PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS
Qingdao (China) - June 15-18, 2016
ISSN 2308-1910

Session
Breeding and Genetics

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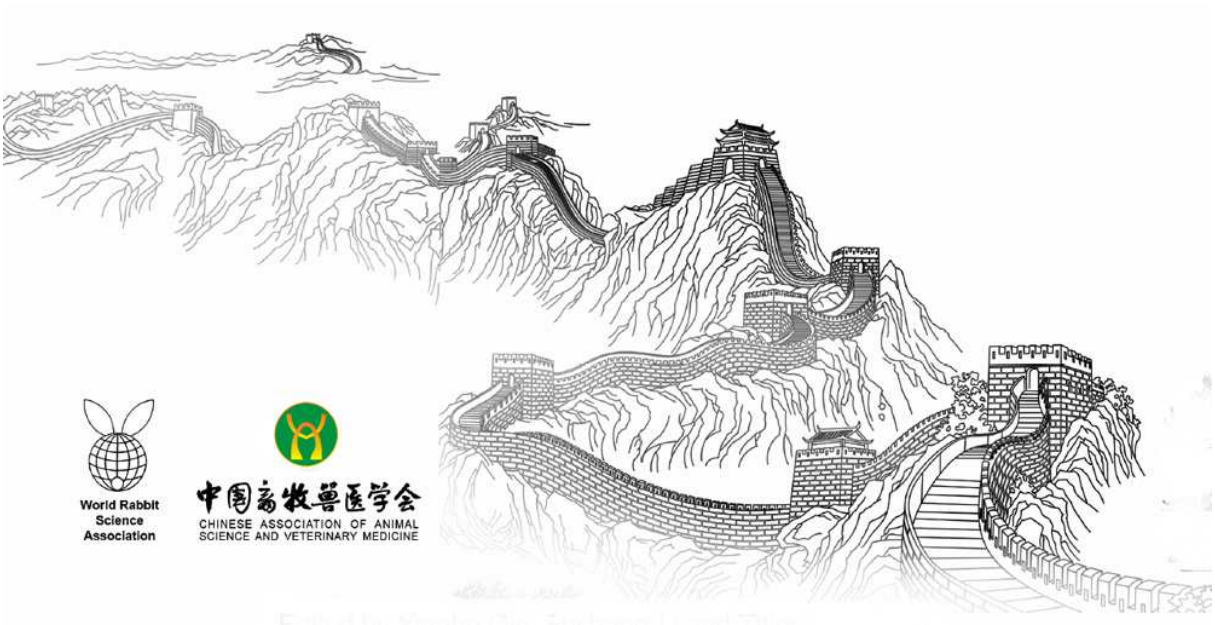
How to cite this paper :
Chen D.J., Sang L., Sun S.K., Chen Y.F., Xie X.P., 2016 - Cloning and sequence analysis of Nramp1 gene in Fujian yellow rabbit.). *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 3-18 + Poster*



CLONING AND SEQUENCE ANALYSIS OF NRAMP1 GENE IN FUJIAN YELLOW RABBIT

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ABSTRACT

The total RNA obtained from the spleen of Fujian yellow rabbit was used to amplify the cDNA for the precursor of Fujian yellow rabbit Nramp1 subunit by RT-PCR, and the opening reading frame of 1635 bps was acquired. The fragments amplified were ligated with T-Vector pMD™20 vector and transformed into the competent *E. coli* Competent cells JM109. The positive clones were verified, sequenced and submitted to GenBank, (accession number: KT943979). Based on the ORF sequence, the upstream and downstream sequences were isolated by 5'- and 3'- RACE, respectively. The full cDNA (2180bp) consists of a 5'-terminal untranslated region (UTR) (103bp), a 3'-terminal UTR (443bp) and an ORF(1635bp). The sequence result was compared with homologous sequences from other animals including human, sheep and mouse. Sequence alignment showed that ranks of their similarities were above 82%.

Key words: Fujian yellow rabbit; RACE; clone; sequence analysis

INTRODUCTION

Nramp1 (Natural resistance-associated macrophage protein 1) gene is a conservative gene with a high degree of amino acid sequence homology and similar secondary structure. It can encode a typical peptide with complete membrane glycoprotein phosphate. 1～8 transmembrane domain which may plays an important role in protein structure and function of Nramp1 family (Hong-mei Wu et al.,2005). The conserved sequence structure of Nramp1 gene and its resistance to disease are non pathogen specific, which is one of the best candidate genes to measure the comprehensive disease resistance of livestock and poultry (Blackwell et al.,1998 ; Govoni et al.,1995). Fujian Yellow Rabbit a Chinese native excellent rabbit breeds show reproductive rate, good carcass quality, wide adaptability, strong stress resistance and many other advantages (Xi-ping Xie et al.,2009). In 2006, it was listed in the Catalogue for protection and conservation of livestock and poultry Genetic Resources in China by the National Commission on livestock and Poultry Genetic Resources. In this study, with Fujian yellow rabbit spleen total RNA as template, rabbits Nramp1 cDNA sequences were determined by RT-PCR and RACE. Thus, the Nramp1 gene might be a candidate gene for wide adaptability and strong stress resistance performance of Fujian yellow rabbit.

MATERIALS AND METHODS

Experiment animals

Animal care and handing were in accordance with the policy on the Care and Use of Animals of the Ethical Committee, Fujian Academy of Agricultural Sciences. Female Fujian Yellow rabbits (90 days) purchased from Yuhuashan Rabbit Breeding Farm of Lianjiang (Fuzhou, China) were raised with a free access to food and water at all time, and maintained on a 12L/12D (light/dark) cycle and in an air-conditioned room.

RNA extraction and quality determination

Total RNA was extracted from Fujian Yellow rabbit spleen tissues by TRIzol (Invitrogen, Carlsbad, CA, USA) according to the standard protocol. The RNA samples were treated with DNase I (TaKaRa,

Japan) for 4 h. RNA was quantified by measuring the absorbance at 260 nm using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., San Jose, CA, USA). The spleen RNA was assessed by the ratio of the absorbance at 260 and 280 nm. The integrity of the RNA samples was examined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Rapid amplification of 5' and 3' cDNA ends (RACE)

Based on the conserved sequence of Nramp1 genes from closely related species, primers（Table 1.）for PCR were designed to amplified an Nramp1 cDNA fragment from rabbit. Cycling parameters for PCR amplification were 94℃ pre denaturation 2 min, 98℃ of denaturation 10 S, 55℃ annealing for 30 S, 72℃ extension for 2 min, 30 cycles，the last 72 ℃ extension for 5 min.The PCR products were gel-purified and sequenced (Fig 1).

Table 1: Primer sequence for RACE.

Primer names	Primer sequence (5′–3′)	Length	Amplification target
F0	CGCAYYVTCCTCTGGCTGAM	20	RT-PCR Outer
R0	GATCKKVGCRTAGTYSTG	18	RT-PCR Outer
F1	TYGTGGGCTCVGAYATGC	18	RT-PCR Inner
R1	SRTTGGTYTKCTKGTAGAA	19	RT-PCR Inner
R2	TCCGCAGTCCGTAGTTGTCG	20	5'RACE Inner PCR
R3	CTCGTAGCCAAAGGTCAAGG	20	5'RACE Outer PCR
F5	CTTCCTCATTGAGGCCAGCAT	21	3'RACE Inner PCR
F6	TTATGCCCCACAACATCTACCTGC	24	3'RACE Outer PCR
F4	AGGTGTGGTGACGGGAAAGGACT	23	RACE sequence verification
R4	AGCAGGTCGTTTAGGCCGGACA	22	RACE sequence verification

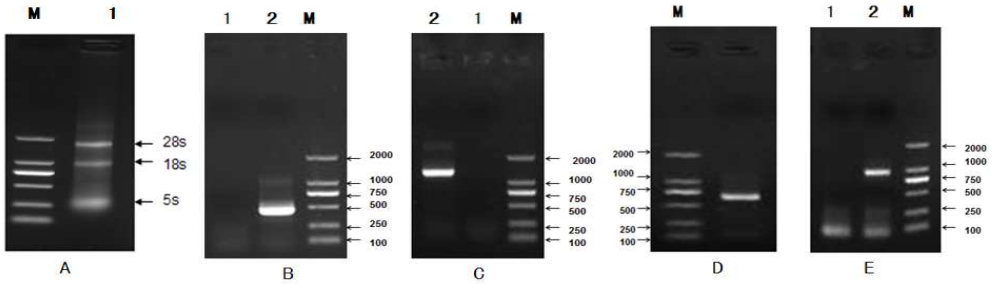

Figure1: Products were detected by gel electrophoresis. A: Total RNA;B: Core sequence of Nramp1. C: 3'RACE of Nramp1; D: 'RACE of Nramp1.; E: Verified PCR products.The first lane is a negative control, and the second lane is the PCR product.

The 3'and 5' ends were obtained by rapid amplification of cDNA ends (RACE) approaches using 3'-Full RACE Core Set with PrimeScript™ RTase and 5'-Full RACE Kit with TAP (TaKaRa, Japan) following the manufacturer's instructions. Primers for 3'-RACE and 5'-RACE were listed in Table 1. The PCR products were ligated into T-Vector pMD^{TM}20 vector (TaKaARa, Japan) and transformed into the competent *E. coli* Competent cells JM109. Positive clones with the expected-size inserts were determined by PCR on clones and DNA sequencing.

Sequence analysis

cDNA sequences of Nramp1 from Fujian Yellow rabbit were blasted to obtain known homologous sequences of Nramp1 genes from other species. Multiple sequences alignment and phylogenetic analysis were performed using MEGA3.1 (Kuwabara et al., 1996).

Figure 2: Sequence of Nramp1 from Fujian Yellow rabbit and its amino acid sequence. There was a classic conserved domains on the Nramp1 protein: A represent MntH_propeptide superfamily ;

RESULTS AND DISCUSSION

The length of rabbit Nramp1 open reading frame was 1635 bp, encoding a protein with 544 amino acids residues. The full-length of nucleotide sequence and the deduced amino acid sequence are shown in Figure 2.

Multiple sequence alignment showed that nucleotide sequence of Fujian Yellow rabbit Nramp1 shared high similarity with other Nramp1 sequence from goat, sheep, cattle, pig, cattle, horse, chicken, mouse and human. Based on the sequence of Nramp1 from different species, a phylogenetic tree was constructed using the software DNAstar MegAlign method (Fig.3.). The relationships of Nramp1 displayed in the phylogentic tree were consistent with the traditional taxonomy of these species. Sequence alignment showed that ranks of their similarities were above 82%, and meanwhile, the results of genetic evolution analysis of the gene showed a fairly high degree of homology between rabbits and big mouse.

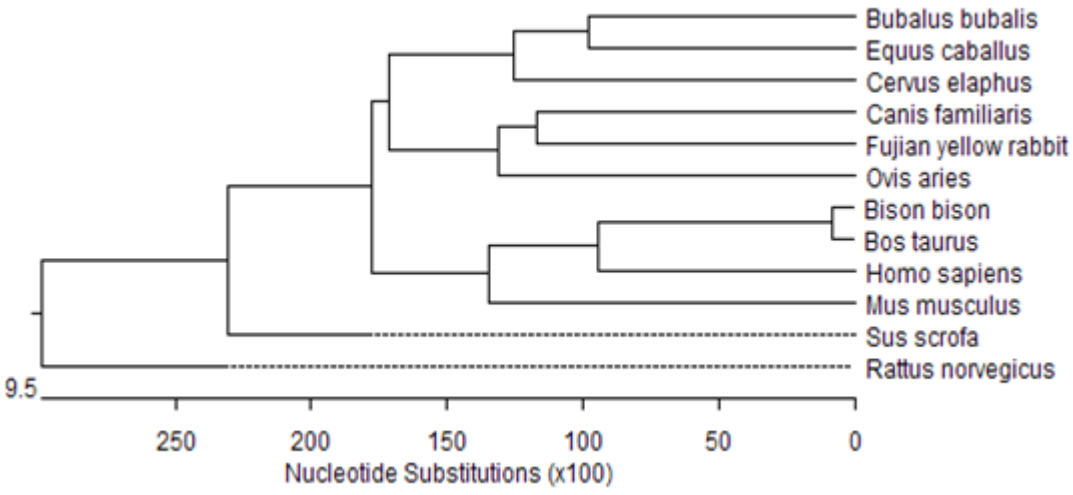

Figure 3: Phylogenetic tree of the nucleotides sequences of from Fujian yellow rabbit and other mammals.

CONCLUSIONS

The full cDNA (2180bp) of Nramp1 gene consists of a 5'-terminal untranslated region (UTR) (103bp), a 3'-terminal UTR (443bp) and an ORF(1635bp). The sequence result was compared with homologous sequences from other animals including human, cattle, sheep, and mouse. Sequence alignment showed that ranks of their similarities were above 86%, and meanwhile, the results of genetic evolution analysis of the gene showed a fairly high degree of homology between rabbits and big mouse.

ACKNOWLEDGEMENTS

This study was supported by National modern agriculture industry technology system (rabbit) (CARS - 44 e - 11), Special scientific research fund of public welfare in Fujian Province（2014R1101002-3）.

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Cloning and Sequence Analysis of Nramp1 Gene in Fujian Yellow Rabbit

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The Message

- Obtain the sequence of cDNA encoding Fujian Yellow Rabbit Nramp1 protein by methods of RT-PCR and RACE.
- Multiple sequences alignment and phylogenetic analysis were performed using MEGA3.1.

Introduction

- The conserved sequence structure of Nramp1 gene and its resistance to disease are non pathogen specific, which is one of the best candidate genes to measure the comprehensive disease resistance of livestock and poultry .
- Fujian Yellow Rabbit a Chinese native excellent rabbit breeds show reproductive rate, good carcass quality, wide adaptability, strong stress resistance and many other advantages.
- Nramp1 gene might be a candidate gene for wide adaptability and strong stress resistance performance of Fujian Yellow Rabbit.

Methods

Experiment animals

Fujian Yellow Rabbits (90 days old) came from Yuhuashan Rabbit Breeding Farm of Lianjiang (Fuzhou, China).

RNA extraction and RT

Total RNA was extracted from Fujian Yellow Rabbit spleen tissues by TRIzol according to the standard protocol. Based on the conserved sequence of Nramp1 genes from closely related species, one pair of primers, were designed to amplified an Nramp1 cDNA fragment from rabbit.

Rapid amplification of 5' and 3' cDNA ends (RACE)

The 3'and 5' ends were obtained by rapid amplification of cDNA ends (RACE) approaches using 3'-Full RACE Core Set with PrimeScript™ RTase and 5'-Full RACE Kit with TAP (TaKaRa, Japan) following the manufacturer's instructions. Primers for 3'-RACE and 5'-RACE were listed in Table 1. The PCR products were ligated into T-Vector pMDTM20 vector (TaKaRa,Japan) and transformed into the competent E. coli Competent cells JM109. Positive clones with the expected-size inserts were determined by PCR on clones and DNA sequencing.

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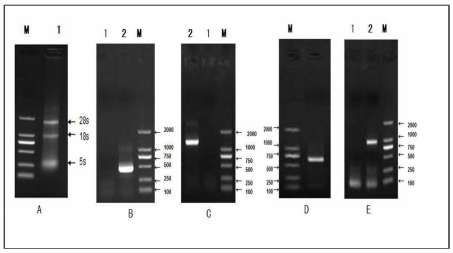
Results

Product of RT-PCR and RACE



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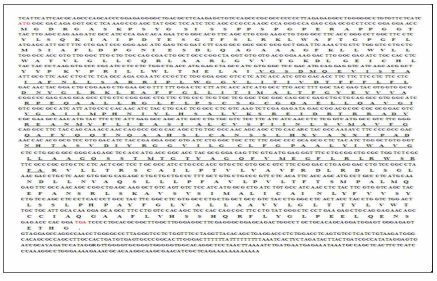

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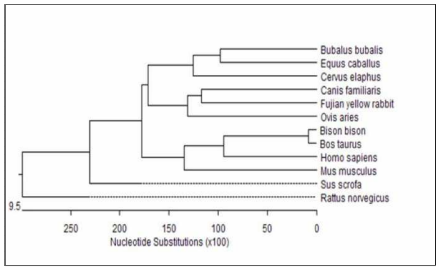

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Acknowledgements

This work was financially supported by Modern Agriculture Technology Research System（CARS-44-E-11）, Fujian Province Public-interest Scientific Institution Basal Research Fund (2014R1101002-3）.