Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2019, Vol. 3 (4), 706-712
https://doi.org/10.29329/ijiaar.2019.217.17
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Review article

# Profile of *b*NRAMP1 Gene Sequence as the Candidate Gene for Pathogenic Bacterial Resistance Trait in Cattle

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**Abstract**

Bovine Natural Resistance Associated Macrophage Protein 1 (*b*NRAMP1) gene is one of the candidate gene that widely used to control the infectious disease in dairy and beef cattle. In mouse, the *b*NRAMP1 gene is important to confer resistance or suspectibility to *Mycobacterium bovis*, Salmonella typhimurium and *Leishmania donovani*. This article was carried out to explain the profile of *b*NRAMP1 gene such as genes structure, number of Single Nucleotide Polymorphisms (SNPs) and its relationship to pathogenic bacterial diseases evidence such as bovine Tuberculosis, mastitis and brucellosis. The mutations (SNPs) in bNRAMP1 gene were occured in intron, exon and 3'UTR regions. Previous studies reported that some SNPs in bNARAMP1 gene were affected to many pathogenic bacterial disease incidences in cattle. Hence, the bNRAMP1 gene can be used as the genetic marker for pathogenic bacterial resistance trait.

**Keywords:** *b*NRAMP1 gene, Genes structure, SNP, Diseases resistance.

**Received:** 14 October 2018 * **Accepted:** 11 July 2019 * **DOI:** https://doi.org/10.29329/ijiaar.2019.217.17

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## INTRODUCTION

The *bovine* Natural Resistance Associated Macrophage Protein 1 (*b*NRAMP1) has been reported to confer resistance and susceptibility to *Mycobacterium bovis, Salmonella typhimurium* dan *Leishmania donovani* in the mouse (*Mus musculus*) (Vidal et al., 1993). Cosivi et al. (1998) reported that *M. bovis* is the most universal pathogen among mycobacteria and affects many vertebrate animals in African (46%), Asian (44%) and South Ameriacan and the Caribbean countries (35%), sporadic and enzootic occurrences of *bovine* Tuberculosis (*b*TB). The host immune response to *M. bovis* infection is complex; following initial exposure, the bacilli are phagocytosed by host macrophages via transporters such as NRAMP1, encoded by Solute Carrier Family 11 member 1 (SLC11A1) gene (Feng et al., 1996).

The Nramp gene expresses exclusively in the monocyte/macrophage lineage and encodes a phosphoglycoprotein (Vidal et al., 1993) that belongs to an ancient family of membrane proteins (Cellier et al., 1995). Recent evidence supports the idea that one of its roles in mouse macrophages resistant to Mycobacterium bovis is to transport metal ions such as iron into the bacteria containing phagosome where it serves as a catalyst of the Fenton/Haber-Weiss reaction to increase the capacity of the cell to limit bacterial growth (Kuhn et al., 1999; Swilling et al., 1999).

Previous studies reported that some SNP in *b*NRAMP1 gene was associated to *b*TB resistance traits (Estrada-Chavez et al., 2001; Kadarmideen et al., 2011; Paixao et al., 2012; Cheng et al., 2015; Baqir et al., 2016; Liu et al., 2016), mastitis (Joo et al., 2003) and brucellosis resistance (Kumar et al., 2005). In addition, many studies reported that SNPs in the *b*NRAMP1 were occured in intron (Schutta, 2006; Kumar et al., 2011; Ranjan et al., 2011; Liu et al., 2016), exon (Schutta, 2006; Baqir et al., 2016) and 3'UTR (Feng et al., 1996; Horin et al., 1999; Kumar et al., 2005; Schutta, 2006; Kadarmideen et al., 2011) regions.

This article was aimed to explain the SNPs in *b*NRAMP1 gene and its association with some pathogenic microbial diseases incidences based on the previous studies. The results to develop Marker Assisted Selection (MAS) program for pathogenic microbial resistance traits in the future.

### Structure and mutation in the *b*NRAMP1 gene

The *b*NRAMP1 gene was located in chromosome 2q43-44 and consisted of 14 introns and 15 exons (Fig. 1) with length 13,543 bp (GenBank: KR002421). Hence, the coding sequence (CDS) of *b*NRAMP1 gene according to sequence reference (GenBank: KR002421) were presented in Table 1.

Previous studies reported that some SNPs in the *b*NRAMP1 gene can be detected with PCR-RFLP (Joo et al., 2003; Kumar et al., 2011; Baqir et al., 2016; Liu et al., 2016), PCR-SSCP (Coussens et al., 2004; Gonzales et al., 2006; Ranjan et al., 2011), qPCR-RFLP (Joo et al., 2003) and sequencing (Ables et al., 2002; Kumar et al., 2005; Schutta, 2006; Gonzales et al., 2006; Paixao et al., 2012; Cheng et al., 2015; Liu et al., 2016) methods. Moreover, SNP in the three exons (exon 5, 9 and 11) and four introns

(intron 5, 6, 9 and 12) regions can be detected through PCR-RFLP analysis (Table 2). In addition, SNP in the CDS regions of *b*NRAMP1 gene were occured in $2^{nd}$, $5^{th}$, $8^{th}$, $9^{th}$, $10^{th}$, $11^{th}$ and $15^{th}$ exons (Table 3). Hence, SNP in the non-coding regions were occured in $2^{nd}$, $3^{th}$, $4^{th}$, $5^{th}$, $6^{th}$, $8^{th}$, $9^{th}$ and $12^{th}$ introns.

Moreover, Ranjan et al. (2011) obtained three type of sequence in the 3'UTR of *b*NRAMP1 gene (*Bos indicus*) consisted of Pattern I (GenBank: FJ236546), Pattern II (GenBank: 236545) and Pattern III (GenBank: FJ236548). Thus, the alignment results among of these sequence showed 6 SNPs of g.29Y; g.140Y; g.257Aindel.; g.261R; g.323Y and g.344K. Dispite, SNP g.11750T/G was changed the microsatellite pattern from g.11748$(GT)_{12}$11773 to g.11750$(GT)_{11}$11773 (Horin et al., 1999).

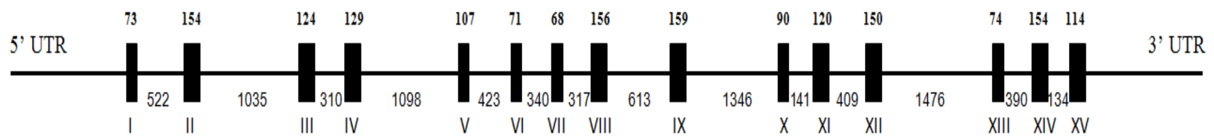


**Figure 1.** Reconstruction of *b*NRAMP1 genes structure. Dark box is the exon region (Schutta, 2006)

**Table 1.** The coding sequences (CDS) of *b*NRAMP1 gene according to GenBank: KR002421

| Coding sequence | Position | Coding sequence | Position |
|---|---|---|---|
| CDS 1 | 1371 - 1377 | CDS 9 | 6927 - 7085 |
| CDS 2 | 1918 - 2051 | CDS 10 | 8437 - 8526 |
| CDS 3 | 3081 - 3203 | CDS 11 | 8668 - 8887 |
| CDS 4 | 3514 - 3633 | CDS 12 | 9197 - 9346 |
| CDS 5 | 4740 - 4846 | CDS 13 | 10823 - 10896 |
| CDS 6 | 5270 - 5340 | CDS 14 | 11287 - 11440 |
| CDS 7 | 5681 - 5748 | CDS 15 | 11575 - 11688 |
| CDS 8 | 6158 - 6313 | | |

**Table 2.** Primers design and restriction enzyme in PCR-RFLP method to detect SNP in *b*NRAMP1 gene

| Primer | Amplicon (bp) | Restriction enzyme | SNP | Region | Amino Acid change | GenBank | Reference |
|---|---|---|---|---|---|---|---|
| F: 5'- TCCGACATGCAGGAAGTCAT -3'<br>R: 5'- GCCGAAGGTCAAGGCCATTATGG -3' | 954 | *Taq*I<br>*Alu*I | g.5008G/T*<br>g.5429A/G* | Intron 5<br>Intron 6 | -<br>- | AY338470 | Kumar et al. (2011) |
| F: 5'- TGGACTGGAGGGTAAGAACG -3'<br>R: 5'- AGGGAGGAATGCAGGTAGATG -3' | 344 | *Bpu*10I | g.6955C/T | Exon 9 | Ala - Val | | |
| F: 5'- ATCTCCTTCCTACTGCCCG -3'<br>R: 5'- CACAAACTGTCCCGCGTAG -3' | 374 | *Pst*I | g.8698C/G | Exon 11 | Pro - Ala | KR002421 | Baqir et al. (2016) |
| F: 5'- TGAGGATCAGTGAGGGAAAAGA -3'<br>R: 5'- AAACTGCTTGCATATTCCCAAC -3' | 998 | *Mae*II | g.6850G/A | Intron 9 | - | | |
| F: 5'- TGTGCTTCACATCTCCTTCCTA -3'<br>R: 5'- AGCACATTGAGCAGGTCGTT -3' | 936 | *Pst*I | g.9106T/A | Intron 12 | - | KR002421 | Liu et al. (2016) |
| F: 5'- CGTGGTGACAGGCAAGGA -3'<br>R: 5'- CGAGGAAGAGGAAGAAGAAAG -3' | 221 | *Msp*I | 4840 to 4843* (mutation type not explained) | Exon 5 | - | U12862 | Joo et al. (2003) |

*SNPs position based on GenBank: KR002421

**Table 3.** Detection SNPs in the *b*NRAMP1 gene based on GenBank: KR002421

| SNP | Region | Amino acid change | Reference | SNP | Region | Amino acid change | Reference |
|---|---|---|---|---|---|---|---|
| g.1925G/A | Exon 2 | Synonymous | 1 | g.5429A/G | Intron 6 | - | 1 |
| g.1997A/G | Exon 2 | His - Arg | 1 | g.6177C/T | Exon 8 | Synonymous | 1 |
| g.2934C/T | Intron 2 | - | 1 | g.6862T/C | Intron 8 | - | 1 |
| g.2995C/T | Intron 2 | - | 1 | g.6955C/T | Exon 9 | Ala - Val | 1 |
| g.3030C/T | Intron 2 | - | 1 | g.8452G/A | Exon 10 | Asp - Asn | 1,4 |
| g.3343G/A | Intron 3 | - | 1 | g.8563Gindel. | Intron 10 | - | 5 |
| g.4694T/G | Intron 4 | - | 2 | g.8566Gindel. | Intron 10 | - | 5 |
| g.4709C/T | Intron 4 | - | 1 | g.8624Gindel. | Intron 10 | - | 5 |
| g.3749G/A | Intron 4 | - | 3 | g.8698C/G | Exon 11 | Pro - Ala | 1,4 |
| g.3774C/T | Intron 4 | - | 3 | g.10810C/G | Intron 12 | - | 1 |
| g.46709C/T | Intron 4 | - | 3 | g.11606C/G | Exon 15 | Arg - Pro | 1 |
| g.4765C/T | Exon 5 | Thr - Ile | 2 | g.11661CAG11665indel. | Exon 15 | - | 4 |
| g.4948T/C | Intron 5 | - | 1 | g.11717A/G | 3'UTR | - | 1 |
| g.5008G/T | Intron 5 | - | 1 | g.11750T/G | 3'UTR | - | 6 |
| g.5010G/A | Intron 5 | - | 1 | 11876(TG)$_n$11903 | 3'UTR | - | 7,8,9 |
| g.5022A/G | Intron 5 | - | 2 | | | | |
| g.1635A/G | Intron 5 | - | 2 | | | | |

[1]Schutta (2006); [2]Ables et al. (2002); [3]Martinez et al. (2008); [4]Paixao et al. (2012); [5]Coussens et al. (2004); [6]Horin et al. (1999); [7]Gonzales et al. (2006); [8]Kumar et al. (2008); Kadarmideen et al. (2011)

**Effect of SNPs in The *b*NRAMP1 gene to Pathogenic bacterial disease resistance trait**

Allen et al. (2010) reported that genetic diversity in the bNRAMP1 gene mainly had association with bTB disease. Cheng et al. (2015) reported that most bTB animals had mutation in the intron 4. Kadarmideen et al. (2011) reported that cattle with SNP g.11876(TG)1511903 had the lowest of bTB incidentes than cattle with SNP g.11876(TG)$_{13}$11903 and SNP g.11876(TG)$_{16}$11903. Baqir et al. (2016) reported that the C allele frequency in SNP g.6955C/T (exon 9) was high in the healthy (0.96) and *b*TB (1.00) cattle. Meanwhile, the G allele frequency in SNP g.8698C/G (exon 11) was high in the healthy (0.71) and *b*TB (0.60) cattle.

Kumar et al. (2011) reported that SNP g.5008G/T (intron 5) and g.5429A/G (intron 6) were not associated with incidences of brucellosis in cattle. Meanwhile, Liu et al. (2016) reported that the G allele frequency in SNP g.6850G/A (intron 9) shows highly in healthy (0.86) and *b*TB (0.52) cattle. Meanwhile, the T allele frequency in SNP g.9106T/A (intron 12) shows highly in healthy (0.66) and *b*TB (0.73) cattle. Paixao et al. (2012) reported that the A allele frequency in SNP g.8452G/A (exon 10) shows highly in healthy (0.57) and *b*TB (0.66) cattle. Meanwhile, the C allele frequency in SNP

g.8698C/G (exon 11) shows highly in healthy (0.67) and *b*TB (0.66) cattle. However, Joo et al. (2003) obtained that the bNRAMP1/MspI gene was monomorphic in healthy and mastitis cattle.

**Conclusion**

The *b*NRAMP1 gene was polymorphic and can be used as genetic marker for pathogenic bacterial resistance trait in cattle. However, the effect of each SNP to many pathogenic bacterial diseases incidences in cattle is important to observe through depth research.

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