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Sequence and Comparative Analysis of Myeloperoxidase (MPO) in River Buffalo (*Bubalus bubalis*)

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Abstract: Precise comparisons of mammalian gene maps require common anchor loci as landmarks for conserved chromosomal segments. One of the comparative anchor tagged sequences (CATS), myeloperoxidase (MPO), has been tested for reaction in the river buffalo [*Bubalus bubalis* (BBU)] using polymerase chain reaction (PCR). The primer pair for MPO reacted successfully with river buffalo DNA after optimization of PCR conditions. River buffalo partial MPO sequence has been submitted to the nucleotide sequences database NCBI/ Bankit/ GenBank with the accession number EU086095. The sequence analysis of the buffalo PCR product (amplicon) and Blast searches with GenBank published sequences revealed detectable sequence homology with MPO sequences of *Bos taurus* (BTA), *Homo sapiens* (HSA), and *Mus musculus* (MMU). MPO, an important enzyme playing a major role in killing pathogens, marked a detectable deviation from the common concept of the remarkable degree of genomic conservation between river buffalo and cattle and implying more inference to the internal chromosomal rearrangements that may exist among them. On the other hand, the results presented here allow the assignment of MPO gene to river buffalo chromosome BBU3p for the first time, based on comparative mapping predictions and the subsequent investigations in this study, therefore extending the river buffalo physical map.

Keywords: MPO, NCBI (EU086095), CATS, River buffalo

INTRODUCTION

The rapid development of the human and mouse gene maps has stimulated the expansion of genetic maps for several additional mammalian species, particularly those of agricultural and companion animal species (Lyons *et al*., 1997). In this respect, augmenting the informational content of gene maps as a tool for developing more efficient breeding strategies has targeted animal improvement by the genomic approach.

Precise comparisons of mammalian gene maps require common anchor loci as landmarks for conserved chromosomal segments. In this respect, Lyons *et al*. (1997) designed a number of evolutionarily conserved primer pair sequences termed comparative anchor tag sequences (CATS), providing PCR-format gene markers that can be used to construct and connect gene maps of different mammalian species.

The river buffalo is an economically important livestock species in many Asian and Mediterranean countries. Its genetic improvement, especially in disease resistance, reproductive performance and quantity of meat and milk production, ranks high among agricultural research needs of these countries. Considering the first factor, disease is one of the major factors contributing to poor livestock productivity in developing countries. In Egypt as in other developing countries an increase in supplies of livestock products is necessary to meet growing demand from burgeoning populations and rapid urbanization. Several projects were conducted in order to increase buffalo productivity.

Myeloperoxidase (MPO) is an enzyme found in the azurophilic granules of neutrophils and in the lysosomes of monocytes. Its major role is to aid in microbial killing. MPO is most abundant in the granules of neutrophils. Monocytes contain only about a third of the MPO present in neutrophils. When neutrophils become activated, which can happen in conjunction with phagocytosis, they undergo a process referred to as a respiratory burst. This respiratory burst causes production of superoxide, hydrogen peroxide, and other reactive oxygen derivatives, which are all toxic to microbes. During respiratory bursts, granule contents are

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released into the phagolysosomes and outside the cell, allowing released contents to come into contact with any microbes present. MPO catalyzes the conversion of hydrogen peroxide and chloride ions (Cl) into hypochlorous acid. Hypochlorous acid is 50 times more potent in microbial killing than hydrogen peroxide. In addition to killing bacteria, the products of the MPO–hydrogen peroxide–Cl system are believed to play a role in killing fungi, parasites, protozoa, viruses and tumor cells (www.emedicine.com/ped/topic1530.htm). On the other hand, Brown *et al.* (2001) identified myeloperoxidase as a component of human Kupffer cells and reported that the oxidative damage resulting from the action of myeloperoxidase may contribute to acute liver injury and hepatic fibrogenesis.

MPO may also have a role in atherosclerosis, carcinogenesis, and degenerative neurological diseases. Support for the role of MPO in atherosclerosis includes a small study that demonstrated that MPO deficiency may protect against cardiovascular disease. The understanding of MPO biology remains incomplete, and much more remains to be discovered (www.emedicine.com/ped/topic1530.htm).

In the present study, river buffalo MPO has been investigated using PCR primer pair from comparative anchored tagged sequences (CATS) [Lyons *et al.,* 1997], sequenced and assigned to river buffalo chromosome BBU3p for the first time by comparative analysis.

MATERIALS AND METHODS

The MPO primer pair used in this study was one of the CATS designed by Lyons *et al.* (1997). The sequences of the forward and reverse primers are:

- F: 5'- CCA CAC CCT CAT CCA ACC -3'
- R: 5'- GCT CCC GGA TCT CAT CC -3'

Genomic DNA Extraction:

Blin and Stafford (1976) protocols for genomic DNA extraction were followed in this study for the isolation of DNA from buffalo leukocytes. Buffalo blood was collected in syringes containing ethylene diamine tetra acetic acid (EDTA). Ten ml blood were transferred to a 50 ml polypropylene tube on ice to which 25 ml of cold 2X sucrose-Triton (0.64M sucrose; 0.02M Tris-base; 0.01M $MgCl_2$; 2% Triton X-100 pH 7.6), and 15 ml dd H_2O were added. The tube was inverted several times and set on ice for 10 min. The mixture was centrifuged at 5000 rpm for 15 min at 4^{o}C and the supernatant was discarded. The cell pellets from buffalo blood, were suspended in 3 ml nuclei lysis buffer, in addition to 1/20 volume of 20% SDS and 1/20 volume of proteinase K (10 mg/ml). The samples were incubated overnight in a shaking waterbath at 37^{o}C.

DNA was extracted once with TE-saturated phenol, then with phenol: chloroform: isoamyl alcohol (25: 24: 1) until there was no protein at the interface, and finally by chloroform-isoamyl alcohol (24:1). For each extraction, the aqueous phase was mixed well with an equal volume of solvent, centrifuged for 10 min at 2000 rpm and the top layer carefully transferred to another falcon tube for the next extraction. To the final aqueous phase, 1/10 the volume of 2.5 M NaOAc (pH 5.5), and 2.5 volume of cold 95% ethanol were added, the tube was agitated to mix. DNA was picked up with a heat-sealed Pasteur pipette and washed briefly in cold 70% ethanol and air dried, then dissolved in an appropriate volume of 1X TE buffer. DNA concentration was spectrophotometrically determined using Pharmacia LKB-Ultrospec.

Polymerase Chain Reaction:

The PCR reaction mixture performed in this study, 25-100 µl, consisted of 0.2 mM dNTPs, 10mM Tris,50 mM KCL, 1.0 to 2.0 mM $MgCL_2$, 1% Triton X-100, 2.5 units *Taq* polymerase and 1.0 µM forward and reverse primers. This was aliquoted into PCR tubes with 100 ng of buffalo genomic DNA. The reaction mixture was overlaid with sterile mineral oil and was run in the PCR machine (Techne Cyclogene). The reaction mixture was cycled for 1 min at 94^{0}C, 2 min at 55^{0}C, and 2 min at 72^{0}C for 30 cycles and finally 1 cycle (10 min) at 72^{o}C. Following the completion of the PCR cycles, 2µl of tracking dye (20 mg bromophenol blue, 1 ml of 0.2 M EDTA, 2 ml of glycerol and sterile distilled water to 20 ml total volume) were added to each PCR tube below the oil layer. The reaction products and an appropriate size marker were electrophoresed on 1.5% agarose in 1X-Tris acetate buffer (TAE) containing 0.8 % of 10 mg/ml ethidium bromide. The gel was examined with a UV transilluminator at 312 nm and photographed using a polaroid MP4$^+$ camera.

Sequence Analysis:

The PCR product of river buffalo DNA was purified and sequenced by Macrogen Incorporation (Seoul, Korea). Sequence analysis and alignment were carried out using NCBI-BLASTN 2.2.14 version (Altschul *et al.,* 1997) and CLUSTAL W 1.83 version for multiple sequence analysis (Gasteiger *et al*., 2003). The river buffalo MPO nucleotide sequence was submitted to GenBank (NCBI, Bankit) with the accession number EU086095.

RESULTS AND DISCUSSIONS

Results:

PCR was performed to test the reaction of MPO primers, one of the comparative anchored tagged sequences, with native river buffalo (*Bubalus bubalis*) DNA. A single distinct band of approximately 900 bp was observed after optimization of the PCR conditions. In order to verify the PCR product, two way sequence analysis of the MPO amplified PCR product of buffalo DNA (amplicon) was conducted, using both the forward and reverse primers. The buffalo amplicon obtained was found to be 532bp (Fig. 1).

GGATGAAATGCTGAAAAAGGTAACCTCACCGTATAGCCGTATGAAAACGGTTT
ACAAGTATGTACAGGATAACATGCAGTGGAACGAGTTCACCAGCATCTGGGCC
ATGGATGGCGTACGCAGTGCCTGGAAAGACAAAAAAGGAACGGTGGGTGAAAT
CAACCTGATACTGGTAAACCTGCTGAAAGATGCGGGCCTGCATGCAAGCCCCAT
CCTGGTCAGCACACATGACAATGGCGTGGTAAATACGGTGGATGCCGGGACCT
ATGAATACCCGGGCTATCACCAGTTCAACAAGGTAATGGCTTACCTGGAGCTCG
ACGGCAAAGCCTTTGTGCTGGATGCTTCGCAAAAAGGAACGCCGGTACACCTG
ATACCGGCCGAAATTCTATATACCCAGGGTTTGGTTATAGAAAAATATGAAACC
TTCGAATGGGGATGGAAGTCCATCTGGAACGAAGAGGCGATGGCGAAGAATAT
TATCCGCATCAACGCAACCATTGATGCTACCGGCAAAATGATCGGTGAAGCCA
AT

Fig. 1: *Bubalus bubalis* MPO sequence.

The following sequence analyses were carried out using the reverse complement of the amplicon. Alignment with GenBank was carried out using NCBI-BLASTN 2.2.14 version (Altschul *et al*., 1997) and CLUSTAL W 1.83 version for multiple sequence analysis (Gasteiger *et al.,* 2003).

The preliminary database search of the buffalo amplicon using NCBI-BLASTN showed no significant similarity. The next step was to check for similarities between the buffalo *(Bubalus bubalis)* nucleotides and those of cattle [*Bos taurus* (a closely related species)], human (*Homo sapiens*) and mouse (*Mus musculus*) MPO using CLUSTAL W 1.83 version for multiple sequence analysis, where the lowest identities can be detected. The results of the search revealed the alignment of the *Bubalus bubalis* (BBU) MPO amplicon with *Bos taurus* (BTA) MPO (Accession number AAFC03085503.1) with a 44% similarity (Fig. 2).

Alignment of *Bubalus bubalis* MPO with *Homo sapiens* (HSA) MPO (Accession number BC130476) and *Mus musculus* (MMU) MPO (Accession number X15313) showed a similarity of 44% and 42% (Fig. 3 & 4, respectively). *Bos taurus* MPO (AAFC03085503.1) showed the same trend as buffalo. It showed a 46% and 45% similarity with *Homo sapiens* MPO (BC130476) and *Mus musculus* MPO (X15313), whereas the *Homo sapiens* MPO showed a notably high homology with *Mus musculus* MPO with a similarity percentage of 88% [Table 1].

The Buffalo MPO nucleotide sequence was submitted to nucleotide sequences database NCBI/ Bankit/ GenBank and has the accession number EU086095.

Discussions:

Genomes of domestic animals are practically unknown when compared with those of both humans and mice. Cattle is considered to be the most studied species, where 4357 loci are mapped with 1507 assigned genes (BovBase, http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/bovmap/Bovmap/main.pl,August,2007). In other bovids, especially in the river buffalo (*Bubalus bubalis*), physical maps are relatively poor.

In this respect, comparative mapping anchored loci (mapping homologous genes in multiple species) provide more information about chromosomal evolution between distantly related species. A remarkable conservation of linkage organization of homologous genes was observed in species from diverse mammalian orders (Copeland, 1993; Johansson *et al.,* 1995; Nadeau, 1995 and Womack and Kata, 1995).

Table 1: Comparison Table of the four investigated MPO nucleotide sequences.

SeqA	Name	Len(nt)	SeqB	Name	Len(nt)	Similarity
1	BBU MPO	532	2	BTA MPO	620	44%
1	BBU MPO	532	3	HSA MPO	840	44%
1	BBU MPO	532	4	MMU MPO	840	42%
2	BTA MPO	620	3	HSA MPO	840	46%
2	BTA MPO	620	4	MMU MPO	840	45%
3	HSA MPO	840	4	MMU MPO	840	88%

CLUSTAL W alignments

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SeqA    Name   Len(nt)             SeqB   Name  Len(nt)        Similarity
================================================================================
 1       BBU     532                  2     BTA    620               44%
================================================================================

BBU             --------------------------------GGCTTCACCGATCATTTTG--CCGGTAGC  27
BTA             GTGGCATCGACCCCATCCTGCGGGGCCTCATGGCCACCCCTGCGAAGCTGAACCGCCAGA  60
                                               ***  * **    *   **  ***  **

BBU             ATCAA----TGGTTGCGTTGATGCGGATAATATTCTTCGCCATCGCCTCTTCGTTCCAGA  83
BTA             ACCAAATCGCGGTGGACGAGATCCGGGAGCGGCTGTTTGAGCAGGTCATGAGGATCGGGC  120
                * ***     *** *    *** ***       * ** *     * *     * **  *

BBU             TGGACTTCCAT---CCCCATTCGAAG----------GTTTCATATTTTTCTATAACCAAA  130
BTA             TGGACCTGCCTGCGCTCAACATGCAGCGCAGCCGCGACCACGGCCTCCCTGGTAAGGGGG  180
                ***** * * *   * * *   * **              *    *      ***

BBU             C---CCTGGGTATATAGAATTTCGGCCGGTATCAGGTGTACCGGCGTTCCTTTTTG----  183
BTA             CTGCCCTGGCCTCACGGG--CCCACCCACCTCCCAATCCAGCGCCTGTCCTCTCCAGTGC  238
                *   *****    *  *     *  **     *   *  * ** *  **** *

BBU             -CGAAGCATCCAG--CACAAAGGCTTTGCCGTCGAGCTCCAGG--TAAGCCATTACCTTG  238
BTA             CCCCAGCTACCGGGGCACCCCACCCCCACCGCCAACCCTCAGAACCTTGCTCTTAGGATG  298
                 *  ***  ** *  ***     *    *** * * *  ***      **  ***   **

BBU             TTGAACTGGTGATAG---CCCGGGTATTCATA----GGTCCCGGCA---TCCACCGTATT  288
BTA             C-GGCCTGAAGTCAGGCCCCTGGGCCCCTCTGCCCCATTTCCAGCAGCTTCCACCTACTT  357
                  *  ***  *  **   ** ***      *       * ** ***   ******   **

BBU             TACCACGCCATTGTCATGTGTGCTGACCAGGATGGGGCTTGCATGCAGGCCCGCATCTTT  348
BTA             TGTGTTGCTGCTGCTAAGTCGCGTCAGTTGTGTCCGACTC-TGTGCGACCCCGTAGATGG  416
                *     **   **  * **    * *   *  *  * **    ***   **** *  *

BBU             CAGC-----AGGTTTACCAGTATCAGGTTGATTTCACCCACCGTTCCTTTTTTGTCTTTC  403
BTA             CAGCCCACCAGGCTTCCCCGTCCCTGGGATTCTCCAGGCAAGAACACTGGAGTGGGTTGC  476
                ****     *** ** ** **  * **     * **  **      **    **  ** *

BBU             CAGGCACTGCGTACGC-CATCCATGGCCCAGATGCTGGTGAACTCGTTCCACTGCATGTT  462
BTA             CATTTCCTTCTCCAATGCATGAAAGGGAAAAGTGAAAGTGAAGTCCCTCAGTCGTGTCCG  536
                **    ** *       ***  * **   *  **   ***** **  **    *  *

BBU             ATCCTGTACATACTTGTAAACCGTT-TTCATACGGCTATACGGT----GAGGTTACCTTT  517
BTA             ACTCTTAGCGACCCCACGGACTGCAGCCCACCAGGCTCCTCAGTCCATGGGATTTCCCAG  596
                *  **   *   *      ** *     **   ****   * **    * * ** **

BBU             TTCAGCATTTCATCC---------  532
BTA             GCAAGAGTACTGGAGTGGGGTGCC  620
                   **  *
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Fig. 2: Sequence alignment file of BBU (*Bubalus bubalis*) MPO and BTA (Bos taurus) MPO (AAFC03085503.1).

Lyons *et al.* (1997) designed 410 evolutionarily conserved primer pairs which are specific for anchor locus gene amplification from DNA of any mammalian species. These sequences are called Comparative Anchor Tagged Sequences (CATS).

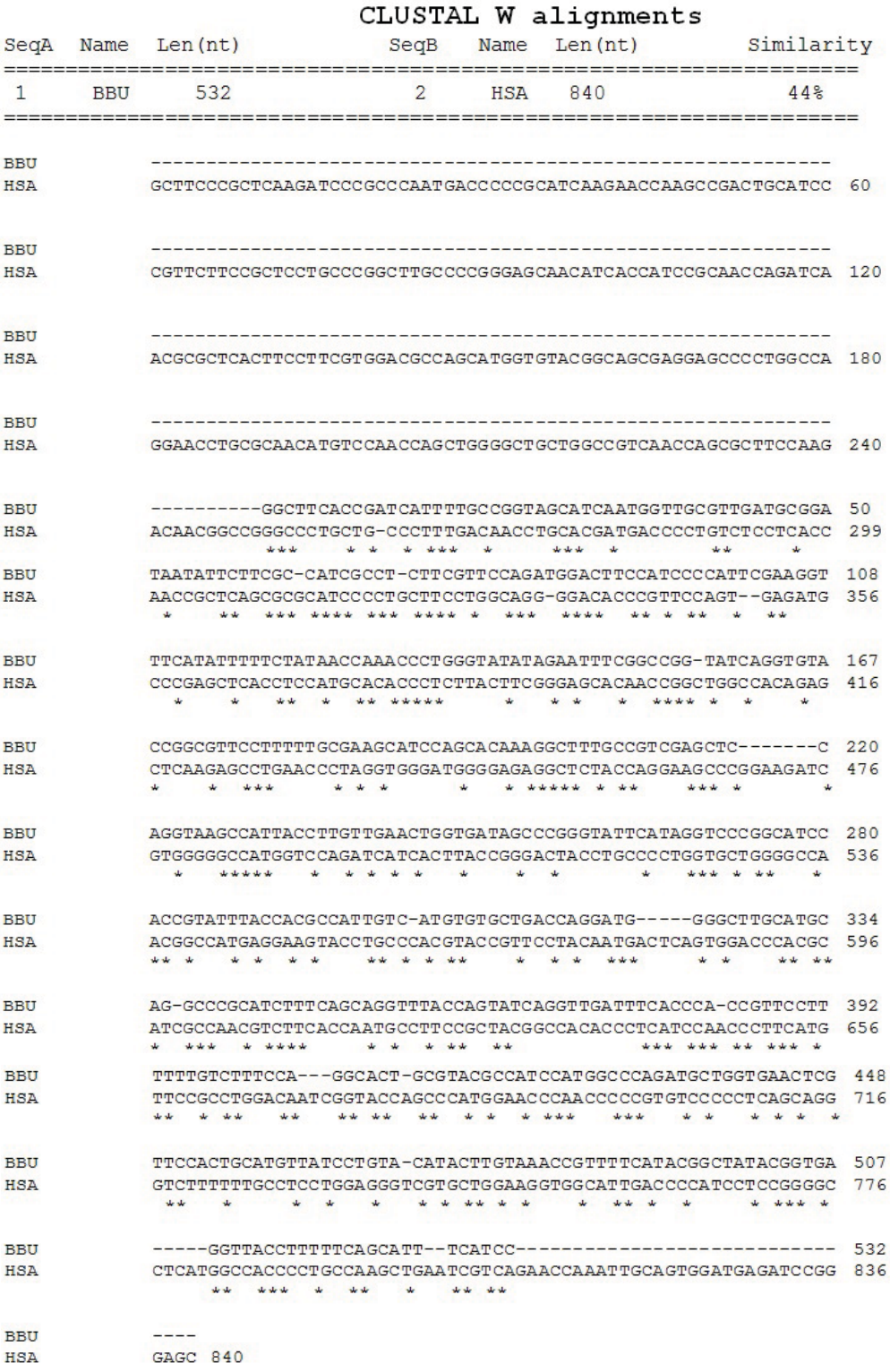

Fig. 3: Sequence alignment file of BBU (*Bubalus bubalis*) MPO and HSA (*Homo sapiens*) MPO (BC130476).

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                              CLUSTAL W alignments
SeqA Name    Len(nt)          SeqB  Name   Len(nt)            Similarity
=========================================================================
1     BBU     532               2    MMU    840                   42%
=========================================================================

BBU         ------------------------------------------------------------
MMU         GCTTCCCCCTCAAGATTCCACCCAATGACCCTCGAATCAAGAACCAAAAGGACTGCATCC  60

BBU         ------------------------------------------------------------
MMU         CCTTCTTCCGCTCCTGCCCGGCATGCACCAGGAACAACATCACCATTCGCAACCAGATCA 120

BBU         ------------------------------------------------------------
MMU         ACGCGCTCACTTCCTTCGTGGACGCCAGTGGGGTGTACGGCAGCGAGGACCCCCTAGCCA 180

BBU         ------------------------------------------------------------
MMU         GAAAGCTGCGCAACCTCACCAACCAGCTGGGGCTGCTGGCTGTCAATACACGATTCCAAG 240

BBU         ------------------------------------------------------------
MMU         ACAATGGCAGGGCCCTGATGCCCTTTGACAGCCTGCACGATGACCCCTGCCTCCTCACCA 300


BBU         -----------GGCTTCACCGATCATTTTGCCGGTAGCATCAATGGTTGCGTTGATGCGG  49
MMU         ACCGCTCCGCCCGCATTCCTTGTTTTCTGGCAGGGGACATGCGCTCCAGCGA-GATGCCG 359
                       ** *  *   *  * * ** **   ***        ***  ***** *

BBU         ATAATATTCTTCGC-CATCGCCTCTTCGTTCCAGATGGACTTCCATCCCCATTCGAAGGT 108
MMU         GAGCTCACCTCCATGCACACCCTCTTTGTTCGAGA-GCATAACCGGCT--GGCCACACAG 416
                 *   ** *   **   ****** **** *** * *   **  *      *  *

BBU         TTCATATTTTTCTATAACCAAACCCTGGGTATATAGAATTTCGGCCGGTA--TCAGGTGT 166
MMU         CTCAAGCGCCTGAATCCTCGA---TGGAATGGGGAGAAGCTCTACCAGGAGGCCCGGAAG 473
             ***      *  **   * *     *  *    ****  **  ** * *   * **

BBU         ACCG-GCGTTCCTTTTTGCGAAGCATCCAGCACAAAGGCT--TTGCCGTCGAGCTCCAGG 223
MMU         ATTGTAGGGGCCATGGTCCAGATCATCACATACCGGGACTACCTGCCCTTGGTGTTGGGG 533
            *  *   *  ** *  * *  * ****    **   * **   **** * *   *   **

BBU         TAAGCCATTACCTTGTTGAACTGGTGATAGCCCGGGTATTCATAGGTCCCGGCAT-CCAC 282
MMU         CCAGCAGCCATGAAGAAGTACCTACCGCAGTACCGATCCTACAACGACTCAGTAGACCCT 593
              ***    *    *  * **       **  * * *  *   * * * * * *  **

BBU         CGTATTTACCACGCCATTGTCATGTGTGCTGACCAGGATGGGGCTTGCATGCAGGCCCGC 342
MMU         CGAATCGCCAATGTCTTCACCAATGCTTTCCGTTATGGCCACACCCTCATCCAACCCTTC 653
            ** **   * * * * *   **    *       * *      *   *** **  **  *
BBU         ATCTTTCAGCAGGTTTACCAGTATCAGGTTGATTTCACCCACCGTTCCTTTTTTGTCTTT 402
MMU         ATGTTCCGCCTGAACAATCAGTACC-GGCCCACAGCGGCCAACCCCCGAGTCCCACTCAG 712
            ** ** *  * *    * ***** * **   *   *  *** *   *   *

BBU         CCAGGCACTGCGTACGCCATCCATGGCCCAGATGCTGGTGAACTCGTTCCACTGCATGTT 462
MMU         CAAGGTCTTTTTT--GCCAGCTGGAGAGTTG-TGCTGGAAG----GTGGCATTG-ACCCC 764
            * ***   *   *  **** *    *    * ******       **  ** ** *

BBU         ATCCTGTACATACTTGTAAACCGTTTTCATACGGCTATACGGTGAGGTTACCTTTTTCAG 522
MMU         ATCCTCCGAGGCCTCATGGCCACTCCTGCCAAACTGAATCGCCAGAATCAAATTGTGGTG 824
            *****       **  *   *  *  *  *    *  **      * *  ** *   *

BBU         CATTTCATCC------ 532
MMU         GATGAGATCCGGGAGC 840
             **   ****
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Fig. 4: Sequence alignment file of BBU (*Bubalus bubalis*) MPO and MMU (*Mus musculus*) MPO (X15313).

CATS primers were previously used for river buffalo mapping of insulin - like growth factor (IGF2); vimentin (VIM); glucose-6-phosphate dehydrogenase (G6PD); glycoprotein hormone, alpha polypeptide (CGA) and glutathione -S-transferase 2, alpha (GSTA2) using somatic cell hybrids [Mahfouz, 2000]. Lactate dehydrogenase B (LDHB) and biglycan (BGN) were sequenced and mapped to river buffalo by Hassan (2004) and Mahfouz (2007), respectively.

In order to extend the usage of CATS in *Bubalus bubalis* (river buffalo) gene mapping, MPO primer pair were tested for reactivity with buffalo DNA. It reacted successfully with river buffalo genomic DNA and showed a single distinct band of approximately 900 bp. A band of approximately 750 bp was shown by Lyons *et al.* (1997), using the same primer pair and other mammalian DNAs, including bat; dog; human; mouse; rabbit and rat. However, it was not possible to assign MPO to buffalo chromosomes using buffalo somatic cell hybrids (unpublished data). Therefore, further analysis of buffalo MPO at the sequence level was thus conducted in this study. It was sequenced and compared with GenBank published sequences. Multiple sequence analysis (Gasteiger *et al.,* 2003) between the buffalo amplicon and *Bos taurus* MPO [AAFC03085503.1], *Homo sapiens* MPO [BC130476] and *Mus musculus* MPO [X15313] was conducted. A detectable homology was seen between *Bubalus bubalis* MPO and: *Bos taurus* MPO [AAFC03085503.1], *Homo sapiens* MPO [BC130476] and *Mus musculus* MPO [X15313], with a percentage of 44% for both the first and second and 42% for the third (Table 1). This low similarity between *Bubalus bubalis* MPO and the other three species may be attributed to the internal molecular rearrangements that are likely to occur between closely as well as between distantly related species**.** *Bos taurus* MPO [AAFC03085503.1] has also shown percentage similarities of 46% and 45% with *Homo sapiens* MPO [BC130476] and *Mus musculus* MPO [X15313], respectively (Table 1) which are not much different from those reported with buffalo.

Lyons *et al*. (1997) reported the remarkable degree of genomic conservation which is apparent when comparing the human genome with that of cow, where only about one-third as many chromosomal rearrangements have occurred as were observed between human and mouse. This is not observed in MPO gene since *Homo sapiens* MPO [BC130476] and *Mus musculus* MPO [X15313] are 88% similar, while *Homo sapiens* MPO and *Bos taurus* MPO [AAFC03085503.1] are only 46% similar (Table 1).

Alignment of buffalo MPO and *Bos taurus* MPO [AAFC03085503.1] showed only 44% similarity. This is an interesting result since most of the studies, conducted so far, were focusing on the close relation and the remarkable degree of chromosome conservation observed between cattle and buffalo (CSKBB, 1994; De Hondt *et al*., 1997; De Hondt & El Nahas, 2001; Di Meo *et al*., 2000; El Nahas *et al*.,1993;1996;1997; 1998;1999; 2001; El Nahta *et al*.,1994; Iannuzzi *et al.* 2000a;2000b; Mahfouz, 2000; Oraby *et al.,* 1998; Othman & El Nahas, 1999; Othman *et al*.,2003 and Othman & Bibars, 2004). It is now required to direct more attention and shed more light on the differences between the two species, especially at the molecular level, in order to detect the genetic differences between them.

MPO has an important role in disease resistance and defense mechanisms, where it is known to be released by phagocytic cells during inflammatory responses (www.emedicine.com/ped/topic1530.htm). The sequence differences found between buffalo MPO and *Bos taurus* MPO may explain the reasons behind the tendency of buffalo to be more resistant to diseases, where certain facts note the stronger immune tolerance of *Bubalus bubalis* compared to cattle. In Egypt, Italy, Bulgaria and other Balkan states, certain herds of *Bubalus bubalis* have surpassed the local cattle in growth, environmental tolerance and health. It was also found to be notably resistant to various diseases like ticks, contagious pleuropneumonia, anaplasmosis and babesiosis (Report of the advisory committee on Technology Innovation, 1981).

Considering the issue of map and chromosomal assignments, MPO was mapped to *Bos taurus* BTA 19 (Yang and Womack, 1995), human HSA 17q23.1 and mouse MMU 11 49.0cM chromosomes which are known to be homologous (Solinas-Toldo, 1995; Womack and Kata, 2000). Cattle chromosome BTA 19 has been reported to be homologous to buffalo chromosome BBU3p (Report of The Committee For The Standardization of Banded Karyotypes of The River Buffalo, 1994 ; El Nahas *et al*., 1997 and De Hondt and El Nahas, 2001), therefore based on the chromosomal homology between cattle and buffalo MPO is expected to be located on BBU3p.

In conclusion: MPO, one of the CATS, an important enzyme playing a major role in killing pathogens, marked a detectable deviation from the common concept of the remarkable degree of genomic conservation in different mammalian species, and therefore implying more inference to the internal chromosomal rearrangements that may exist among them. On the other hand, the results presented here allow the assignment of MPO gene to BBU3p for the first time, based on comparative mapping predictions and the subsequent investigations in this study, thus extending the river buffalo physical map.

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