATHL-4 cDNA (AB 294198, AB 294375, gi: 126149290) and Frisian crossbred cattle trachea CATHL-4 cDNA (AB 294209, gi: 126149291) respectively. This alignment showed that native buffalo CATHL-4 trachea cDNA possesses a copy of the forward primer from 22 to 40bp (the forward

primer and its duplicate were assessed by naked eye, they include some mutations) corresponding to the copy of the forward primer which was found in Egyptian native cattle (AB 294198, AB 294375, gi: 126149290) from 17-37bp and in Frisian crossbred cattle (AB 294209, gi: 126149291) from 16-35 bp. These duplicates exist at the 5' end of exon II. The alignment also revealed that native buffalo CATHL-4 trachea cDNA includes intron I sequence ranging from 55-150bp which corresponds to the intronic segment in Egyptian native cattle (AB 294198, AB 294375, gi: 126149290) from 47 to 137bp and in Frisian crossbred cattle (AB 294209, gi: 126149291) from 55-152 bp (Fig. 4).

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GGAGTTCTGGTTTGCATGACAGACAGTAGCCCAGCGACACAAGTGCAGATTTCTCTGGTGAGGATTAATC
AAGGAGGAAAGGGGTTAGGGTTTTTTCTTTTTTGTGTTTGLATCCTTGCCCTTAACTGGGGCGGTTCCCTT
CCCCAATTCAATTCTTTGGGGGGGGGGGTTAGTTCGGGGAGGGGTAAAATTTAATTTTTTTGAGGCCCG
TTCATTGAGTTGTTCCACGGAAGGAGGAAGGGTTTCCTTTTAGTTGAGGTCTGGGGGCTTGCGAAGGA
AACCAATGGCCCCGGAAGAATTAGCACCGGCCCA
    
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Figure 3: Sequence of the downstream strand of cathelicidin-4 amplified fragment of native buffalo trachea cDNA. The forward primer for CATH-4 are underlined and indicated in bold typeface and its duplicate are underlined and indicated in bold typeface and italic (the 2 sequences have some mutations) and intron-1 indicated in bold typeface and italic.

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## DISCUSSION

Bovine cathelicidin-4 gene spans approximately 1262 base pairs in genomic DNA, the coding sequence is distributed into 4 exons ranging from 56 (exon 4) to 198 (exon 1) base pairs. Intron sizes range from 102 (intron 1) to 586 (intron 3) base pairs [<http://www.hgsc.bcm.tmc.edu/prijects/bovine>].

The primer pair of bovine cathelicidin-4 was designed to amplify a segment of 247bp, from 48 to 294bp of *Bos taurus* CATHL-4 mRNA (gi|31341226|ref|NM\_174827.2|). This primer pair also amplifies a segment of 349bp (from 37 to 358bp) of *Bos taurus* CATHL-4 genomic DNA located on BTA 22 (gi: 76649266) which covers exon I and exon II and intron I (102bp, from 199-301bp) [<http://www.hgsc.bcm.tmc.edu/prijects/bovine>]. In the present study these primers amplified two different segments in native buffalo at the level of mRNA: An amplified product of 247 bp was obtained in lung cDNA only and an amplified product of 349 bp was obtained in cDNA of trachea, mammary gland, ovary, colon, testis and intestine tissues. The amplified sequence of native buffalo CATHL-4 lung cDNA (247bp) showed 94% and 85% alignment with a segment of *Bos taurus* CATHL-4 mRNA (gi: 31341226 ref |NM174827.2|) and a larger segment of *Bubalus bubalis* myeloid cathelicidin mRNA (gi: 51950347| emb AJ 812216.11|), respectively.

Moreover, it showed 77% alignment with three segments: a segment of Egyptian native cattle from 175 to 301 bp (AB 294198, AB 294375, gi: 126149290); Frisian crossbred cattle from 190-316 bp (AB 294209, gi: 126149291) and of CATHL-4 genomic DNA on BTA22 from 8390 to 8264 bp (gi: 76649266). All the three segments do not include intron I sequence.

These alignments indicated that buffalo lung cDNA have a spliced form of CATHL-4 mRNA. This result agrees with the result of Das *et al.* (2006) who reported that comparison of buffalo cathelicidin with known *Bos taurus* cathelicidin show 70.8-92.9% identity at nucleotides level. On the other hand, the alignment of the amino acid translated sequence CATHL-4 cDNA of lung buffalo showed 58 - 67% identities with both amino acid translated sequence CATHL-4 mRNA of *Bos taurus* (gi: 31341226 ref |NM174827.2|) and *Bubalus bubalis* (gi: 51950347| emb AJ 812216.11|). Das *et al.* (2006) have also reported that buffalo cathelicidin and *Bos taurus* cathelicidin show 65-88.3% identity at amino acids level, leading to the conclusion that buffalo possess the ancestral gene of cathelicidin similar to bovine species.

CLUSTAL-W analysis showed that native buffalo CATHL-4 trachea cDNA (349bp) possess a copy of the forward primer from 22 to 40 bp





corresponding to the copy of the forward primer found in CATHL-4 trachea cDNA of Egyptian native cattle (AB 294198, AB 294375, gi: 126149290) and Frisian crossbred cattle (AB 294209, gi: 126149291) (Abou Mossallam *et al.*, 2007). These duplicates existing at the 5' end of exon II may be the cause of intron retention, as a form of alternative splicing. It was observed that mutation of a 5' splice site depressed the removal of the intron-1 (Talerico & Berget, 1990). The alignment showed that native buffalo CATHL-4 trachea cDNA includes intron I sequence ranging from 55-150bp which corresponds to the intronic segment in Egyptian native

cattle CATHL-4 trachea cDNA (AB 294198, AB 294375, gi: 126149290) from 47 to 137bp and in Frisian crossbred cattle CATHL-4 trachea cDNA (AB 294209, gi: 126149291) from 55-152 bp (Abou Mossallam *et al.*, 2007). This means that native buffalo possesses unspliced form (intron retention) of CATHL-4 in trachea mRNA. Intron retention, as a form of alternative splicing, has been observed to occur in a number of different genes although constitutive splicing of intronic sequences from RNA is the dominant form of gene expression.

### Scores Table

	SeqA	Len(nt)	SeqB	Len(nt)	Score
	CatF	316	CatN	303	89
	CatF	316	BufT	322	66
	CatN	303	BufT	322	65

CatF	<u>CGGAGGGTCTCGGGC</u> <b><u>GCAGT</u></b> <u>AGCCACAGCGACCAC</u> GAAGTGCCAGATC--TTCATTCTG	57
CatN	-----ATTCTTTCGTGATCA <b><u>GCACAGT</u></b> <u>AGC-ACAGCGACCACA</u> ATC--AGTTTITG	50
BufT	--GGAGTTCTGGTTTGCATGAC <b><u>AGACAGT</u></b> <u>AGC-CCAGCGACACA</u> AGTGCAGATTTCTCTG	57
	* * * **** * * *	* **
CatF	GTGACGGATTGATAGAGGGGGGACTGGGTTCAAGGCTCATTTTTCCCTTCTGATTGT--C	115
CatN	G-----GATATGTAAGAGA-TGGGTTCAAGGCTCATTTTTCCCTTCTGATTITTTTC	100
BufT	<u>GTGA-GGATTAATCAAGGAGGAAAGGGTTAGGGT</u> ---TTTTTCTTTTTTTGTTTTGTA	113
	* ** * * **** ** ***** ** * * * * *	
CatF	TCCCTGCCCTCACCTAGACAGTCCCTCCCAACTCACATCCTTGGGAGGTGGGTCTAG	175
CatN	TCCCTGCCCTCACCTAGACAGTCCCTCCCAACTCACATCCTTGGGAGGTGGGTCTAG	160
BufT	TCCTTGCCCTTAACITGGGCGGTTCTTCCCAATTC AATTC TTTGGGGGGGGTTTAG	173
	*** ***** * ** * * * * * ***** ** * * * * * * * *	
CatF	CTCCAGGAGGCGGTAGAGATTAGCTTCT--GAGGACAGCTCATTGAGCTGATCCACAGCA	233
CatN	CTCCAGGAGGCGGTAGAGATTAGCTTCT--GAGGACAGCTCATTGAGCTGATCCACAGCA	218
BufT	TTCCGGGAGGGTAAAAATTTAATTTTTTTGAGGCCCGTTTATTGAGTTGTTCCACGGAA	233
	*** ***** *	
CatF	CGAAGCACGGCCCTCCTGTAGCTGAGGGCTTGGGCGCTGGCCGAGGGCACCACTAGTCCC	293
CatN	CGAAGCACGGCCCTCCTGTAGCTGAGGGCTTGGGCGCTGGCCGAGGGCACCACTAGTCCC	278
BufT	GGAGGAAGGTTTCCTTTAGTTGAGGCTTGGGGCTTGGCGAAGGAAACCAATGGCCCC	293
	** *	
CatF	<b><u>AGCAGCAGTAGCCACAGCGACCA</u></b> ----- 316	
CatN	<b><u>GGCAGCAGTAGCCACAGCGACCA</u></b> ---- 303	
BufT	<b><u>GGAAGATTAGCACCGGCGCCAAAAAATT</u></b> 322	
	* *	

Figure (4): CLUSTAL W (1.83) multiple sequence alignment of CATH4 Trachea cDNA of native Buffalo (BufT) ; Egyptian native cattle (CatN; AB 294198, AB 294375, gi: 126149290) and Frisian crossbred cattle (CatF; AB 294209, gi: 126149291). The forward primers for CATH-4 are underlined and indicated in bold typeface whereas their duplicates are underlined and indicated in bold typeface and italic and intron-1 sequences are underlined.

Examples of intron retention have been found in bovine growth hormone, in the transcripts encoding fibronectin, platelet-derived growth factor A chain, and during the developmental control of *Drosophila* genes (Kienzle *et al.*, 1999). CLUSTAL-W alignment of the whole sequence of native buffalo CATHL-4 trachea

cDNA (349bp) showed identities of 65 and 66% with Egyptian native (AB 294198, AB 294375, gi: 126149290) and Frisian crossbred cattle (AB 294209, gi: 126149291), respectively.

These results are in line with those of Nurtdinov *et al.* (2003) showing that to account for lower conservation of alternatively spliced regions (cassette exons, retained introns, or exon extensions generated by alternative splicing sites), the threshold for accepting alignments of such regions was 60% identity, whereas for constitutive exons it was 70%. Although in their case the quality of obtained alignments was assessed by eye, in no case was any ambiguity encountered.

The results indicated that an amplified product of 247 bp which was obtained in cDNA of buffalo lung did not retain intron I sequence (spliced form) or other copy of the forward primer, whereas an amplified product of 349 bp which was obtained in cDNA of buffalo trachea retained intron I sequence (unspliced form) as well as a copy of the forward primer. The expression of different isoforms (spliced and unspliced) in buffalo agreed with the result of Modrek and Lee (2003) who revealed that alternative splicing allows for generation of novel proteins without sacrificing old ones. If a new isoform proves to be beneficial, its fraction increases by subtle regulatory changes. On the other hand, unlike gene duplication, alternative splicing does not lead to dramatic changes in protein concentrations (Ermakova, 2006). The presence of intron-1 retention isoforms in different tissues of buffalo (trachea, mammary gland, ovary, colon, testis and intestine), which could not be detected in the lung is similar to the observations of Mansilla *et al.* (2005) who reported that the embryonic proinsulin mRNA isoform (intron 1 retention in the 5' untranslated region) was tissue specific in embryonic chick and mouse.

The results revealed that CATH-4 mRNA of native buffalo and cattle as well as Frisian crossbred cattle retain intron-1 whereas *Bos taurus* and *Bubalus bubalis* CATH-4 mRNA did not retain this intron. To

explain similar findings, Ermakova (2006) reported that alternative isoforms are often genome-specific. There is a large number of gene expression studies, both in tissue culture cells and in transgenic animals, which show that intron-containing genes are expressed more efficiently than intron less ones (Li & Garoff, 1998).

In conclusion, the results of this study indicate that native buffalo showing two isoforms of CATH4 (247 bp and 349 bp) express different cathelicidin-4 transcripts (spliced and unspliced forms) which may be used to produce different functional proteins. Thus native buffalo could express cathelicidin-4 gene more efficiently than Egyptian native and Frisian crossbred cattle which have one isoform. These Egyptian species retaining intron -1 (unspliced form) in their CATH4 mRNA could express cathelicidin-4 gene more efficiently than *Bos taurus* or *Bubalus bubalis* reared abroad which have spliced forms. Knowledge of the multiple functions and structures of cathelicidin provide a new lead in the design of potent antimicrobial peptides with therapeutic application. This cathelicidin may be a good candidate antibiotic for preventing disease outbreaks in domestic livestock and a starting point for the development of novel synthetic antimicrobial agents for these animals.

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## REFERENCES

- Abou Mossallam AA, Mahfouz ER, Babars MA, El Nahas SM, 2007. Retention of Cathelicidin-4 Intron-1 in mRNA of Egyptian Cattle. *J Apple Sci Res* 3(11): 1400-1406.
- Altschul SF, Stephen F, Maden TL, Shaffer AA, Jinghui Z, Zhang Z, Miller W, Lipman DJ, 1997. Gapped BLAST and PSI-BLAST a new generation of protein database search programs. *Nucleic Acids Res* 25: 3389-3402.
- Babiker MA, Abdel-Banat, Koga D, 2002. Alternative Splicing of the Primary Transcript Generates Heterogeneity within the Products of the Gene for *Bombyx mori* Chitinase. *J Biol Chem* 277(34): 30524-30534.
- Bals R, Wang X, Zasloff M, Wilson JM, Wilson JM, 1998. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc Natl Acad Sci USA* 95: 9541-9546.
- Chang DD, Sharp PA, 1989. Regulation by HIV Rev depends upon recognition of splice sites. *Cell* 59: 789-795.
- Das H, Sharma B, Kumar A 2006. Cloning and characterization of novel cathelicidin cDNA sequence of *Bubalus bubalis* homologous to *Bos taurus* cathelicidin-4. *DNA Seq* 17 (6): 407-414.
- Dorschner RA, Pestonjamas VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B, Gudmundsson GH, Gallo R L, 2001. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active

- against group A Streptococcus. *J Investig Dermatol* 117: 91-97.
- Ermakova E, Nurtdinov R N, Gelfand MS, 2006. Fast rate of evolution in alternatively spliced coding regions of mammalian genes. *BMC Genomics* 7: 84.
- Falla TJ, Karunaratne DN, Hancock REW, 1996. Mode of action of the antimicrobial peptide indolicidin. *J Biol Chem* 271: 19298–19303.
- Frohman M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, Gudmundsson GH, 1997. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem* 272: 15258–15263.
- Gallo RL, Murakami M, Ohtake T, Zaiou M, 2002. Biology and clinical relevance of naturally occurring antimicrobial peptides. *J Allergy Clin Immunol* 110: 823-831.
- Ganz T, 2002. Antimicrobial polypeptides in host defense of the respiratory tract. *J Clin Invest* 109: 693-697.
- Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A, 2003. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res* 31:3784-3788.
- Grubor B, Gallup JM, Meyerholz DK, Crouch EC, Evans RB, Brogden KA, Lehmkühl HD, Ackermann MR, 2004. Enhanced surfactant protein and defensin mRNA level and reduced viral replication during parainfluenza virus Type 3 pneumonia in Neonatal lamb. *Clin Diagn Lab Immunol* 11: 599-607.
- Gutsmann T, Hagge S O, Larrick J W, Seydel U, Wiese A, 2001. Interaction of CAP18-derived peptides with membranes made from endotoxins or phospholipids. *Biophys J*. 80: 2935-2945.
- Hsu Ch-H, Chen Ch, Jou M-L, Lee A Y-L, Lin Y-Ch, Yu Y-P, Huang W-T, Wu Sh-H, 2005. Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: evidence for multiple conformations involved in binding to membranes and DNA. *Nucleic Acids Research* 33(13): 4053-4064.
- Hwang DY, Cohen JB, 1997. A splicing enhancer in the 3'-terminal c-H-ras exon influences mRNA abundance and transforming activity. *J Virol* 71: 6416-6426.
- Li, Ke-Jun, Garoff H, 1998. Packaging of intron-containing genes into retrovirus vectors by alphavirus vectors. *Cell Biology* 95 (7): 3650-3654.
- Kienzle N, Young DB, Liaskou D, Buck M, Greco S, Sculley TB, 1999. Intron Retention May Regulate Expression of Epstein - Barr virus Nuclear Antigen 3 Family Genes. *Journal of Virology* 73 (2): 1195-1204.
- Legrain P, Rosbash M, 1989. Some cis- and trans-acting mutants for splicing target pre-mRNA to the cytoplasm. *Cell* 57: 573-583.
- Malm J, Sørensen OE, Persson T, Frohm-Nilsson M, Johansson B, Bjartell A, Lilja H, Ståhle-Bäckdahl M, Borregaard N, Egesten A, 2000. The human cationic antimicrobial protein (hCAP-18) is expressed in the epithelium of human epididymis, is present in seminal plasma at high concentrations, and is attached to spermatozoa. *Infect Immun* 68: 4297–4302.
- Mansilla A, López-Sánchez C, de la Rosa EJ, García-Martínez V, Martínez-Salas E, de Pablo F, Hernández-Sánchez C, 2005. Developmental regulation of a proinsulin messenger RNA generated by intron retention. *EMBO reports* 6 (12): 1182–1187.
- Marone M, Mozzetti S, De Ritis D, Pierelli I, Scambia G, 2005. Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. *Biol Proceed Online* 3 (1): 19-25.
- Martin E, Ganz T, Lehrer RI, 1995. Defensins and other endogenous peptide antibiotics of vertebrates. *J Leukocyte Biol* 58: 128–136.
- Modrek B and Lee CJ, 2003. Alternative splicing in the human, mouse and rat genomes is associated with an increased frequency of exon creation and/or loss. *Nat Genet.* 34: 177–180.
- Niyonsaba F, Ushio H, Nagaoka I, Okumura K, Ogawa H, 2005. The Human beta -Defensins (-1, -2, -3, -4) and Cathelicidin LL-37 Induce IL-18 Secretion through p38 and ERK MAPK Activation in Primary Human Keratinocytes. *Journal of Immunology* 175: 1776-1784.
- Nizeti V and Gallo RL, 2003. Cathelicidins and Innate Defense Against Invasive Bacterial Infection. *Scand. J Infect Dis* 35: 670-676.
- Nurtdinov RN, Artamonova II, Mironov A A, Gelfand MS, 2003. Low conservation of alternative splicing patterns in the human and mouse genomes. *Human Molecular Genetics* 12 (11): 1313-1320.
- Ramanathan B, Davis EG, Ross CR, Blecha F, 2002. Cathelicidins: microbicidal activity, mechanisms of action, and roles in innate immunity. *Microbes Infect* 4: 361-372.

- Singh J, Nanda A, Adams G, 2000. The reproductive pattern and efficiency of female buffaloes. *Animal Reproduction Science* 60-61: 593-604.
- Talerico M, Berget SM, 1990. Effect of 5' splice site mutations on splicing of the preceding intron. *Mol Cell Biol* 10:6299-63050.
- Tomasinsig L, Zanetti M, 2005. The Cathelicidins – Structure, Function and Evolution. *Current Protein and Peptide Science* 6: 23-34.
- White SH, Wimley WC, Selsted ME, 1995. Structure, function, and membrane integration of defensins. *Curr Opin Struct Bio* 5: 521–527.
- Zanetti M, Gennaro R, Scocchi M, Skerlavaj B, 1995. Structure and biology of cathelicidins. *Adv. Exp Med Biol* 479: 203-218.
- Zanetti M, Gennaro R, Skerlavaj B, Tomasinsig L, Circo R, 2002. Cathelicidin peptides as candidates for a novel class of antimicrobials. *Curr Pharm Des* 8: 779-793.
- Zanetti M, Gennaro R, Romeo D, 1995. Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. *FEBS Lett* 374: 1-5.

