Journal of Applied Sciences Research, 3(11): 1400-1406, 2007

Retention of Intron-1 in Cathelicidin-4 mRNA of Egyptian Native and Frisian Crossbred Cattle

Ahlam A. Abou Mossallam, Eman R. Mahfouz, Mona A. Bibars and Soheir M. El Nahas

Department of Cell Biology, National Research Centre, Cairo, Egypt.

Abstract: The antimicrobial peptide gene cathelicidin-4 (CATHL-4) was investigated in native and Frisian crossbred cattle reared in Egypt. The gene was tested for reaction with cDNA of various cattle tissues such as blood; lung; trachea; intestine; muscle; lymph and liver using PCR. A positive PCR product was seen in blood, lung, trachea, liver and lymph tissues, but not in intestine of both native and crossbred cattle. NCBI-Blast analysis of native and crossbred cattle CATHL-4 cDNA sequences (303 and 316bp, respectively) with *Bos taurus* CATHL-4 mRNA (gi: 31341226) and BTA22 (gi: 76649266) were almost the same, they both showed high sequence identity. Analysis of PCR products of CATHL-4 cDNA revealed the retention of intron-1 in both native and crossbred cattle, which is absent in *Bos taurus* CATHL-4 mRNA (gi: 31341226). This may have resulted from alternative splicing which increases the genetic diversity of the genome without increasing the overall number of genes. It may play a role in increasing their innate immunity by increasing gene expression, since intron-containing genes are expressed more efficiently than intronless ones. The results indicate that both native and crossbred cattle reared in Egypt may express cathelicidin gene more efficiently than *Bos taurus or Bubalus bubalis* reared abroad.

Keywords: Antimicrobial peptides- Cathelicidin-4 –Egyptian cattle- Intron retention

INTRODUCTION

Antimicrobial peptides are natural antibiotics that function as a first line of defense in host innate immunity. They are gene-coded with potent and broad antimicrobial capabilities. Cathelicidins and Defensins are the two major families of mammalian anti-microbial peptides. In addition to their antimicrobial functions they provide important physiological role by providing connecting signals between innate and adaptive immunity[1,49,50]. Defensins and cathelicidins have been shown recently to have chemotactic effects on host cells[7]. A single cathelicidin gene is present in humans, mice, rat and other mammals (although exhaustive investigations, or the complete sequencing of their genomes, may reveal further family components in some of these species) however other species such as pigs[58], cows and sheep[40,26] and horses[39] have 11, 7 and 3 member of the cathelicidin family, respectively and possibly more gene family members. The corresponding genes are usually arranged in clusters[59]. Species that have several cathelicidins such as pigs and cow have duplicated the cathelicidin gene to produce several unique C-terminal antimicrobial peptides with structures that vary from α-helical to β-sheet to rich in single amino acids[53,31,15]. Although the benefit of the multiple cathelicidin genes is not well established, it was reported that inhibition of antimicrobial peptides increases bacterial growth in pigs and mice[8,47] Also mice deficient in cathelicidin-related 48 antimicrobial peptide (mCRAMP) develop significantly larger group of A Streptococcus (GAS) skin lesions, compared with their wild-type littermates[36].

Cathelicidins are synthesized in the bone marrow as prepropeptides, stored in neutrophil granules as propeptides, and released as active, mature peptides upon neutrophil degranulation[25] where they contribute to oxygen-independent mechanisms of antimicrobial activity[19,9]. The cathelicidins of mammalian origin are synthesized in precursor form, requiring proteolytic processing to release the mature C-terminal antimicrobial peptide[30,59] that have broad-spectrum 54 antimicrobial activities[20,30,44,54]. They are expressed in myeloid and epithelial cells, the latter indicates their role in host defense at body surfaces[14,20,21,3,13].

Antimicrobial peptides that belong to the Cathelicidin gene family have been found to be important to the mammalian immune response. They are reported to be expressed in several tissues and increased in neonatal skin, possibly to compensate for an immature adaptive immune response. Cathelicidin secreted in mammary gland and human milk can contribute to the anti-infectious properties of milk[34]). The mechanism by which the peptides kill bacteria is by thinning and disrupting the bacterial membrane[37].

Corresponding Author: Ahlam A. Abou Mossallam, Department of Cell Biology, National Research Centre, Cairo, Egypt.

In the search for new classes of antibiotics, there has been interest in naturally occurring antimicrobial peptides. Bacterial resistance to conventional antibiotics has resulted in escalating numbers of life-threatening infections[12,47,38,17,22]. However these native host defense moleculeshave maintained broad-spectrum antimicrobial activity[57,52], which make them an attractive alternatives to current antibiotic regimens in selected disease situations[29].

MATERIALS AND METHODS

RNA Isolation and First-strand cDNA Synthesis: Tissue samples including blood; lung; trachea; intestine; mammary gland; colon and liver, from native and crossbred cattle, were subjected to RNA extraction according to Grubor *et al.,*[18]. No PCR products were detected in the absence of reverse transcriptase, which indicated the lack of contaminating genomic DNA.

RT- PCR Ready-to-go kit (Amersham Biosciences) was used for cDNA synthesis according to manufacture instructions.

Primer Design: Primers specific for the antimicrobial peptide gene cathelicidin-4 were designed using known cDNA sequences of *Bos taurus*, published in database with different accession numbers. The sequence of the forward and reverse primers was determined using the software Primer 3[33], which is available free online at http://www.genome.wi.mit.edu. PCR primers were selected on the basis that the 5` and 3' ends span exons I and II. The primers were synthesized by Amersham Pharmacia Biotech.

Polymerase Chain Reaction (PCR): Amplification reactions (100 µl) contained 5 µl of first-strand cattle cDNAs, 0.2 mM dNTPs, 10 mM Tris, 50 mM KCl, 1.5 mM MgCl2, 0.01% galatin (W/V), 1.25 units Taq polymerase and 1 µM upper and lower primers. The reaction mixture was overlaid with sterile mineral oil. PCR was performed using MJ research PTC-100 thermocycler using 1 cycle (3 min.) at 94°C, followed by 30 cycles for (1 min. at 94°C, 2 min. at 63°C, and 2 min. at 72°C) and finally 1 cycle (10 min.) at 72°C. The reaction products were electrophoresed on 1.5% agarose in 1X- Tris acetate buffer (TAE) containing 0.8 µl of 10 mg/ml ethidium bromide. Primer sequences, annealing temperature, product size and accession number, are shown in Table 1.

Sequence Analysis: The PCR products were purified and sequenced at the Center of Genetic Engineering; Ain Shams University; Cairo; Egypt. Sequence analysis and alignment were carried out using NCBI-BLASTN 2.2.14 version[2] and CLUSTAL W analysis[16].

RESULTS AND DISCUSSIONS

Results: Egyptian native and crossbred Frisian cattle reared in Egypt were investigated. In an attempt to identify and analyze the presence of CATHL-4 antimicrobial peptide in native and crossbred cattle in Egypt. Total RNA was extracted from samples of different tissues (blood; lung; trachea; intestine; mammary gland; colon and liver) from which cDNAs were prepared. No PCR products were detected in the absence of reverse transcriptase, which indicated the lack of contaminating genomic DNA. The presence or absence of CATHL-4 gene in cDNA of different tissues was investigated. CATHL-4 PCR product gave a single sharp band of 349 bp in both native and crossbred cattle cDNA. Positive PCR products were seen in blood, lung, trachea, liver and lymph tissues, but not in intestine of both native and crossbred cattle (Fig 1 and 2, respectively).

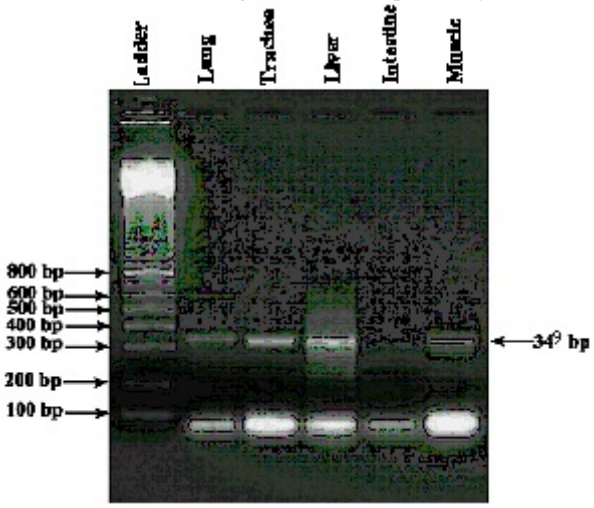

Fig. 1: Representative ethidium bromide-stained gel of amplified PCR products of Cathelicidin- 4 using cDNA extracted from different tissues of native cattle, L-100 ladder and the right arrow indicate the amplified fragments size.

A one way sequence of CATHL-4 PCR amplified segments of both native and crossbred cattle trachea are presented in figures 3 and 4, respectively. These segments include the forward primer since only one way sequence was performed. The nucleotide sequence data were submitted to nucleotide sequences database DDBJ/ EMBL/ GenBank with the accession numbers AB 294198, AB 294375 (gi: 126149290) for native and AB 294209 (gi: 126149291) for crossbred cattle.

NCBI-Blast analysis of the CATHL-4 amplicons showed a 98% alignment between native (from 65 to 301bp) and crossbred cattle (from 82 to 316 bp).

Table 1: DNA sequence of the primer tested

Name	Sequence	Accession no.	Ann. Temp.	Size (bp)
Cathelicidin-4 (CATHL-4)	GTGGTCGCTGTGGCTACTG CTGCCTCCGCGGGCTGCTGAATCG	31341226	63	247

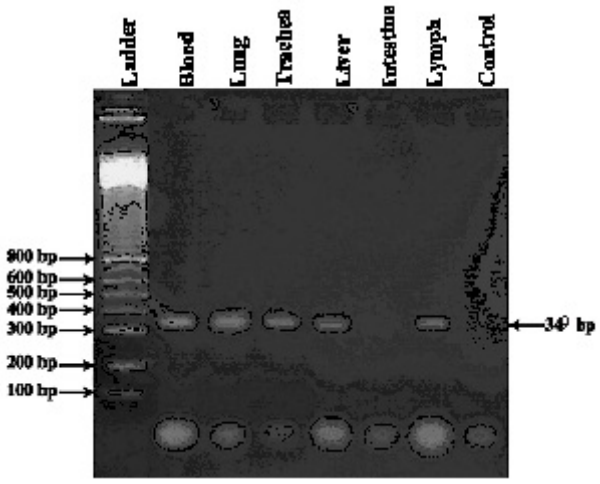

Fig. 2: Representative ethidium bromide-stained gel of amplified PCR products of Cathelicidin- 4 using cDNA extracted from different tissues of Frisian crossbred cattle, L-100 ladder and the right arrow indicate the amplified fragments size.

However using CLUSTAL W[16] analysis the (whole sequence of both cattle cDNA showed an 89% homology. Blast analysis of CATHL-4 cDNA sequences for native and crossbred cattle revealed high sequence identity with *Bos taurus* CATHL-4 mRNA (gi: 31341226 and gi|462|emb|X67340.1) and bovine chromosome 22 (BTA 22) genomic DNA sequence (gi: 76649266) to which CATHL-4 is assigned[6]. The latter segment included exon I and II and intron-I.

Native cattle CATHL-4 cDNA sequence (303bp) showed 99% homology with *Bos taurus* CATHL-4 mRNA, covering a segment from 140 to 302bp in native cattle and from210 to 48 bp in *Bos taurus* CATHL-4 mRNA (gi: 31341226), which includes the forward primer (from 67-48 bp in *Bos taurus* and from 283-302 bp in native cattle). In addition, a 90% alignment from 67 to 48bp of *Bos taurus* (forward primer) and from19 to 39bp in native cattle was also revealed. This indicates that the native cattle amplicon sequence possesses a second copy of the forward primer (Fig 3). The duplication exists at the 5' end of exon II. The native cattle cDNA amplicon also showed a 97% homology (from 65 to 302bp) with BTA22 (gi: 76649266) (from272 to 36bp) (Fig 3). The genomic DNA (BTA22) segment includes exon I and II and intron- I.

Results of Blast analyses of crossbred cattle CATHL-4 cDNA sequence (316bp) with *Bos taurus* CATHL-4 mRNA (gi: 31341226) and BTA22 (gi: 76649266) were almost the same as those found in native cattle. A 100% homology between crossbred cattle (from 155 to 316bp) and *Bos taurus* CATHL-4 mRNA (from 210 to 49 bp) was found, in addition to the presence of a duplicate of the forward primer in crossbred cattle from16-35bp at the 5' end of exon II (Fig 4). Also a 96% homology with BTA22 (Fig 4 76649266) was revealed. Alignment extended from 42 to 316bp in crossbred cattle and from 314 to 37bp in BTA 22 genomic DNA that includes intron-I.

Blast analysis of native and crossbred cattle CATHL-4 cDNA amplicons sequences revealed 93% and 94% sequence identity with buffalo *Bubalus bubalis* CATHL-4 mRNA (gi:51950347). The aligned segments were in native cattle from 139 to 302bp in crossbred from154 to 316bp and in *Bubalus bubalis* from 204 to 42bp. *Bubalus bubalis* CATHL-4 mRNA (gi: 51950347) is 517bp, it showed 93% sequence identity with *Bos taurus* CATH-4 mRNA gi: 31341226 ;524 bp) covering the whole sequence of *Bubalus bubalis* and from 8 to 524bp of *Bos taurus.*

It is worth mentioning that alignment between sequences of native and crossbred cattle CATHL-4 and native river buffalo CATHL-4 cDNA PCR-products which was also found to retain intron-I (unpublished data) revealed a reasonable homology.

Discussion: Cathelicidin is present in cattle as several members[40]. However, it is present in human and other mammals such as mice and rat as a single gene. The presence of species-related abundance and combinations of distinct family members is a characteristic feature of host defense peptide families and has been proposed to be associated to differential selective pressures resulting from exposure to differing microbial pathogens or biota[55].

Primer pair specific for cathelicidin-4 reacted positively with cDNA of different tissues of native and crossbred cattle and gave a band at 349 bp. Some of these tissues are epithelial. The expression of antimicrobial peptides at epithelial surfaces plays a role in host defense at body surfaces[[14,21,20,3,13]]. Cathelicidins present in the oral cavity, in the salivary system, in some oral epithelia, and in saliva, contribute to broad-spectrum defense of the oral cavity[35].

Homology between native and crossbred cattle CATHL-4 cDNA sequences and that of *Bos taurus* CATHL-4 mRNA (gi: 31341226) (from 48-210bp) were100% and 98%, respectively. Sequence analysis of

ATTTCTTTCGTGATCA**GCACAGTAGCACAGCGACCAC**AATCAGTTTT***TTGGGATATGTAA***
GAGATGGGTTCAGGCTCATTTTTCCTTCTCTGATTTTTTCTCCCTGCCCCTCACCTAGACAGT
CCCCTCCCCAACTCACATCCTTGGGAGGTGGGTCTAGCTCCAGGAGGCGGTAGAGATTAGC
TTCTGAGGACAGCTCATTGAGCTGATCCACAGCACGAAGCACGGCCTCCCTGTAGCTGAG
GGCTTGGGCGCTGGCCGAGGGCACCACTAGTCCCG**GCAGCAGTAGCCACAGCGACCAC**
A

Fig. 3: Sequence of the downstream strand of Cathelicidin-4 amplified fragment of native cattle cDNA. The forward primer is in bold typeface, its duplication is underlined and retained Intron-I is in italic bold typeface.

CGGAGGGTCTCGGGC**GCAGTAGCCACAGCGACCAC**GAAGTGCCAGATCTTCATT***CTGGT***
GACGGATTGATAGAGGGGGGACTGGGTTCAGGCTCATTTTTCCTTCTCTGATTGTCTCCCTG
CCCCTCACCTAGACAGTCCCCTCCCCAACTCACATCCTTGGGAGGTGGGTCTAGCTCCAGG
AGGCGGTAGAGATTAGCTTCTGAGGACAGCTCATTGAGCTGATCCACAGCACGAAGCACG
GCCTCCCTGTAGCTGAGGGCTTGGGCGCTGGCCGAGGGCACCACTAGTCCCA**GCAGCAGT**
AGCCACAGCGACCA

Fig. 4: Sequence of the downstream strand of Cathelicidin-4 amplified fragment of crossbred cattle cDNA. The forward primer is in bold typeface, its duplication is underlined and retained Intron-I is in italic bold typeface.

native and crossbred cattle CATHL-4 cDNA amplicons revealed the presence of a duplication of the forward primer, which resides at the 5' end of exon II with a single base deletion in native cattle.

The Cathelicidin genes are approximately 2kb in size and show a conserved IV exons / III introns organization[40]. Exon I to exon III code for the signal peptide and the cathelin domain[43]. We are here concerned with the bovine cathelicidin-4 gene which is located on bovine *Bos taurus* chromosome 22 (BTA22) (gi: 76649266), 1262bp[. It contains IV exons (198; 108; 72 and 56bp) with III introns (102; 136 and 586bp) (http://www.hgsc.bcm.tmc.edu/projects/bovine.). The primer pair of bovine CATHL-4 used in this investigation was designed from exon I and exon II. They are designed to amplify a segment of 247bp, from 48 to294bp of *Bos taurus* CATHL-4 mRNA (gi: 31341226). However in the present study these primers amplified a larger than expected segment (349bp) in both native and crossbred cattle cDNA, in all tissues tested. 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/projects/bovine.). BTA 22 (gi: 76649266) showed a sequence identity of 97% (65-302bp) and 96% (42-316bp) with native and crossbred cattle CATHL-4 cDNA sequences, respectively. The results thus indicate that both native and crossbred cattle CATHL-4 cDNAs retain intron-I in their CATHL-4 transcripts, which is absent in *Bos taurus* CATHL-4 mRNA (gi: 31341226).

Blast analysis of native and crossbred cattle CATHL-4 cDNA with native buffalo (member of the bovine) *Bubalus bubalis* CATHL-4 cDNA {which was also found to retain intron-I (unpublished data) }revealed a reasonable homology. The sequence homology between native and crossbred cattle includes exon I and II and the retained intron-I. It should be pointed out that *Bubalus bubalis* CATHL-4 mRNA (gi|51950347; 517bp) of the GenBank is intronless since it showed 93% sequence identity with *Bos taurus* CATHL-4 mRNA (gi:31341226; 524bp), covering the whole sequence of *Bubalus bubalis.*

The high sequence identity found between cattle and buffalo is not surprising since the cathelicidins sequences in *Bovidae* were found to be more closely related between than within species[43]. Also the 5' region of the bovine cathelicidin genes (to which cattle and buffalo belong) appears to be fairly well conserved not only at the exon but also at the intron levels[40].

Retention of intron-I in cDNAs encoding CATHL-4 in native and crossbred cattle may have resulted from alternative splicing[41]. Alternative splicing increases the genetic diversity of the genome without increasing the overall number of genes. By altering the pattern of exons, from a single primary transcript that are spliced together, different proteins can arise from the processed mRNA from a single gene[11]. At least 40% of genes in

both multicellular plants and animals are alternatively spliced[5], and possibly as many as 70% in the human genome[27]. Up to 60% of alternative splice variants are conserved between mouse and human[42], suggesting that many of these splice forms are necessary components of the genomic toolkit.

Alternative splicing may be one of the most extensively used mechanisms that accounts for the greater macromolecular and cellular complexity of higher eukaryotic organisms[4]. It occurs more often in transcripts from genes expressed within individual cell types that have undergone selection to provide diverse functions, such as in the immune system[51,46].

Recent global analyses of splicing shed more light on the role of splicing defects in human disease. A more detailed knowledge of the identity and distribution of splicing enhancer and silencer sequences in exons and introns provide valuable information for improving the ability to predict which disease mutations have the potential to disrupt splicing[45,56].

Intron retention observed in CATHL-4 cDNA native and crossbred cattle may play a role in increasing their innate immunity by increasing the gene expression. Intron-containing genes are expressed more efficiently than intronless ones. The presence of an intron-1 In the pre-mRNA appears to stimulate accumulation of mRNA in the cytoplasm through its interaction with the nuclear splicing machinery[28]. Addition of introns of a mouse TS gene to an intronless TS minigene in transgenic mice had significant effects on the level of expression of the minigene. Ten to 100 times more mRNA was produced from minigenes that contained introns than from those that lacked introns[10].

The results indicate that both native and crossbred cattle could express cathelicidin gene more efficiently than *Bos taurus or Bubalus bubalis* reared abroad.

REFERENCES

1. Agerberth, B., J. Charo, J. Werr, B. Olsson, F. Idali, L. Lindbom, R. Kiessling, H. Jomvall, H. Wigzell and G.H. Gudmundsson, 2000. The human anti-microbial and chemotactic peptides LL-37 and α-defensins are expressed by specific lymphocyte and monocyte populations. Blood, 96: 3086-3093.
2. Altschul, S.F., F. Stephen, T.L. Maden, A.A. Shaffer, Z. Jinghui, Z. Zhang, W. Miller and D.J. Lipman, 1997. Gapped BLAST and PSI-BLAST a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-3402.
3. Bals, R., X. Wang, M. Zasloff and J.M. Wilson 1998. The peptide antibiotic LL-37/ hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the air way surface. Proc. Natl. Acad. Sci. USA, 95: 9541-9546.
4. Blencowe, B.J., 2006. Alternative Splicing: New insights from global analyses. Cell, 126: 37- 47.
5. Brett, D., H. Pospisil, J. Valcarcel, J. Reich and P. Bork, 2002. Alternative splicing and genome complexity. Nat. Genet, 30: 29-30.
6. Castiglioni, B., M. Scocchi, M. Zanetti and L. Ferretti, 1996. Six antimicrobial peptide genes of the cathelicidin family map to bovine chromosome 22q24 by fluorescence in situ hypridization. Cytogenet. Cell Genet, 75(4): 240-242.
7. Chertov, D.O. and J.J. Oppenheim, 2001. Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). Journal of Leukocyte Biology, 69: 691-697.
8. Cole, A.M., J. Shi, A. Ceccarelli, Y.H. Kim, A. Park and T. Ganz, 2001. Inhibition of neutrophil elastase prevents cathelicidin activation and impairs clearance of bacteria from wounds. Blood, 97: 297-304.
9. Cowland, J., A. Johnsen and N. Borregaard, 1995. hCAP-18, a cathelin/pro-bacteneci-like protein of human neutrophil specific granules. FEBS Lett., 368: 173-176.
10. Deng, T., Y. Li and L.F. Johnson, 1988. Thymidylate synthase gene expression is stimulated by some (but not all) introns. Nucleic Acids Research, 2(17): 645- 658.
11. Dirksen, E.R., J.A. Felix and M.J. Sanderson, 1995. Preparation of explant and organ cultures and single cells from airway epithelium. Methods Cell Biol., 47: 65-74.
12. Fernandez, Guerrero M.L., J.M. Ramos, J. Marrero, M. Cuenca, R. Fernandez Roblas and M. de Gorgolas, 2003. Bacteremic pneumococcal infections in immunocompromised patients without AIDS: the impact of beta-lactam resistance on mortality. Int. J. Infect. Dis., 7: 46-52.
13. Frohm, M., B. Agerberth, G. Ahangari, M. Stahle-Backdahl, S. Liden, H. Wigzell and G.H. Gudmundsson, 1997. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammation disorders. J. Biol. Chem., 272: 15258-15263.
14. Gallo, R.L., K.J. Kim, M. Bernfield, C.A. Kozak, M. Zanetti, L. Merluzzi and R. Gennaro, 1997. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J. Biol. Chem., 272: 13088-13093.

15. Gallo, R.L., M. Murakami, T. Ohtake and M. Zaiou, 2002. Biology and clinical relevance of naturally occurring antimicrobial peptides. J. Allergy Clin. Immunol, 110: 823-831.
16. Gasteiger, E., A. Gattiker, C. Hoogland, I. Ivanyi, R.D. Appel, A. Bairoch, 2003. ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res., 31: 3784-3788.
17. Gonzalez, A., T. Bischoff, S. Tallent, G. Sheke, B. Ostrowsky, M.B. Edmond and R.P. Wenzel, 2003. Antibiotic resistance in the community. J. Hosp. Infect., 55: 156-157.
18. Grubor, B., J.M. Gallup, D.K Meyerholz, E.C. Crouch, R.B. Evans, K.A. Brogden, H.D. Lehmkuhl and M.R. Ackermann, 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.
19. Gudmundsson, G.H., B. Agerberth, J. Odeberg, T. Bergman, B. Olsson and R. Salcebo, 1996. The human gene FALL39 and processing of the cathelin precursor to the antimicrobial peptide LL-37 in granulocytes. Eur. J. Biochem, 238: 325-332
20. Hancock, R.E.W., 1997. Peptide antibiotics. Lancet, 349: 418-422.
21. Hancock, R.E.W. and R. Lehrer, 1998. Cationic peptides: a new source of antibiotics. Trends Biotechnol, 16: 82-88.
22. Healy, V.L., I.S. Park and C.T. Walsh, 1998. Studies on the Bacterial D-Ala-D-X-Ligase Family: Active Site Mutants of the Van C2 D-Ala-D-Ser Ligase Reverted Back Towards D-Ala-D-Ala Ligases Chem. Biol., 5: 197-207.
23. http://www.genome.wi.mit.edu.
24. http://www.hgsc.bcm.tmc.edu/projects/bovine. Baylor College of Medicine, Human Genome Sequencing Center as Btau 3.1, August 2006.
25. Hua, Y.J., Y. Tanaka, K. Nakamura, M. Sakakibara, S. Nagata and H. Kataoka, 1999. Identification of a prothoracicostatic peptide in the larval brain of the silkworm, Bombyx mori. J. Biol. Chem., 247: 31169-1173.
26. Huttner, K.M., M.R. Lambeth, H.R. Burkin, D.J. Burkin and T.E. Broad, 1998. Localization and genomic organization of sheep antimicrobial peptide genes. Gene, 206: 85-91.
27. Kampa, D., J. Cheng, P. Kapranov, M. Yamanaka, S. Brubaker, S. Cawley, J. Drenkow, A. Piccolboni, S. Bekiranov, G. Helt, H. Tammana and T.R. Gingeras, 2004. Novel RNAs identified from an in-depth analysis of the transcriptome of human chromosomes 21 and 22. Genome Res., 14: 331-342.
28. Kurachi, S., Y. Hitomi, M. Furukawa and K. Kurachi, 1995, Role of intron-I in expression of the human factor IX gene. J. Biol. Chem., 270: 5276– 5281.
29. Lee, P.H.A., T. Ohtake, M. Zaiou, M. Murakami, J.A. Rudisill, K.H. Lin and R.L. Gallo, 2005. Expression of an additional cathelicidin antimicrobial peptide protects against bacterial skin infection. PNAS, 102(10): 3750-3755.
30. Lehrer, R.I., A. Barton and T. Ganz, 1988. Concurrent assessment of inner and outer membrane permeabilization and bacteriolysis in *E. coli* by multiple-wavelength spectrophotometry. J. Immunol. Methods, 108: 153-158.
31. Lehrer, R.I. and T. Ganz, 2002. Cathelicidins: a family of endogenous antimicrobial peptides. Curr. Opin. Hematol, 9: 18-22.
32. Li, Ke-Jun and H. Garoff, 1998. Packaging of intron-containing genes into retrovirus vectors by alphavirus vectors. Cell Biology, 95(7): 3650-3654.
33. Marone, M., S. Mozzetti, D. De Ritis, I. Pierelli and G. Scambia, 2001. Semiqualitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. Biol. Proceed. Online, 3(1): 19-25.
34. Murakami, M., R.A. Dorshner, L.J. Stern, K.H. Lin and R.L. Gallo, 2005. Expression and Secretion of Cathelicidin Antimicrobial Peptides in Murine Mammary Glands and Human Milk. Pediatric Research, 57: 10-15.
35. Murakami, M., T. Ohtake, R.A. Dorschner and R.L. Gallo, 2002. Cathelicidin Antimicrobial Peptides are Expressed in Salivary Glands and Saliva. J. Dent. Res., 81(12): 845-850.
36. Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R.A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner and R.L. Gallo, 2001. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature, 414: 454-457.
37. Saiman, L., S. Tabibi, T.D. Starner, P.S. Gabriel, P.L. Winokur, H.P. Jia, P.B. Mc Cray Jr. and B.F. Tack, 2001. Cathelicidin Peptides Inhibit Multiply Antibiotic-Resistant Pathogens from Patients with Cystic Fibrosis. Antimicrobial Agents and Chemotherapy, 45(10): 2838-2844.
38. Schwenger, V., E. Mundlein, E.E. Dagrosa, A.M. Fahr, M. Zeier, G. Mikus and K. Andrassy, 2002. Treatment of life-threatening multiresistant staphylococcal and enterococcal infections in patients with end-stage renal failure with quinupristin/ dalfopristin: Preliminary report. Infection, 30: 257-261.

39. Scocchi, M., D. Bontempo, S. Boscolo, L. Tomasinsig, E. Giulotto and M. Zanetti, 1999. Novel cathelicidins in horse leukocytes. FEBS Lett. 457: 459-464.
40. Scocchi, M., S. Wang and M. Zanetti, 1997. Structural organization of the bovine cathelicidine gene family and identification of a novel member. FEBS Lett., 417: 311-315.
41. Sun, Q., R.K. Hampson and F.M. Rottman, 1993. In vitro analysis of bovine growth hormone pre-mRNA alternative splicing. Involvement of exon sequences and trans-acting factor(s). J. Biol. Chem,. 268: 15659-15666.
42. Thanaraj, T.A., F. Clark and J. Muilu, 2003. Conservation of human alternative splice events in mouse. Nucleic Acids Res., 31: 2544-2552.
43. Tomasinsig, L. and M. Zanetti, 2005. The cathelicidins-Structure, function and evolution. Current Protein and Peptide. Science, 6: 23-34.
44. Travis, S.M., N. Anderson, W.R. Forsyth, C. Espiritu, B.D. Conway, E.P.G. Greenberg, P.B. McCray Jr., R.I. Lehrer, M.J. Welsh and B.F. Tack, 2000. Bactericidal activity of mammalian cathelicidin peptides. Infect. Immun, 68: 2748-2755.
45. Wang, Z., M.E. Rolish, G. Yeo, V. Tung, M. Mawson and C.B. Burge, 2004. Systematic identification and analysis of exonic splicing silencers. Cell, 119: 831-845.
46. Watson F.L., R. Puttmann-Holgado, F. Thomas, D.L. Lamar, M. Hughes, M. Kondo, V.I. Rebel and D. Schmucker, 2005. Extensive diversity of Ig-superfamily proteins in the immune system of insects. Science, 309: 1874-1878.
47. Wilson B.A. and A.A. Salyers, 2002. Ecology and physiology of infectious bacteria--implications for biotechnology. Curr. Opin. Biotechnol. 13: 267-274.
48. Wilson C.L., A.J. Ouellette, D.P. Satchell, T. Ayabe, Y.S. López-Boado, J.L. Stratman, S.J. Hultgren, L.M. Matrisian and W.C. Parks, 1999. Regulation of intestinal a-defensin activation by the metalloproteinase matrilysin in innate host defense. Science, 286: 113-117.
49. Yang D., Q. Chen, O. Chertov and J.J. Oppenheim, 2000. Human neutrophil defensins selectively chemoattract naïve T and immature dendritic.
50. Yang D., O. Chertov, S.N. Bykovskaia, Q. Chen, M.J. Buffo, J. Shogan, M. Anderson, J.M. Schroder, J.M. Wang, O.M.Z. Howard and J.J. Oppenheim, 1999, ß-Defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science, 286: 525-528.
51. Yeo G., D. Holste, G. Kreiman and C.B. Burge, 2004. Variation in alternative splicing across human tissues. Genome Biol., 5: R74.
52. Zaiou M., and R.L. Gallo, 2002. Cathelicidins, essential gene-encoded mammalian antibiotics J. Mol. Med., 80: 549-561.
53. Zaiou M., V. Nizet, R.L. Gallo, 2003. Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. J. Invest. Dermatol, 120: 810-816.
54. Zannetti M., R. Gennaro and D. Romeo, 1995. Cathelicidins; a novel protein family with a common proregins and a variable C-terminal antimicrobial domain. FEBS Lett., 374: 1-5.
55. Zasloff M., 2002. Antimicrobial peptides of multicellular organisms. Nature, 415: 389-395.
56. Zhang X.H. and L.A. Chasin, 2004. Computational definition of sequence motifs governing constitutive exon splicing. Genes Dev., 18: 1241-1250.
57. Zhang G., C.R. Ross and F. Blecha, 2000. Porcine antimicrobial peptides: New prospects for ancient molecules of host defense. Vet. Res., 31: 277-96.
58. Zhao C., T. Ganz and R.I. Lehrer, 1995a. The structure of porcine protegrin genes. FEBS Lett. 368: 197-202.
59. Zhao C., T. Ganz and R.I. Lehrer, 1995b. Structures of genes for two cathelin-associated antimicrobial peptides: prophenin-2 and PR-39. FEBS Lett., 376: 130-134.