

Genomic Characterization of Bovine Beta-defensins

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ABSTRACT

Mastitis continues to be one of the most detrimental diseases to profitable dairy farming; it not only reduces animal health and productivity, but also poses threats to milk quality and safety. Consequently, it has received considerable attention with regard to the development of informative genetic markers that will allow identification of cows and sires more resistant to disease. The overall objective of this project is to develop a marker informative for mastitis susceptibility, and the project will test the hypothesis that polymorphisms in beta-defensin candidate genes are informative for mastitis. Beta-defensins represent a family of closely related genes whose products exhibit antimicrobial activity and have been associated with general immune function. Very little is known regarding the genomic sequence of bovine beta-defensins. As a preliminary experiment, primers were designed to specifically amplify regions of known bovine beta-defensin genes in a pool of bovine genomic DNA representing 12 Holstein cows by means of the polymerase chain reaction (PCR). The resultant amplicons were sequenced to verify identity and to identify single nucleotide polymorphisms (SNP). One 821bp product appears to contain a portion of the coding region of a novel beta-defensin. This amplicon *in silico* mapped to a GNOMON-predicted gene with 99% nucleotide identity; the only variants were 6 confirmed SNP within the amplicon. One of the SNP (position 290) results in an amino acid change of the predicted protein product (serine to phenylalanine). Primers were then designed to span the remaining portions of the predicted gene (including 3 exons and two introns). Work is underway to further characterize the genomic structure of the novel beta-defensin and to identify SNP within the gene. A panel of genomic DNA from cows of known susceptibility/resistance to mastitis will be used to test for informative associations between SNP and mastitis. The identification of an as-yet uncharacterized beta-defensin provides new information regarding the bovine genome; the SNP associated with this gene may prove invaluable for the development of a marker-assisted selection program to improve resistance against mastitis and potentially other diseases.

INTRODUCTION

Mastitis, an umbrella term for any inflammation of mammary tissue, often refers to a bacterial infection which costs the dairy industry two billions dollars each year in the US (Wall 2005). Beta-defensins are genes that code for peptides which have antimicrobial properties (Swanson 2004). There are many beta-defensins that are common to all bovines, and are likely the result of a duplication event (Luenser 2005); however, the genomic structure of these genes remains largely unknown. SNP found within beta-defensins which change the amino acid sequence of the peptide gene product may affect the function of the beta-defensin proteins. Because beta-defensins have been associated with the bovine immune reaction to mastitis (Goldammer 2004, Strandberg 2005), they can be investigated as candidate genes for the development of potential genetic markers for mastitis resistance and susceptibility. SNP found within beta-defensin genes can be evaluated for use as genetic markers, depending on factors such as allele frequency and the strength of any relationship to mastitis found.

OBJECTIVES

- Obtain sequence from regions of key beta-defensin genes
- Identify and characterize any SNP present

MATERIALS AND METHODS

DNA isolated from a pool of 96 Holstein cows was used as template for amplification by use of PCR (polymerase chain reactions), utilizing primers designed to amplify known bovine beta-defensins. It was anticipated that there would be non-specific amplification of related beta-defensin gene segments due to the number of highly related beta-defensin genes. Agarose gel electrophoresis was used to separate multiple amplicons produced. Isolated amplicons were purified from gel bands and sequenced, and Sequence analysis of pooled genomic DNA using Sequencher software allowed for rapid initial SNP

discovery. Sequences were compared to known bovine genomic sequence to identify the specific gene fragment amplified; subsequent sequencing of PCR products generated from genomic DNA of individual Holstein cows allowed for further characterization of SNP of interest.

RESULTS

A novel genomic sequence was generated through an attempt to amplify a member of the beta-defensin family. Sequencing revealed that the 821bp amplicon generated was not a known beta-defensin. A portion of the sequence strongly resembled the coding region of beta-defensins, but it did not share high homology through the noncoding regions; it did, however, share a 99% homology with an unmapped genomic contig sequence (Genbank NW_940027.1). Further analysis revealed that a portion of this sequence (144 consecutive bp) shared a 100% homology to a 474bp GNOMON-predicted cDNA sequence (hmm330156) within the genomic contig. All of the deviations from sequence identity to NW_940027.1 arise from these putative SNP sites. One SNP (position 290) creates an amino acid change in the predicted gene product which may alter function. All three possible genotypes for the pos290 SNP were readily detected. Analysis of 25 Holsteins revealed an allele frequency of .70 for the “G” allele; $2pq = .42$.

>novel defensin contig sequence 821bp SNPS at 71, 290, 365, 396, 402, 760 and 763

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TCTGTCATGGGACAAAATCAAATTTCTTGCTTTTATGAAAAAGAAAAAAGGAGAGTCACTTAACAGAGRCA
TATTCTCTTTCTCCTCATGAAACCAATGAGGAGACGGGCTCAGAAGCTTTGACTCGATAGAGTGGCTTAGA
AACACCAGGAATTTGTCAGGTCAGTTCTCAGCCCCCTCTCACTACTGAGGACTCAGCACTGGCTTCTCAGA
AGCATCAAATATGGTGAGTTTAGTCTGACCTCGTGTGGATGGGGGTAGCAGGACAGAAGGAGGTAGAAACAG
RAGAGAGAGCCCGCAGCTCCCCCGGGGCTCCACGAGCTCACCTGACCCAGCAGACAGGACCAGGAAGAGG
AGCGK GAGGAGCAGGTGATGGAGCCTCATGCTGGYGTCCCRAGCTCTATGGCTGACGCTGGAGAAGACGCTG
CACTTGCCACTTTATAAAGGTCCAGGTTCCAGGAGGAATTCGCTCTGGCTGTGGATTTGGTTATTAGGAA
CTGGTTAACTGAGCCTTTCCCTGTATGTCATGGGAGTGTCTTTATAGCATAAAGCAGGGGCACTCGTATG
CTAAGAGGCTGTGGAAAGGCCAGGAATAGCTTTCCTGCTGCCCTTTTGACCAGAAGCTTCTCCCTGGCAGTC
AGTTCAGCTGATGGGCAAGGGTGTGGCAAGGGGCACAGACGTGCCTGTTTTCCCTGGCAGGGGCACAGACTT
CTTGCTGCACCTTTGCTTACCCGCTCTCCAGCCCCACCSTCRCCCTTGAGGGCTTTGAATCTGCCCACTGT
TTTCTGTG CAGAAGGAAATGGTAACCCA
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R = A/G; K = G/T; Y = C/T; S = C/G

Figure 1. Sequence of putative beta-defensin amplicon. Primers are highlighted in yellow; SNP are highlighted in pink.

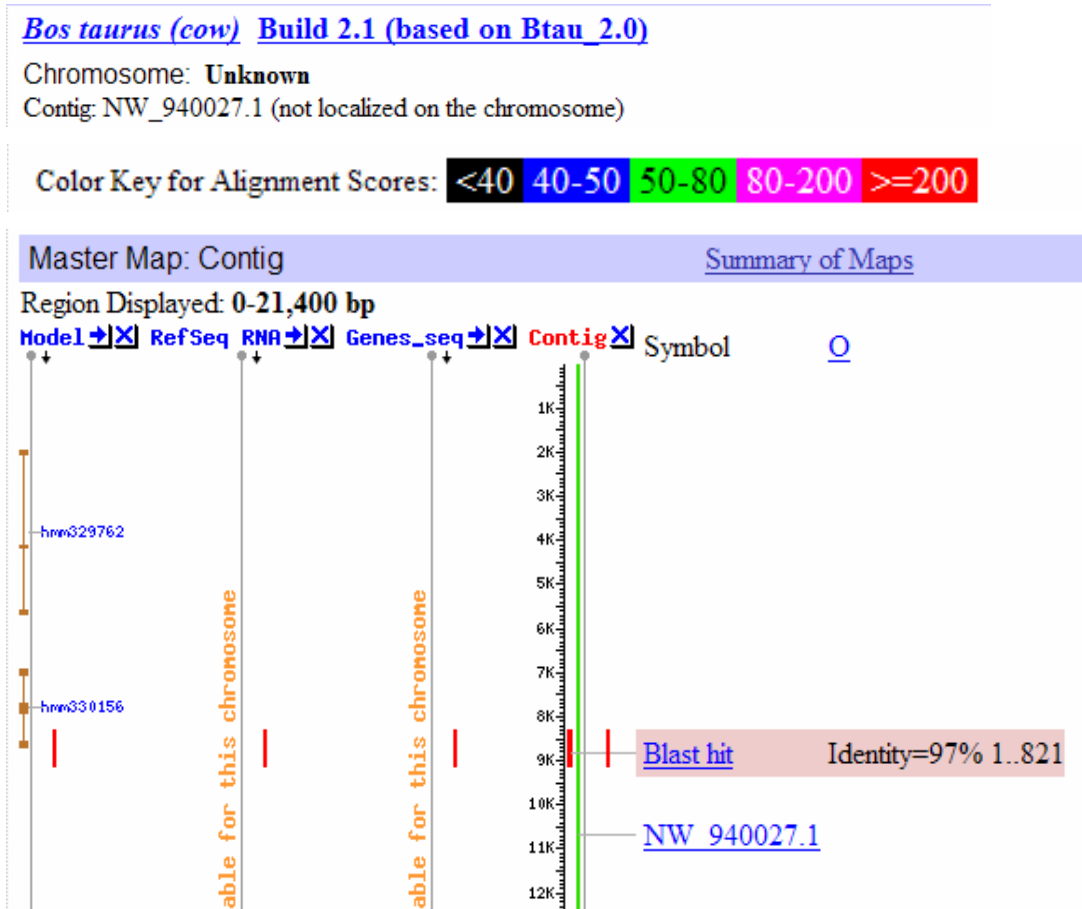


Figure 2. Localization of novel sequence to GNOMON-predicted gene (NCBI Map Viewer).

Gnomon model in Map Viewer: [hmm330156](#)

Gene predicted by Gnomon on *Bos taurus* Btau_2.0 genomic contig BtUn_WGA15424_2

CDS:

>lcl|hmm330156 157 amino acids

MRLHLLLLALLFLVLSAGSGELVGPAGGAGSL^SCFYLLLSYPHPHEHSGRERERGQCKEGLSVHGARVQSLDGEVERDQRCLVVK
RKKACEEDGKAPGQACERTGRPLVPWKHETDWHLFRAPSKMLQVVVKEGEDAAGTDAEIEIETAPFDKASKI

mRNA:

>lcl|hmm330156 Shaded portion is included in the amplicon; highlight is SNP290

ATGAGGCTCCATCACCTGCTCCTCGCGCTCCTCTTCCTGGTCCTGTCTGCTGGGTGAGCTCGTGGGAGCCCCGGGGGAGCT
GCGGGCTCTCTCT^CCTGTTTCTACCTCCTTCTGTCTGCTACCCCCATCCACACGAGCACAGTGGCAGGGAGAGGACAGGGGTGT
AAGGAATGTGGCCTTTTCAGTGCACGGGGCCCGGGTTCAATCCCTGGACGGGGAAGTAGAGAGGGACCGGTCCTTGTGGTCAAG
AGGAAAAAGGCCTGTGAGGAAGACGGGAAGGCCCTTGGTCAGGCGTGTGAGAGACGGGAAGGCCTCTGGTGCCTGGAAGCATGAG
ACAGATTGGCACCTGTTTTCGGGCCCCGAGTAAAATGCTGCAGGTCGTGGTAAAAGAAGGCGAAGATGCGGCCGGGACTGATGCGGAG
ATAGAAACTGCGCCCTTTGACAAAGCATCTAAAATTTAA

Figure 3. Comparison of novel sequence to the coding region of the predicted gene. The SNP highlighted (SNP 290) results in a major amino acid substitution of serine to phenylalanine.

from guarantees the usefulness of that gene as a genetic marker; further work would need to be done to test the informativeness of those SNP for mastitis resistance.

IMPLICATIONS

The development of a novel candidate gene marker for mastitis resistance in cattle would result in the opportunity to use marker assisted selection to genetically improve the nation's dairy herds and render them less susceptible to this economically important disease.

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