



Molecular characterization of mannose-binding lectin protein in chickens

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Mannose-binding lectin (MBL), a serum lectin protein, is an important element of the innate immune system. Upon binding to carbohydrate ligands, MBL is able to activate the complement system via the lectin pathway leading to opsonization and lysis of the microorganisms (Jack *et al.* 2005). Thus it plays a major role in the innate immune defense. Lowered levels of serum MBL were detected after experimental infection of chickens with different viral, bacterial and parasitic organisms (Schou *et al.* 2010). Similarly, some novel SNPs were discovered in the promoter and the 52 untranslated region of the chicken MBL gene (Rikke *et al.* 2013). However, the MBL coding sequence and the expression of MBL protein in serum has not been studied in our indigenous chicken breeds. Considering the gaps in knowledge on chicken-MBL and its relative role in disease resistance in the germplasm available in India, this study was designed to detect the serum level of MBL protein in chickens of divergent breeds/lines, and to investigate the possible sequence variations of MBL2 gene in chickens.

Detection of serum MBL level in chickens by ELISA: Blood samples were collected from 12 chickens of same age and sex (females). These birds were belonging to 3 divergent strains/breeds (Aseel, Rhode Island Red and White Leghorn). The serum MBL level was quantified using the chicken mannose binding lectin C (MBL-C) ELISA kit. The ELISA readings were subjected to one way ANOVA to determine the breed effect. The readings between the breeds were compared using zero order correlation.

Total RNA extraction and PCR (polymerase chain reaction): Fresh tissue liver samples from the experimental birds were collected after euthanizing the birds and total RNA was extracted using Trizol method. The cDNA was synthesized using first strand cDNA synthesis kit, after checking the total RNA concentration (quantity) and quality.

PCR was carried out in a 50 µl reaction volume using 2

sets of primers, one published primer-pair (Rikke *et al.* 2013) specifying an amplicon size of 544 bp (5'-GTTACAACAATTCCACGTTCTCCT-3' and 5'-GTTACAACAATTCCACGTTCTCCT-3') and one pair of designed primer with product size of 835 bp (5'-GGTAAAGGTGCTGATCTGTGG-3' and 5'-TGAGAGAAGAAAGTTGGATTGATT-3'). All PCR products were visualized by agarose gel electrophoresis and photographed using gel Doc.

Cloning of PCR products: The purified PCR product was cloned using pGEM-T easy vector in DH5α strain of *E. coli* as per Sambrook and Russel (2001) and recombinant clones were selected based on a blue/white screening method and finally confirmed by PCR. The custom sequenced clones and corresponding coding sequence of MBL gene from 12 different species, downloaded from NCBI Genbank, were analyzed by BLASTn and megalign software of DNASTAR.

Detection of serum MBL level in chickens by ELISA: The concentration of MBL in serum was measured in the 3 chicken breed lines, viz. Rhode Island Red, White Leghorn and Aseel. The mean serum MBL concentration did not differ significantly among the breeds/strains of chickens included in this study. This finding is consistent with the report of Laursen *et al.* (1998) and Schou *et al.* (2010) where the mean serum MBL concentration showed no difference among the 14 different breeds of chickens.

The serum levels of MBL in chickens vary from less than 1 to more than 35 µg/mL (Laursen *et al.* 1998, Laursen and Nielsen 2000, Norup *et al.* 2009) due to genetic variation. The result of this study also showed large variation in serum MBL levels in the 42 chickens with mean concentration of 7.9 µg/ml, and range of 4.02 to 17.99 µg/mL. Chickens of the WLH strain line showed slightly lower mean serum MBL concentration (7.3±0.8 µg/mL) as compared to that of Aseel (8.1±0.7 µg/mL) and RIR (8.3±0.5 µg/mL).

Chickens strains with low or high serum MBL concentrations have been produced by Juul-Madsen *et al.* (2007) through selective breeding. The apparent importance of MBL to the susceptibility to infectious diseases in chickens and the ease of breeding for MBL expression together suggest

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the obvious possibility of the use of MBL gene as a selection parameter for developing chicken breeds which are resistant to infectious organisms. This could probably be of particular significance in organic production systems where potentially high levels of disease, together with regulations on prophylactic treatment call for alternative disease control methods.

Introducing chicken lines with high MBL concentrations could similarly be a possible measure in poverty alleviation in developing countries, where disease control through treatment or vaccination programs in rural areas often are lacking or simply too expensive for smallholder farmers. However, human studies indicate that high serum MBL levels in concern with the complement system may have a reverse effect in certain infections and thus contribute to the enhanced uptake of intracellular organisms, like *Mycobacterium tuberculosis* and *Leishmania* species (Garred *et al.* 1994, Soborg *et al.* 2003). Before implementing breeding programs for high serum MBL, the possibility of a similar negative effect in chickens should therefore be considered and a strategy where only chickens with very low levels of serum MBL are eliminated from the breeding stock would probably be a more feasible approach.

Cloning and sequence analysis of MBL2 gene from chickens: The coding sequence of chicken MBL2 gene from three chicken breed lines were cloned and sequenced. The sequence data have been submitted to GenBank with accession numbers KF469208-KF469210.

The cloned sequence was compared with the reference sequence (accession number AF231714.1) using MegAlign of DNASTAR. A total of 21 mismatches and 5 gaps were found. These mismatches may be explained by the presence of SNPs in the coding sequence of chicken MBL2 gene. Previous studies of human MBL gene have also shown that point mutations in the MBL coding region are partly responsible for the observed low MBL serum concentration (Garred *et al.* 1992). However, Rikke *et al.* (2013) found only one SNP in the 5' UTR and non SNP in the coding region. Indications of SNPs in the coding region of the nucleotide sequence of the chicken MBL gene have previously been suggested by Laursen *et al.* (1998). They found discrepancies between the amino acid sequence by peptide sequencing and the deduced amino acid from cDNA. In MBL1 gene of Chinese native cattle, Changfa *et al.* (2011) also reported three SNPs that are associated to mastitis resistance.

The phylogenetic analysis (MegAlign, DNASTAR) of the coding sequence of MBL2 gene (Fig. 1) from the 3 strains of chickens indicated that the CDS of MBL2 gene from Aseel chicken strains might have different evolutionary development compared to the other strains of chickens. BLASTx analysis of the coding sequences from Aseel chicken strain showed only one non-conserved substitution of amino acids. RIR showed absence of any difference

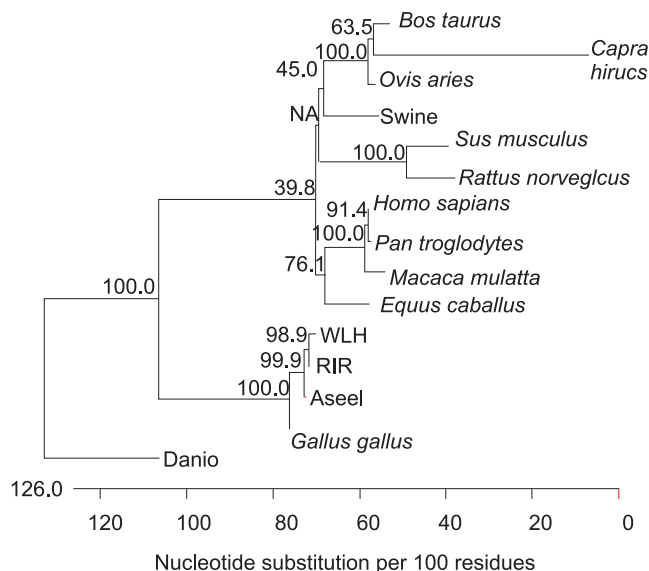


Fig. 1. Phylogenetic tree with 1,000 bootstrap resampling (MegAlign, DNASTAR) of the CDS of MBL gene in divergent eukaryotic species.

compared to the reference amino acid sequence. Furthermore, the MBL amino acids from WLH showed a non-conserved substitution from reference amino acid sequence.

Further studies need to be done to identify the potential SNPs that are responsible for lowered serum MBL levels in different strains of chickens. Limited works have been reported on MBL in chicken. The present work highlights the preliminary findings on MBL in serum and the cds in Aseel breed in comparison to the commercial synthetic chicken breeds.

Though the mean serum MBL concentration did not differ significantly among the three different breeds of chickens included in this study, the presence of varying serum MBL concentrations within breeds indicate the importance of MBL2 gene in chicken innate immunity. The mismatches present in the MBL2 sequence of these chickens may represent SNP that might be found in the CDS of MBL2 gene.

SUMMARY

Mannose-binding lectin (MBL), upon binding to carbohydrate ligands, is able to activate the complement system via the lectin pathway leading to opsonization and lysis of the microorganisms. Thus, it plays a major role in the innate immune defense and holds the potential to be used in selection for disease tolerance. This study was carried out to evaluate and profile the serum level of MBL protein in divergent breeds/lines of chickens, viz. Rhode Island Red, White Leghorn and Aseel vis-à-vis to investigate the possible sequence variations of MBL2 coding sequence in chickens. The serum MBL concentration was determined by Quantitative ELISA. The mean MBL concentration among

the three breeds of chickens did not differ significantly but the serum MBL concentration of the individual chickens showed variation from 4.02 µg/ml to 17.99 µg/ml. The coding sequence of the MBL 2 gene from liver tissues of the 3 breeds was cloned and custom sequenced. The sequence results were compared among breeds and the reference sequence (GenBank accession number AF231714.1) using MegAlign of DNASTAR (Lasergene). The mismatches among the sequences may represent single nucleotide polymorphisms (SNPs) in the CDS of MBL2 gene. Evolutionarily, the MBL in RIR and WLH breeds of chicken was found closer than that of Aseel.

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