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Cloning and Sequence Analysis of Glyceraldehyde-3-Phosphate Dehydrogenase Gene in Yak*

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ABSTRACT : In order to study the biological function of *gapdh* gene in yak, and prove whether the *gapdh* gene was a useful intra-reference gene that can be given an important role in molecular biology research of yak, the cDNA sequence encoding glyceraldehyde-3-phosphate dehydrogenase from yak was cloned by the RT-PCR method using gene specific PCR primers. The sequence results indicated that the cloned cDNA fragment (1,008 bp) contained a 1,002 bp open reading frame, encoding 333 amino acids (AAs) with a molecular mass of 35.753 kDa. The deduced amino acids sequence showed a high level of sequence identity to *Bos Taurus* (99.70%), *Xenopus laevis* (94.29%), *Homo sapiens* (97.01%), *Mus musculus* (97.90%) and *Sus scrofa* (98.20%). The expression of yak's *gapdh* gene in heart, spleen, kidney and brain tissues was also detected; the results showed that the *gapdh* gene was expressed in all these tissues. Further analysis of yak GAPDH amino acid sequence implied that it contained a complete glyceraldehyde-3-phosphate dehydrogenase active site (ASCTTNCL) which ranged from 148 to 155 amino acid residues. It also contained two conserved domains, a NAD binding domain in its N-terminal and a complete catalytic domain of sugar transport in its C-terminal. The phylogenetic analysis showed that yak and *Bos taurus* were the closest species. The prediction of secondary structures indicated that GAPDH of yak had a similar secondary structure to other isolated GAPDH. The results of this study suggested that the *gapdh* gene of yak was similar to other species and could be used as the intra-reference to analyze the expression of other genes in yak. (**Key Words :** Yak, Glyceraldehyde-3-Phosphate Dehydrogenase gene, Cloning, Housekeeping Gene)

INTRODUCTION

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key glycolytic enzyme that catalyzes the glyceraldehydes-3-phosphate to 1,3-diphosphoglycerate and generates $NADP^+$ that can enter the respiratory chain and generates an ATP in the process of glycolysis. Owing its role in the glycolysis pathway, GAPDH was regard merely as a housekeeping gene (Viscogliosi and Ller, 1998). In recent years, more and more researchers found that GAPDH contains many isomers, the function is not only involved in energy metabolism, but also related with many cellular processes of life, such as apoptosis, neuronal disorders, viralpathogenesis, phosphotransferase activity, membrane fusion, cell endocytosis regulating, microtubule binding, RNA output, DNA replication and DNA repair (Robbins et al., 1995; Sirover, 1999). In the past, people often use GAPDH gene as the intra-reference to analyze the structure of other proteins and the expression of other genes, rarely arouse people's attention. But the latest study found that the mRNA levels of *gapdh* can be extensively regulated, it was found that the expression of *gapdh* gene increased in a variety of cancers, such as breast cancer, liver cancer, pancreatic cancer and renal cell carcinoma (Katherine et al., 1992; Vila et al., 1992; Gong et al., 1996; Yamagata et al., 1998; Schek et al., 1988; Haque et al., 2000).

The Yak (*Bos grunniens*) is a remarkable animal developed on the Qinghai-Tibetan Plateau that often called the "roof of the world" and was described by Miller (Miller, 1994) as the most extensive high-elevation region on earth. It is clear that the yak is different from *Bos taurus* but close to the American bison and because its adaptation to life on the plateau many adaptability changes may influence its genetic material (Gerald et al., 2003). It implies that some genes of yak may be differs from *Bos Taurus* in many ways. Although the GAPDH gene has been cloned in *Bos taurus*, but there is no report related to the cloning of Yak *gapdh* cDNA fragment. Here we report the cloning of cDNA

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sequence of Yak *gapdh* gene, the bioinformatics analysis of this sequence and the expression detection of *gapdh* gene in heart, liver, spleen, kidney and brain tissues.

MATERIALS AND METHOD

Yak tissues

Liver, heart, spleen, kidney and brain tissue samples of yak were collected from a newly slaughtered 3.5 years old healthy male yak in northwest plateau of Sichuan province. Each tissue sample was cut into 0.5-1.0 cm^3 cubes, rinsed by DEPC treated water, put the samples into 1.5 ml tube, and add 1 ml Sample Protector (Takara Biotechnology Co., Ltd.) into each tube. To avoid RNA degradation samples were frozen in liquid nitrogen immediately until further treatment.

Extraction of total RNA

Total RNA was extracted from yak liver, heart, spleen, kidney and brain tissue samples by using RNA reagent kit (Takara Biotechnology Co., Ltd.) according to the instruction. The extracted RNA were treated with DNaseI to avoid contamination of total DNA that may disturb the results and all the RNA samples were dissolved in DEPC water, stored at -80°C. The purity and concentration of each RNA sample were detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

Reverse transcription and polymerase chain rection

Total RNA (400 ng) were reversely transcribed by SMART technology (Takara Biotechnology Co., Ltd.) according to the instruction of the kit. The primer F: 5'-GACAAGATGGTGAAGGTCGGA-3' and primer R: 5'-TTACTCCTTGGAGGCCATGTG-3' were designed and used to amplify the cDNA of yak's *gapdh* gene according to the *gapdh* gene mRNA sequence of cattle (*Bos Taurus*, accession number: BC102589). The primary synthesized ds-cDNA from liver was used as template for PCR amplification. The reaction system were as follows: template 1 μl, 10×Taq buffer 2 μl, dNTP Mix (2.5 mmol) 1 μl, primer F 1 μl, primer R 1 μl, Taq DNA polymerase 0.2 μl, ddH_2O 11.8 μl, the total volume was 20 μl. PCR was performed with the following program: 94°C for 2 min followed by 31 cycles of 94°C 1 min, 63°C 1 min, 72°C 90 s, and a final extension for 5 min at 72°C.

Cloning of *gapdh* gene

The amplified DNA fragments were isolated by 1.0% agarose gel electrophoresis and then were purified by using Agarose Gel DNA Purification Kit (Takara Biotechnology Co., Ltd.). The purified PCR product was cloned into the pMD18-T vector (Takara Biotechnology Co., Ltd.) and then transferred into the *E. coli* JM109. The recombinant plasmid was named as pMD18-T-GAPDH and then the inserted cDNA fragment was sequenced by Invitrogen (Shanghai) company.

Detection of *gapdh* gene in different tissues

The expression pattern of *gapdh* gene was detected in different tissues by using RT-PCR method. Total RNA of heart, spleen, kidney and brain were extracted and then synthesized into ds-cDNA by the same method used in previous step. Each ds-cDNA sample was used as the template in RT-PCR analysis and the reaction system and PCR procedure were the same as *gapdh* amplification procedure in liver tissue. β-actin (*Bos Taurus*, accession number: BT030480) was used as loading control in this experiment. β-actin primer F: 5′- GATGTGGATCAG CAAGCA-3′; primer R: 5′- CCTTCACCGTTCCAGTTT-3′. PCR was performed with the following program: 94°C for 2 min followed by 30 cycles of 94°C 30 s, 52°C 30 s, 72°C 30 s, and a final extension for 5 min at 72°C.

Sequence analysis of *gapdh* gene

The sequenced cDNA fragment was compared to nonredundant (nr) database at the National Center for Biotechnology Information (NCBI; Bethesda, MD) using the Blastx algorithm (Altschul et al., 1997). Function-point analysis was conducted by online tools motifs-PROSITE (http://cn.expasy.org/tools/scanprosite/). The conserved domain was searched against the NCBI database (Http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The secondary structure of GAPDH was predicted by online service (http://imtech.res.in/raghava/apssp/). The phylogenetic tree map was drawn by DNAMAN software on the basis of amino acid sequence of GAPDH in 6 species.

RESULT

Amplification and cloning of *gapdh* gene

The synthesized ds-cDNA products from liver tissue were used as template for PCR reaction and *gapdh* gene fragment was amplified by using gene specific primers. The expected length of the amplified *gapdh* gene fragment is 1,008 bp. The results showed that a 1,000 bp length fragment was obtained, and it was inserted into pMD18T vector. The recombinant plasmid was digested by *Eco*RI and *Hind*III, and detected by PCR amplification to determine whether the PCR product was ligated into T-vector. The results indicated that both restriction enzyme digestion and PCR amplification can produce a 1,000 bp length DNA fragment. The subsequent sequencing and analysis showed that the inserted fragment was *gapdh* gene and its length was 1,008 bp. An open reading frame spans

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1      ATGGTGAAGGTCGGAGTGAACGGATTCGGCCGCATCGGGCGCCTGGTCACCAGGGCTGCT
1       M  V  K  V  G  V  N  G  F  G  R  I  G  R  L  V  T  R  A  A
                  β1       c        α1
61     TTTAATTCTGGCAAAGTGGACATCGTCGCCATCAATGACCCCTTCATTGACCTTCACTAC
21      F  N  S  G  K  V  D  I  V  A  I  N  D  P  F  I  D  L  H  Y
              c     β2          c
121    ATGGTCTATATGTTCCAGTATGATTCCACCCACGGCAAGTTCAACGGCACAGTCAAGGCA
41      M  V  Y  M  F  Q  Y  D  S  T  H  G  K  F  N  G  T  V  K  A
           α2          c           α3
181    GAGAACGGGAAGCTCGCCATCAATGGAAAGGCCATCACCATCTTCCAGGAGCGAGATCCT
61      E  N  G  K  L  A  I  N  G  K  A  I  T  I  F  Q  E  R  D  P
        c     β3          β4          c
241    GCCAACATCAAGTGGGGTGATGCTGGTGCTGAGTATGTGGTGGAGTCCACTGGGGTCTTC
81      A  N  I  K  W  G  D  A  G  A  E  Y  V  V  E  S  T  G  V  F
           c        β5       c     β6       c
301    ACTACCATGGAGAAGGCTGGGGCTCACTTGAAGGGTGGCGCCAAGAGGGTCATCATCTCT
101     T  T  M  E  K  A  G  A  H  L  K  G  G  A  K  R  V  I  I  S
           c     α4       c     α5       c     β7
361    GCACCTTCTGCCGATGCCCCATGTTTGTGATGGGTGTGAACCACGAGAAGTATAACAAC
121     A  P  S  A  D  A  P  M  F  V  M  G  V  N  H  E  K  Y  N  N
                 c                 c     α6
421    ACCCTCAAGATTGTCAGCAATGCCTCCTGCACCACCAACTGCTTGGCCCCCCTGGCCAAG
141     T  L  K  I  V  S  N  A  S  C  T  T  N  C  L  A  P  L  A  K
           c     β8       c                 α7
481    GTCATCCATGACCACTTTGGCATCGTGGAGGGACTTATGACCACCGTCCACGCCATCACT
161     V  I  H  D  H  F  G  I  V  E  G  L  M  T  T  V  H  A  I  T
                                      β9
541    GCCACCCAGAAGACTGTGGATGGCCCCTCCGGGAAGCTGTGGCGTGACGGCCGAGGGCT
181     A  T  Q  K  T  V  D  G  P  S  G  K  L  W  R  D  G  R  G  A
           c                 c                 β10
601    GCCCAGAATATCATCCCTGCTTCTACTGGCGCTGCCAAGGCCGTGGGCAAGGTCATCCCT
201     A  Q  N  I  I  P  A  S  T  G  A  A  K  A  V  G  K  V  I  P
              c        β11      c        α8
661    GAGCTCAACGGGAAGCTCACTGGCATGGCCTTCCGCGTCCCCACTCCCAACGTGTCTGTT
221     E  L  N  G  K  L  T  G  M  A  F  R  V  P  T  P  N  V  S  V
           c  α9       c           β12            c
721    GTGGATCTGACCTGCCGCCTGGAGAAACCTGCCAAGTATGATGAGATCAAGAAGGTGGTG
241     V  D  L  T  C  R  L  E  K  P  A  K  Y  D  E  I  K  K  V  V
              β13          c                 α10
781    AAGCAGGCGTCAGAGGGCCCTCTCAAGGGCATTCTAGGCTACACTGAGGACCAGGTTGTC
261     K  Q  A  S  E  G  P  L  K  G  I  L  G  Y  T  E  D  Q  V  V
                       c              β14         c
841    TCCTGCGACTTCAACAGCGACACTCACTCTTCTACCTTCGATGCTGGGGCTGGCATTGCC
281     S  C  D  F  N  S  D  T  H  S  S  T  F  D  A  G  A  G  I  A
              α11          c              β15         c
901    CTCAACGACCACTTTGTCAAGCTCATTTCCTGGTACGACAATGAATTCGGCTACAGCAAC
301     L  N  D  H  F  V  K  L  I  S  W  Y  D  N  E  F  G  Y  S  N
           β16     c           β17          c
961    AAGGTGGTGGACCTCATGGTCCACATGGCCTCCAAGGAGTAA
321     K  V  V  D  L  M  V  H  M  A  S  K  E  *
                    α12
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Figure 1. The conding sequence, deduced amino sequence and the secondary structure of yak *gapdh* gene. The secondary structure was showed in amino sequence: Bold-Strand; Underline-Helix; Italic-Coil. The asterisk (*): Stop codon.

over 1,002 bp, encodes a protein of 333 amino acids (Figure 1). The deduced GAPDH molecular mass was 35.753 kDa with an isoelectric point of 8.57. Then the DNA sequence of *gapdh* gene was deposited in GenBank (accession no. EU195062).

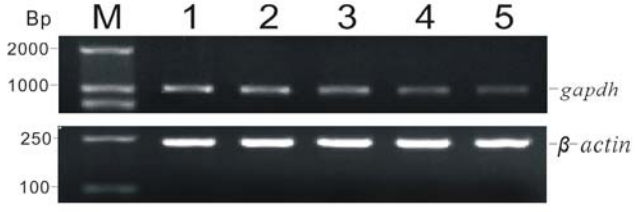

Figure 2. Expression of *gapdh* gene in different tissues. Lane M: Marker; Lanes 1, 2, 3, 4 and 5: RT-PCR products amplified from liver, heart, kidney, spleen and brain, respectively by using *gapdh* and β-actin specific primers.

Analysis of *gapdh* gene expression in different tissues

To characterize *gapdh* gene expression patterns in different tissues, RNA of heart, spleen, kidney and brain tissue samples were also isolated and the ds-cDNA synthesis were performed by the same method performed in liver samples, and *gapdh* gene specific primer was used for PCR amplification. The results showed that the expression of *gapdh* gene can be detected in all of the samples, but the expression level was varied in different tissues. As shown in Figure 2, the gene expressed in different development tissues, including the liver, heart, spleen, kidney and brain. The *gapdh* expression was, however, substantially weaker in spleen and brain, although it is difficult to precisely quantify the amount of RNA by RT-PCR. The load control

Table 1. The different nucleic acids and amino acid between *Bos grunniens* and *Bos Taurus*

Items	Nucleic acids site					Amino acid site
	196	395	524	947	961	65
Bos grunniens（EU195062）	C	T	C	C	A	A
Bos Taurus（BC102589）	T	C	T	T	G	N

β-actin PCR product was 232 bp. This observation suggests that the *gapdh* gene may play different roles in different tissues.

Sequence analysis of *gapdh* gene

The derived sequence was compared with the public nucleotide and protein database (GenBank) by using the Blastn and Blastx algorithms (Altschul et al., 1997). Blastn analysis showed that the nucleic acid sequences similarity between yak and *Bos Taurus* GAPDH was 99.40%, only 5 nucleic acids were different (Table 1). Blastx analysis compared with other species indicated that there has high sequence similarity (99.70%) between yak GAPDH and cattle, only 1 amino acid was different (Table 1). The deduced amino acid sequence analysis showed that the identity of yak GAPDH amino acid sequence with *Bos taurus, Xenopus laevis, Homo sapiens, Mus musculus* and *Sus scrofa* were 99.70%, 94.29%, 97.01%, 97.90% and 98.20%, respectively (Figure 3). All proteins are 333 amino acids in length except *Xenopus laevis*. A rooted

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BOS_GRUNNIENS..MVKVGVNGFGRIGRLVTRAAFNSGKVDIVAINDPFIDLHYMVYMFQYDSTHGKFNGTV    58
BOS_TAURUS    ..MVKVGVNGFGRIGRLVTRAAFNSGKVDIVAINDPFIDLHYMVYMFQYDSTHGKFNGTV    58
HOMO_SAPIENS  MGKVKVGVNGFGRIGRLVTRAAFNSGKVDIVAINDPFIDLNYMVYMFQYDSTHGKFHGTV    60
MUS_MUSCULUS  ..MVKVGVNGFGRIGRLVTRAAICSGKVEIVAINDPFIDLNYMVYMFQYDSTHGKFNGTV    58
SUS_SCROFA    ..MVKVGVNGFGRIGRLVTRAAFNSGKVDIVAINDPFIDLHYMVYMFQYDSTHGKFHGTV    58
XENOPUS_LAEVIS.MVKVGINGFGRIGRLVTRAAFDSGKVQVTAINDPFIDLDYMVYMFKYDSTHGRFKGTV    58
Consensus       vkvg ngfgrigrlvtraa  sgkv  aindpfidl ymvymf ydsthg f gtv

                  65th AAS
                     ↓
BOS_GRUNNIENSKAENGKLAINGKAITIFQERDPANIKWGDAGAEYVVESTGVFTTMEKAGAHLKGGAKRVI   118
BOS_TAURUS    KAENGKLVINGKAITIFQERDPANIKWGDAGAEYVVESTGVFTTMEKAGAHLKGGAKRVI   118
HOMO_SAPIENS  KAENGKLVINGNPITIFQERDPSKIKWGDAGAEYVVESTGVFTTMEKAGAHLQGGAKRVI   120
MUS_MUSCULUS  KAENGKLVINGKPITIFQERDPTNIKWGEAGAEYVVESTGVFTTMEKAGAHLKGGAKRVI   118
SUS_SCROFA    KAENGKLVINGNPITIFQERDPAKIKWGDAGAEYVVESTGVFTTMEKAGAHLKGGAKRVI   118
XENOPUS_LAEVISAAENGKLIINDQVITVFQERDPSNIKWGDAGAVYVVESTGVFTTTEKASLHLKGGAKRVV   118
Consensus     kaengkl in  it fqerdp  ikwg aga yvvestgvftt eka  hl ggakrv

                                  148-155 AAS
                                  ┌──────┐
BOS_GRUNNIENSISAPSADAPMFVMGVNHEKYNNTLKIVSNASCTTNCLAPLAKVIHDHFGIVEGLMTTVHA   178
BOS_TAURUS    ISAPSADAPMFVMGVNHEKYNNTLKIVSNASCTTNCLAPLAKVIHDHFGIVEGLMTTVHA   178
HOMO_SAPIENS  ISAPSADAPMFVMGVNHEKYDNSLKIISNASCTTNCLAPLAKVIHDNFGIVEGLMTTVHA   180
MUS_MUSCULUS  ISAPSADAPMFVMGVNHEKYDNSLKIVSNASCTTNCLAPLAKVIHDNFGIVEGLMTTVHA   178
SUS_SCROFA    ISAPSADAPMFVMGMNHEKYDNSLKIISNASCTTNCLAPLAKVIHDNFG VEGLMTTVHA   178
XENOPUS_LAEVISSAPSADAPMFVVGVNHEKYENSLKVVSNASCTTNCLAPLAKVINDNFGIVEGLMTTVHA   178
Consensus     isapsadapmfv g nheky n lk  snascttnclaplakvi d fgiveglmttvha

                                  ┌──────┐
                                  active site
BOS_GRUNNIENSITATQKTVDGPSGKLWRDGRGAAQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTPNV   238
BOS_TAURUS    ITATQKTVDGPSGKLWRDGRGAAQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTPNV   238
HOMO_SAPIENS  ITATQKTVDGPSGKLWRDGRGALQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTANV   240
MUS_MUSCULUS  ITATQKTVDGPSGKLWRDGRGAAQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTPNV   238
SUS_SCROFA    ITATQKTVDGPSGKLWRDGRGALQNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTANV   238
XENOPUS_LAEVISTATQKTVDGPSGKLWRDGRGAGQNIIPASTGAAKAVGKVIPELNGKITGMAFRVPTPNV   238
Consensus      tatqktvdgpsgklwrdgrga qniipastgaakavgkvipelngk tgmafrvpt nv


BOS_GRUNNIENSVVDLTCRLEKPAKYDEIKKVVKQASEGPLKGILGYTEDQVVSCDFNSDTHSSTFDAGAG   298
BOS_TAURUS    VVDLTCRLEKPAKYDEIKKVVKQASEGPLKGILGYTEDQVVSCDFNSDTHSSTFDAGAG   298
HOMO_SAPIENS  VVDLTCRLEKPAKYDDIKKVVKQASEGPLKGILGYTEHQVVSSDFNSDTHSSTFDAGAG   300
MUS_MUSCULUS  VVDLTCRLEKPAKYDDIKKVVKQASEGPLKGILGYTEDQVVSCDFNSNSHSSTFDAGAG   298
SUS_SCROFA    VVDLTCRLEKPAKYDDIKKVVKQASEGPLKGILGYTEHQVVSSDFNSDTHSSTFDAGAG   298
XENOPUS_LAEVISVVDLTCRLQKPAKYDDIKAAIKTASEGPMKGILGYTQDQVVSTDFNGDTHSSIFDADAG   298
Consensus     svvdltcrl kpakyd ik   k asegp kgilgyt  qvvs dfn   hss fda ag


BOS_GRUNNIENSIALNDHFVKLISWYDNEFGYSNRVVDLMVHMASKE                          333
BOS_TAURUS    IALNDHFVKLISWYDNEFGYSNRVVDLMVHMASKE                          333
HOMO_SAPIENS  IALNDHFVKLISWYDNEFGYSNRVVDLMAHMASKE                          335
MUS_MUSCULUS  IALNDNFVKLISWYDNEYGYSNRVVDLMAYMASKE                          333
SUS_SCROFA    IALNDHFVKLISSYDNEFGYSNRVVDLMVHMASKE                          333
XENOPUS_LAEVISIALNENFVKLVSWYDNECGYSHRVVDLMCHMASKE                          333
Consensus     ialn  fvkl s ydne gys rvvdlm  maske
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Figure 3. The alignment of *Bos grunniens* GAPDH with known GAPDH in other species. The amino acids are numbered at the right side of each row. Identical amino acids are indicated by 'Consensus letter'. The different amino acid between yak and cattle was showed by number upside the amino acid residue. The GAPDH active site is underlined (ranged from 148 to 155 AAS).

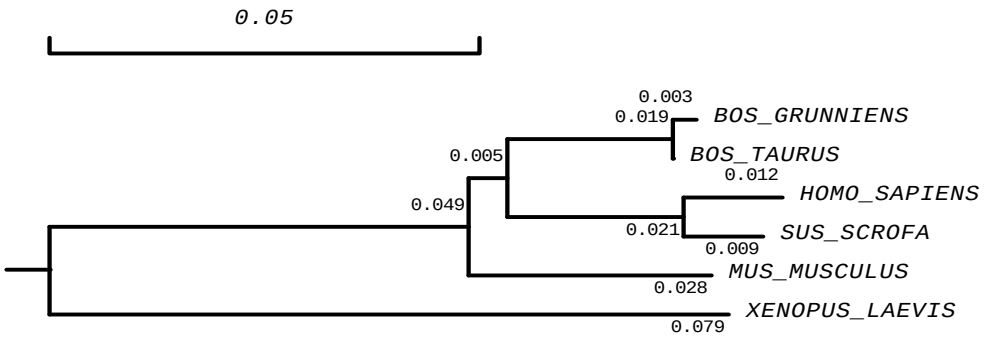

Figure 4. Analysis of phylogeny (the number indicate branch length). A rooted phylogenetic tree calculated by DNAMAN software showed that the genetic distance of *Bos Tarrus* and *Bos Grunniens* were the closet. Conversely, the distance between Bos Grunniens and Xenopus Laevis was the farthest.

phylogenetic tree was calculated based on an alignment of amino acid sequences by using the maximum likelihood method implemented in the DNAMAN software, and phylogenetic tree (Figure 4) analysis found the phylogenetic relationship between GAPDH of yak and *Bos taurus* was closest.

Function-point analysis showed that the predicted 'ASCTTNCL' (148-155 sites) polypeptide amino acid sequence was an active site of yak GAPDH to be as a further proof of the cloned sequence was *gapdh* gene (Figure 3). Further analysis of yak GAPDH amino acid sequence implied that it contains a complete NAD (P) binding domain in its N-terminal, a complete catalytic domain of sugar transport in its C-terminal (Figure 5). Secondary structure prediction indicated that there are several α-helix and β-sheet in yak GAPDH protein sequence (Figure 1).

DISCUSSION

In recent years, gene expression pattern from glycolytic pathway of cattle has been thoroughly studied (Hocquette et al., 1996; Kim et al., 2001; Lee, 2007). As a key glycolytic enzyme in glycolysis GAPDH has also been thoroughly studied. However, in addition to its function in glycolysis, GAPDH was shown in various tissues of other animals to be involved in functions unrelated to glycolysis (Martin et al., 1993). It may play an important role in gene transcription, DNA replication, DNA repair (Sirover, 1999), and nuclear RNA exportation, when GAPDH existing in nucleus (Singh and Green, 1993; Zang et al., 1998), and the other functions of phosphate group transfer of this enzyme were also reported (Engel, 1998; Tisdale, 2002). Although GAPDH was a highly conserved protein, its subcellular localization (Mazzola and Sirover, 2003) determines its different biological function. GAPDH distributed in the cell membrane of the endosome mainly reacted in the macromolecules transport, the cytoplasm GAPDH involved in glycolysis metabolism and mainly produced energy (ATP), this kind of GAPDH can bind with the 5'UTR, 3'UTR mRNA in order to control gene expression through the transcriptional regulation and the endonuclear GAPDH can also control gene expression through the regulation of transcriptional, even more the GAPDH can transmit the genetic information through the replication of DNA and also can be passed on DNA repair maintain genomic stability (Sirover et al., 1997). Here we report the cloning, sequences analysis and expression detecting of yak *gapdh* gene. Its cDNA sequence contains a 1002bp open reading frame. Sequence data were searched against the GenBank database using BlastX. As expected, the results showed that there was high level of sequence identity between yak *gapdh* gene and cattle, Xenopus, humans, mice and pigs. It indicated that GAPDH is a conserved gene.

Sequence analysis of yak GAPDH showed its molecular mass was 33.6 KDa and the isoelectric point was 8.57; Evolutionary tree analysis indicated that the pro-source relationship between Yak and cattle GAPDH was closest, indicated that they had the same evolutionary ancestor. The predicted proteins structures compose mainly by the Helix,

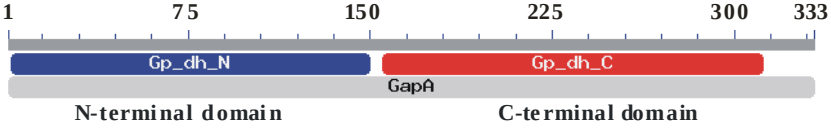

Figure 5. The conserved domain of GAPDH. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a tetrameric NAD-binding enzyme involved in glycolysis and glyconeogenesis. N-terminal domain is a Rossmann NAD (P) binding fold that range from 2-150 amino acids and C-terminal domain is a mixed alpha/antiparallel which range from 155-312 amino acids.

Sheet and Coil. The N-terminal protein contains a complete NAD binding domain (2-150 AAS); C-terminal protein contains a complete catalytic domain of sugar transport and metabolism (155-312 AAS). It implied that the GAPDH of yak perform the same function just like it act in another mammalian animals.

In this study, the expression patterns of *gapdh* gene in different tissue were detected by RT-PCR method. RT-PCR is a high sensitive method to detect the expression level of mRNA (Gianni et al., 2005). But, it is important to select a control gene that is stably expressed in different tissues or in both processed and unprocessed samples when we detected the gene expression level using RT-PCR method. *Gapdh* gene has been always considered and used as an internal standard gene. This study confirmed that *gapdh* gene was expressed in liver, heart, spleen, brain and kidney tissues of yak, but the results also showed some variations of its expression level in different tissues. It reflected such a fact that *gapdh* gene may play different roles in different tissues. Although it still can be use as an intra-reference gene when study the gene expression pattern in the same tissue under different treatment, whether it can be use as a reference gene to compare the gene expression level among different tissues for semi-quantitative RT-PCR in yak needs further discussing. However, we noted that GAPDH gene was deemed a useful model (Berry and Boulton, 2000) to control gene expression and the relationship between proteins functions and amino acid sequences; which did not attract sufficient attention. So far, the GAPDH biological function of yak has not yet been fully clarified. Future work in our lab will aim at studying the biological function *gapdh* gene in yak.

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