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Molecular cloning and characterization of glucose transporter 1 (glut1) and citrate synthase cDNA in buffalo (*Bubalus bubalis*)

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Glucose transporter type-1 (glut1) and citrate synthase plays crucial role in glucose transport and regulation of tricarboxylic acid cycle (TCA) cycle in mammalian energy metabolism. The present study was aimed to clone and characterize glut1 and citrate synthase cDNA in water buffalo (*Bubalus bubalis*). Total of 90 cumulus oocyte complexes (COCs) were used for mRNA isolation and reverse transcribed to cDNA, which was further used in polymerase chain reaction (PCR) amplification of glut1 and citrate synthase. PCR products of glut1 and citrate synthase were cloned by T/A cloning using pGEM-T easy vector and further sequenced. Gene sequence analysis of glut1 and citrate synthase revealed that they have open reading frame of 1479 (encoding 492 aa) and 1401 bp (encoding 466 aa), respectively. Further phylogenic analysis of gene and deduced amino acid sequences suggests that bubaline glut1 shares ~ 89 to 98% and ~ 97 to 99% similarity at nucleotide and amino acid level respectively whereas citrate synthase shared ~ 89 to 99% at nucleotide and ~ 96 to 99% at amino acid level respectively with other domestic species and human. Predicted protein structures of buffalo glut1 protein accentuate the presence of crucial amino acids involved in glucose transport moreover the essential catalytic residues are highly conserved in buffalo citrate synthase.

Key words: Buffalo, cloning, characterization, Glut1, citrate synthase.

INTRODUCTION

Buffalo (*Bubalus bubalis*) constitutes the major pillar of the dairy and meat industry of India contributing a large share to agricultural gross domestic product (GDP), maintenance and improvement of such an important domestic species requires an elaborate database. The gene sequence data, sequence homology and phylogenetic relationship with different domestic species clearly depict the functional differences at gene and protein level. Citrate synthase is a key regulatory metabolic enzyme that catalyzes the first step in tri-carboxylic acid (TCA) cycle, a potential regulator of aerobic energy production in highly dividing cells and is present in virtually all the cells capable of oxidative metabolism, therefore, a critical enzyme in cellular biosynthesis (Weitzman et al., 1976). Porcine cardiac tissue derived form of citrate synthase is most widely studied (Bloxham et al., 1981 and Evans et al., 1988) and it has now been isolated from numerous sources. Most common form of citrate synthase consists a dimer of molecular weight 90,000 to 100,000 (Srere, 1975; Singh et al., 1970) however, a tetrameric form with larger subunit of molecular weight (55,000 to 60,000) was also reported in some prokaryotic organisms (Weitzman, 1976). Bovine heart derived citrate synthase cDNA sequence encodes a 466 amino acids containing protein

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Table 1. Oligonucleotide primers for gene specific RT-PCR amplification.

Gene of interest	Primer sequence	Annealing temp (°C)	Fragment size (bp)	Reference /Gene bank accession number
Glut-1 primer1	5'AGAGGGAGGCCAAGAGAGTC 3' CCTTCACTGTCGTGTCGCTA	54	1091	M60448.1
Glut-1 primer 2	5'GTCACCATCCTGGAGCTGTT 3' TACCCCAAGAGGTGGCTATG	54	1161	M60448.1
Citrate synthase	5'GCCATGGCTTTACTCACTGC 3' CCTCCCCATCTTCAGTTTCA	60	1421	NM_001044721.1
β-Actin	5'GACGATGCTCCCCGGGCCGTCT 3'ATGGGGTACTTGAGGGTCAGGA	51	143	BT030480

and it shares 95.1 and 96.3% similarity with human and porcine sequences respectively (Winger et al., 2000). Earlier reports showed that the alteration in several catalytic residues within citrate synthase leads to decrease in catalytic efficiency of the enzyme (Remington, 1992; Evans et al., 1996; Kurz et al., 1998; Mulholland and Richards, 1998).

Glucose transporter 1 (glut1), also known as solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1) is a protein that in humans is encoded by the *SLC2A1* gene (Mueckler et al., 1985)]. Glut-1 is the most ubiquitous glut isoforms present in mammalian cells and tissues (Scheepers et al., 2004; Wood and Trayhurn, 2003) like erythrocytes, placenta, fetal tissues, brain, endothelia and many immortalized cell lines. GLUT1 facilitates the transport of glucose across the plasma membranes of mammalian cells (Olson and Pessin, 1996). Currently, 13 members of the facilitated glucose transporter family (GLUTs) have been identified in mammals (Joost and Thorens, 2001; Zhao and Keating, 2007). Earlier cloning and sequencing study of glut1 cDNA in chicken showed that it shares 95% amino acid sequence similarity to mammalian gluts (Wagstaff et al., 1995).

In our laboratory, heat shock protein HSP70.1 (*HSPA-1A*) was cloned and characterized in buffalo embryos (Sharma et al., 2012). The present study aimed the cloning and characterization of glut 1 and citrate synthase cDNA sequences in buffalo (*B. bubalis*).

MATERIALS AND METHODS

Ooocyte collection and mRNA extraction

Ovaries at random stages of the estrous cycle were collected from local abattoir and transported at 32 to 37°C in 0.9% normal saline to the laboratory within 2 h. Ovaries were rinsed well in pre-warmed phosphate buffer saline (PBS) fortified with antibiotics (75 mg/L penicillin-G, 50 mg/L streptomycin sulphate). Cumulus oocytes complexes (COCs) were collected by aspiration of antral follicles (5 to 8 mm diameter) with 18 gauge needle adapted to 5 ml syringe. COCs (with three to four layer of cumulus) were collected in RNAlater solution (Ambion, Inc, USA) until RNA isolation.

The mRNA was isolated from ninety (90) morphologically good quality oocytes (cumulus oocytes complexes) briefly, cells were lysed in lysis solution and cell lysate was incubated with dynabeads oligo $(dT)_{25}$ for formation of 'Dynabead-mRNA complex'. The 'Dynabead-mRNA complex' was washed twice in 100 µl of each with washing buffer (A) and (B) supplied with the kit and finally eluted in 20 µl of elution volume. For removal of DNA, the eluted mRNA sample was treated with DNAase I as per the manufacturer's protocol (Quigen, GmbH Hilden, Germany). Quality and concentration of isolated mRNA was assessed by Nano-drop (Thermo Scientific, USA).

cDNA synthesis

Reverse transcription was carried out using revert aid kit (Fermentas, Maryland, USA) according to the manufacturer's instructions. Briefly, cDNA was synthesized in a total of 20 µl reaction volume; using 800 ng of RNA template and oligo-dT primers. Reaction was reverse-transcribed using Molony–Murine Leukemia Virus Transcriptase (MMLV-RT) (Fermentas, Maryland, USA) by incubating at 70°C for 5 min followed by incubation at 25°C for 5 min, 42°C for 60 min and finally reaction was stopped by incubating the reaction for 10 min at 70°C. The quality of cDNA was assessed by an amplification reaction for a housekeeping gene β-actin, and amplified product was resolved on 1.5% agrose gel.

Primer designing for RT-PCR

A set of primers for RT-PCR amplification of glut1, citrate synthase and β-actin gene were designed from the conserved coding region of *Bos taurus* sequences. All the primers for RT-PCR were synthesized using primer 3 (v. 0.4.0) software available online through slight adjustment in default parameters set up; quality was checked on primer select software (DNA STAR, USA). Primer sequences, amplicon size, annealing temperature and gene bank accession are listed in Table 1.

Cloning, sequencing, sequence homology and phylogenetic analysis

Primers were synthesized as two primer pairs and single primer pair from bovine (*B. taurus*) complete coding sequences to get amplification of glut1 and citrate synthase respectively. Amplification was carried out in a 50 µl reaction volume containing 10 pmol of each forward and reverse primers, 3 µL template cDNA, 200 µM of dNTPmix, 1.0 mM $MgCl_2$, and 3U proofreading DNA polymerase (MBI, Fermentas, USA) in 1 × Taq buffer. Amplification reaction

Figure 1. Buffalo ovaries and cumulus oocyte complexes (COCs). (A) Buffalo ovaries and (B) Immature oocytes showing 3 to 4 layers of cumulus cells (original magnification of photomicrographs was ×200).

was performed in a thermal cycler (Bioer Technology Co., China) following reaction condition: Initial denaturation at 95°C for 3 min followed by 35 cycles of 45 s at 95°C, at different annealing temperatures (as mentioned in Table 1), extension at 72° C (@1 min/kb), and a final extension of 10 min at 72° C. The amplified products were resolved by agarose gel (1.5 to 2.5%) electrophoresis and visualized over a gel documentation system (Alpha Imager-2200, Alpha Innotech Corporation, Germany) by ethidium bromide (Promega, Madison, USA) staining under UV light.

The amplicons were purified using gel cleanup kit (Advanced microdevices (mdi), Ambala Cant, India) and cloned into pGEMT Easy vector (Promega, Madison, USA), following manufacturer's instructions. Positive recombinant clones were tested for presence of insert by colony and plasmid PCR. Stab culture prepared from three positive clones was sent to Imperial Life Sciences (ILS Bioservices, India) for sequencing. Sequences were subjected to BLAST analysis (www.ncbi.nlm.nih.gov/BLAST) and submitted to gene bank. Nucleotides as well as deduced amino acid sequences were aligned to evaluate their homology with other mammalian sequences using the Clustal W method of MegAlign Programme of Lasergene Software (DNASTAR, USA). Phylogenetic analysis was carried out using MEGA version 4 (Tamura et al., 2007), and buffalo citrate synthase and glut1 structure was predicted using SWISS-MODEL (Arnold et al, 2006).

RESULTS

A total 90 cumulus oocyte complexes collected from 98 buffalo ovaries at 1.12/ovary recovery rate were utilized for RNA isolation (Figure 1). The quality of RNA was assessed by nanodrop (Thermo Scientific, USA) and optical density at 260/280 was found within the acceptable range. Further, the quality of cDNA was assessed by PCR amplification of housekeeping gene, β-actin and a solitary intact band was observed under UV transilluminator (Figure 2E).

Characterization of glut1 and citrate synthase gene

The amplification reaction for complete coding sequence of glut1 and citrate synthase gene was performed using buffalo oocyte RNA (concentration 247.2 ng/µl and OD of 1.906), and final reaction volume 3 µl per 50 µl was used for PCR amplification of gene of interest. Glut1 amplicons of 1091 bp (for primer 1) and 1161 bp (for primer 2) was observed on 1.5% agarose gel and as single amplicon of 1421 bp for citrate synthase which is further confirmed by plasmid PCR (Figures 2A to D). Sequences of complete coding region of glut1 and citrate synthase genes for water buffalo are now available in public domain (Genbank, accession numbers: glut1; HM025989.2, Citrate synthase; JN039302).

Homology and phylogenetic analysis

The nucleotide sequence and predicted amino acid sequences of glut1 and citrate synthase were aligned and compared with available sequences of different mammalian species. Glut1 sequence was compared with cattle (*Bos taurus*), dog (*Cannis lupus familiaris*), horse (*Eqqus caballus*) rabbit (*Oryctolagus cuniculus*), rat (*Rattus norvegicus*) and human (*Homo sapiens*) which revealed the nucleotide substitutions. Buffalo glut1 sequences showed 99% homology with bovine (*B. taurus*), 92% with dog (*C. lupus familiaris*), 92% with horse (*E. caballus*), 92% with human (*H. sapiens*), 89% with rabbit (*O. cuniculus*), and 89% with rat (*R. norvegicus*) and this indicates close evolutionary relationship (Figure 3A). Phylogenetic analysis revealed that *glut1* is a highly conserved gene (89-98% homology among mammalian species) having an open reading

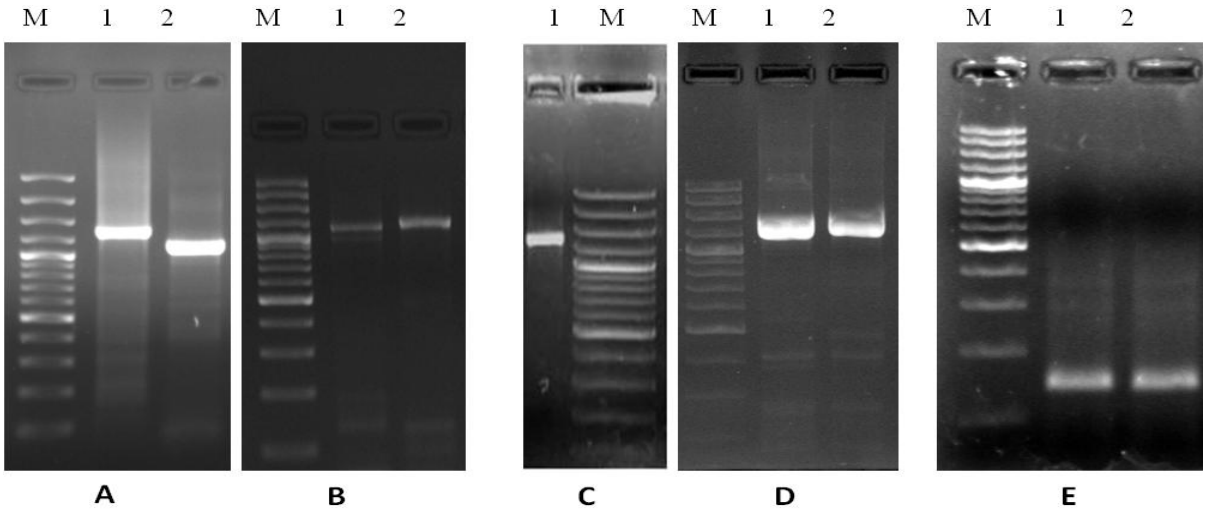


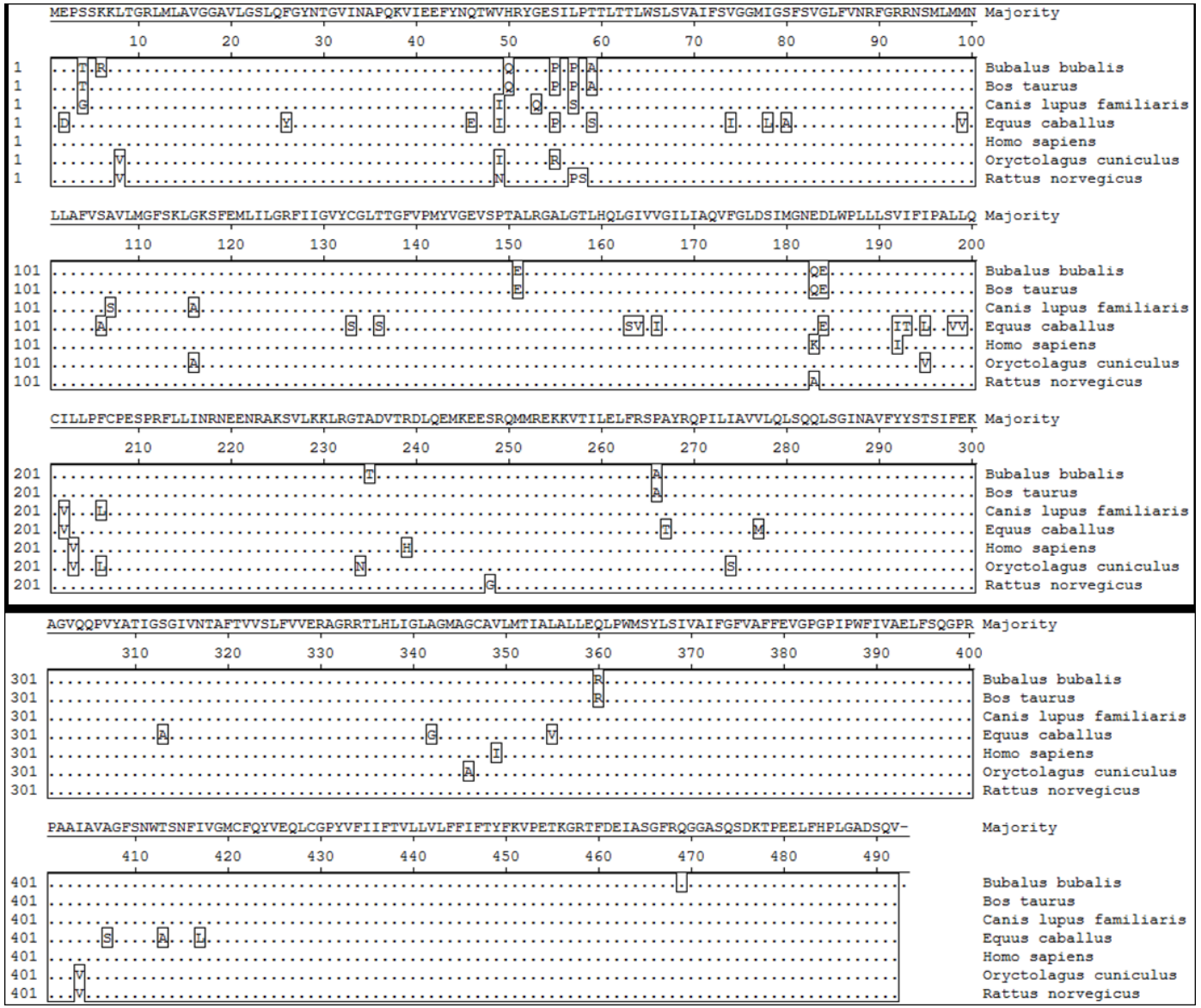
Figure 2. (A) PCR amplification of glut1 complete coding sequences: Lane M, 100bp+ molecular weight marker; Lane 1 (1161 bp) and Lane 2 (1091 bp) amplicons amplified through primer 1 and primer 2 respectively. (**B)** Confirmation of glut1 cloned product through plasmid PCR: Lane 1 (1091 bp) and Lane 2 (1161 bp) for primer 2 and 1, respectively. **(C)** Amplification of Citrate synthase complete coding sequences: Lane M, 100 bp+ molecular weight marker; Lane 1, citate synthase amplicon (1421bp). **(D)** Confirmation of Citrate synthase cloned product through plasmid PCR: Lane M, 100 bp+ molecular weight marker; Lane 1 (1421bp) and Lane 2 (1421 bp) showing plasmid PCR products for citrate synhase. **(E)** Confirmation of cDNA quality through PCR amplification of β-Actin gene: Lane M, 100 bp + molecular weight marker; Lanes 1 and 2, COCs.

frame (ORF) of 1476 bp encoding 492 amino acid. Phylogenetic analysis showed that bovine, rat, mice and humans were derived from different ancestors according to their closer evolutionary relationship (Figure 4A). Among these, cattle and buffalo might have evolved from a common ancestor as expected, pig positioned in between and diverged early from the bovid ancestors, and buffalo *glut1* gene had an individual place, closer to bovine but in different lineage. Further predicted protein structure of buffalo glut1 shows the presence of crucial amino acids for glucose transport (Figure 5A). Similarly, citrate synthase sequences were compared with Cow (*B. taurus*), mice (*Mus musculus*), rabbit (*O. cuniculus*), pig (*Sus scrofa*), rat (*R. norvegicus*) and Human (*H. sapiens*). Buffalo citrate synthase sequences showed ~ 99% homology with bovine (*B. taurus*), 94% with horse (*E. caballus*), 94% with human (*H. sapiens*), 88% with mouse (*M. muscullus)*, 94% with rat (*R. norvegicus*) and 89% with pig (*S. scrofa*) which also shows close evolutionary trend among different domestic specie sand human (Figure 3B). Sequence alignment and phylogenetic analysis shows that citrate synthase is a highly conserved gene (89-99% homology among domestic species) having an open reading frame (ORF) of 1398 bp encoding 466 amino acid. Phylogenetically, bovine, rat, mice and humans were derived from different ancestors according to their closer evolutionary relationship (Figure 4B). Further catalytic amino acid residues in predicted protein structure of buffalo citrate synthase were found conserved (Figure 5B).

DISCUSSION

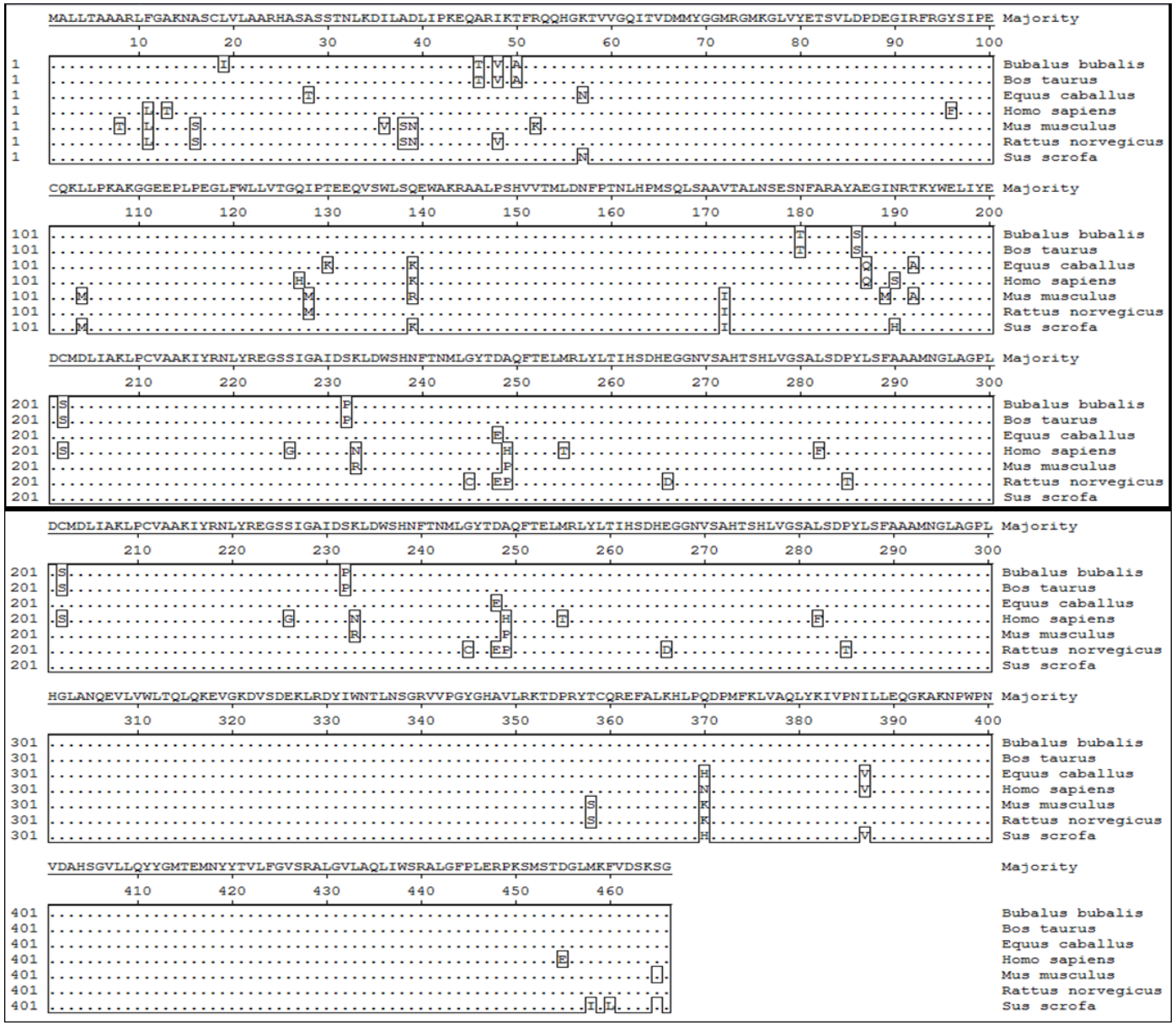
In the present study, we for the first time characterized buffalo cDNA encoding glut1 and citrate synthase genes. The complete coding sequences of buffalo glut1 and citrate synthase genes were analyzed for sequence similarity as well as their phylogenetic relationship amongst other domestic species. Earlier, cDNA encoding the glut1 protein have already been isolated from human, rat, mouse, rabbit, and pig tissues (Mueckler et al., 1985; Birnbaum et al., 1986; Kaestner et al., 1989, Baldwin 1993; Asano 1988) and all exhibits a high level of amino acid identity (97%). Sequence analysis of buffalo glut1 also showed 89 to 98 and 97 to 99% homology at nucleotide and protein level respectively with domestic species and human.

Predicted bubaline glut1 protein has 54060.94 Daltons molecular weight having 491 amino acids; 38 of them are strongly basic (K, R) whereas 32 strongly acidic (D, E), 217 Hydrophobic (A, I, L,F, W, V) and 114 Polar (N, C, Q, S, T, Y) amino acids. Mueckler and colleagues (1985) using hydropathy analysis, predicted that glut1 consists of 12 transmembrane-spanning-helices with the N and C termini and a large loop between transmembrane helices 6 and 7 located on the cytoplasmic side of the membrane (Mueckler et al., 1985, Cairns et al., 1987; Davies et al., 1987; Davies et al., 1990). A smaller loop between transmembrane helices 1 and 2 was predicted to be extracellular (Asano et al., 1991). The bulk of experimental evidence to date supports this model. Cope (1994) also



A

Figure 3. Alignment of predicted amino acid sequence of buffalo (A) *glut1* and (B) *citrate synthase* with different domestic species and human. Identical sequence is indicated by a dot and differences by the corresponding one-letter symbol of the amino acid.



B

Figure 3. Contd.

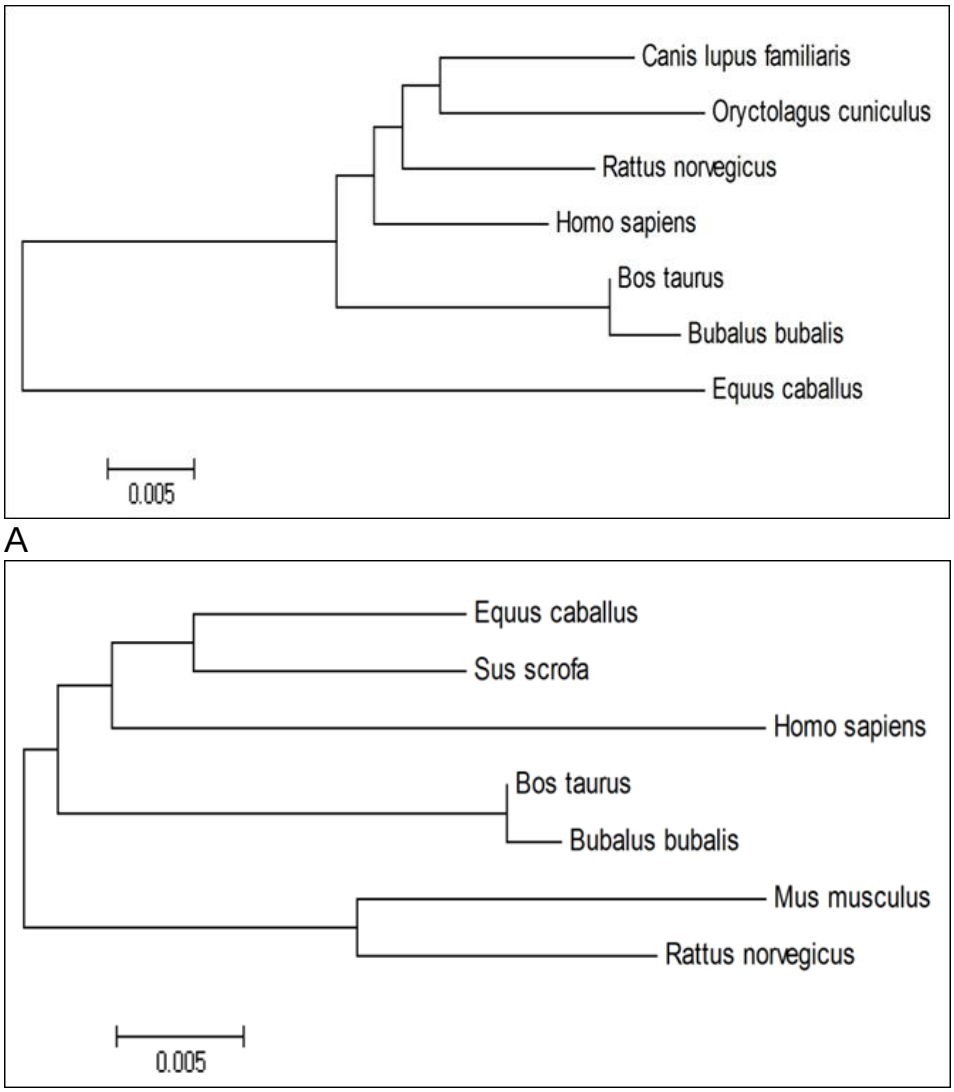


Figure 4. Phylogenetic relationship of (A) *glut1* and (B) *citrate synthase* nucleotide sequences from different species using Mega version 4.1, following the alignment of the ORF sequences using clustal W and neighbour-joining method (nucleotide p distance).

supported the concept of a bilobular structure for the intact glucose transporters in which separate C- and N-domain halves pack together to produce a ligand-binding conformation. Our results of predicted protein structure also support this structure for buffalo glut1. Certain amino acid residues plays critical role in glucose transport. Amongst those G75, G76, G79, N288, and A289 (Olsowski et al., 2000); Q161 (Seatter et al., 1998; Mueckler et al., 1994); V165 (Mueckler and Makepeace, 1997); N317,T321, and P387 (Mueckler and Makepeace, 2002); Q282(Hruz and Mueckler, 1999; Olsowski et al., 2000); I287 (Hruz and Mueckler, 1999); W388 (Kasahara and Kasahara,1998; Garcia et al., 1992); W412 (Garcia et al., 1992) are the crucial amino acids for glucose transport function. These residues were also present in the bubaline predicted structure of glut1 translation.

Sequence homology results for buffalo citrate synthase showed 89 to 99% and 95 to 99% homology at nucleotide and protein level respectively. Earlier citrate synthase was characterized in bovine sequence and was found 92.1 and 93.8% identical to the human and porcine coding sequence, respectively (Winger et al., 2000). The amino acid sequence predicted from the bovine sequence is 95.1% identical to the human sequence and 96.3% identical to the porcine sequence. The porcine sequence contains a stop codon that results in a peptide truncated by 2 amino acids.

Predicted bubaline citrate synthase protein has 51771.66 Daltons molecular weight having 466 amino acids; 47 of them are strongly basic (+) (K, R) whereas 45 are strongly

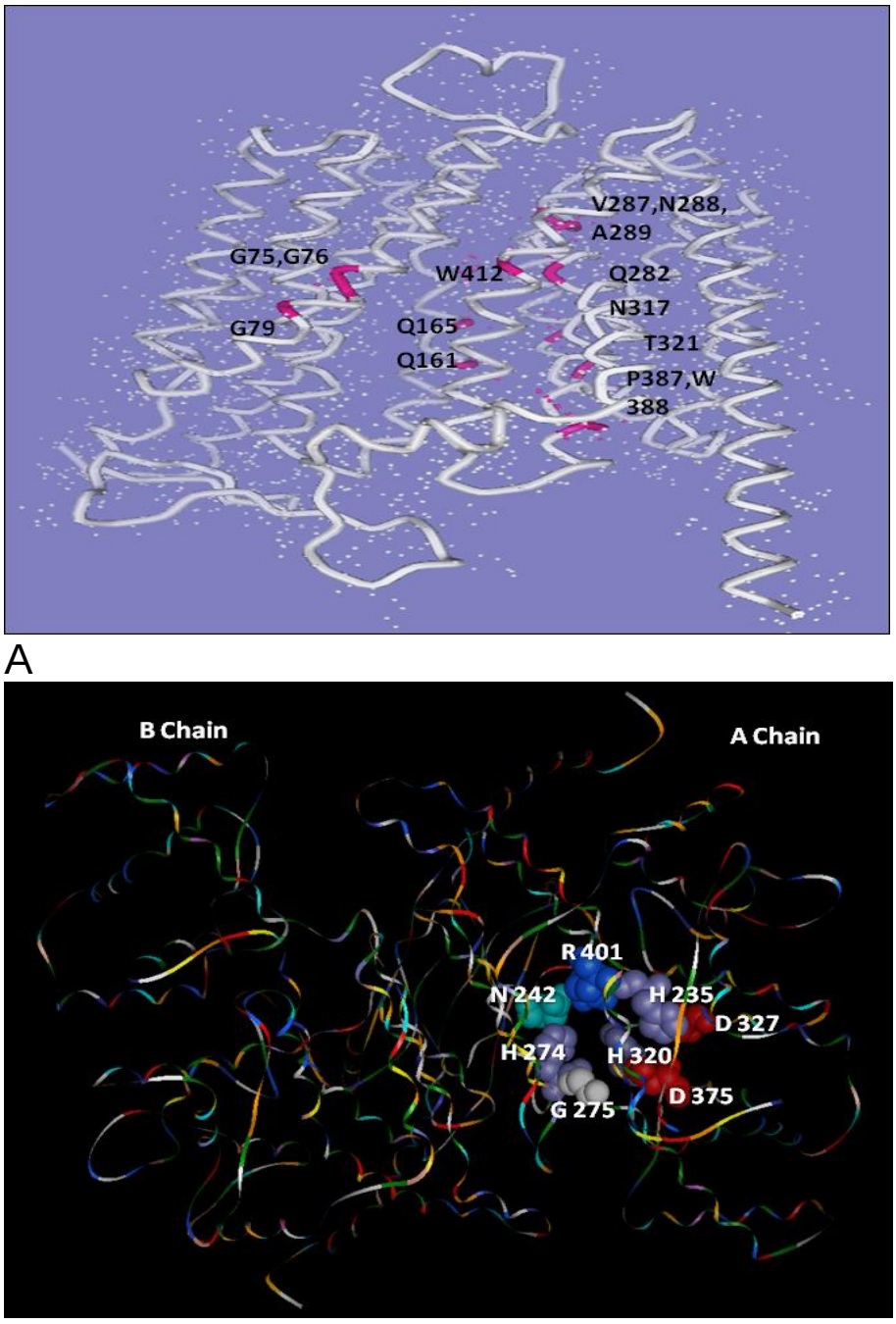


Figure 5. Predicted protein structure of bubaline (A) glut1 and (B) citrate synthase protein (SWISS MODEL).

acidic (-) (D, E), 169 hydrophobic (A, I, L, F, W, V) and 118 Polar (N, C, Q, S, T, Y) amino acids. Predicted structure of buffalo citrate synthase translation showed the presence of highly conserved catalytic residues. Earlier, several studies have been conducted focusing on the same residues, the three catalytic residues (His320, Asp375, His274) and the residues Asp327, Arg401, Gly 275, His235, and Asn242 believed to be involved at the active site or in the conformational change (Evans et al., 1996; Kurz et al., 1998; Mulholland and Richards, 1998). The buffalo amino acid sequences were found conserved with bovine and human amino acid sequences at these eight residues.

This study concludes that *glut1* (89 to 98% homology at

nucleotide and 97 to 99% at amino acid level) and citrate synthase (89 to 99% homology and 95 to 99% at amino acid level) are highly conserved gene among domestic species and human. These sequences were also found conserved in their predicted protein structure for critically active (W412, A289, Q161, V165, N317, T321, P387, Q282, I287, W388, G75, G76, G79 and N288) and catalytic amino acid (His320, Asp375 and His274) residues of buffalo glut1 and citrate synthase, respectively.

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REFERENCES

Arnold K, Bordoli L, Kopp J, Schwede T (2006). The SWISS-MODEL Workspace: A web-based environment for protein structure homology modeling. Bioinfo. 22:195-201.

Asano T, Katagiri H, Takata K, Lin JL, Ishihara H, Inukai K, Tsukuda K, Kikuchi M, Hirano H, Yazaki Y (1991). The role of N-glycosylation of GLUT1 for glucose transport activity J. Biol. Chem. 266:24632-24636.

Asano T, Shibasaki Y, Kasuga M, Kanazawa Y, Takaku F, Akanuma Y, Oka Y (1988). Cloning of a rabbit brain glucose transporter cDNA and alteration of glucose transporter mRNA during tissue development. Biochem. Biophys. Res. Commun. 154: 1204-1211.

Baldwin SA (1993). Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. Biochim. Biophys. Acta. 1154:17-49.

Birnbaum MJ, Haspel HC Rosen OM (1986). Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein. Proc. Natl. Acad. Sci. U.S.A. 83:5784-5788.

Bloxham DP, Parmelee DC, Kumar S, Wade RD, Ericsson LH, Neurath H, Walsh KA, Titani K (1981). Primary structure of porcine heart citrate synthase. Proc. Natl. Acad. Sci. U.S.A. 78: 5381–5385.

Cairns MT, Alvarez J, Panico M, Gibbs AF, Morris HR, Chapman D, Baldwin SA (1987). Investigation of the structure and function of the human erythrocyte glucose transporter by proteolytic dissection. Biochim. Biophys. Acta. 905:295-310.

Cope DL, Holman GD, Stephen T, Baldwint A, Wolstenholme AJ (1994). Research communication domain assembly of the glut1 glucose transporter. Biochem. J. 300:291-294.

Davies A, Meeran K, Cairns MT, Baldwin SA (1987). Peptide-specific antibodies as probes of the orientation of the glucose transporter in the human erythrocyte membrane. J. Biol. Chem. 262:9347-9352.

Davies A, Ciardelli L, Lienhard GE, Boyle JM, Whetton AD, Baldwin SA (1990). Site-specific antibodies as probes of the topology and function of the human erythrocyte glucose transporter. Biochem. J. 266:799-808.

Evans CT, Owens DD, Sumegi B, Kispal G, Srere PA (1988). Isolation, nucleotide sequence and expression of a cDNA encoding pig citrate synthase. Biochem. 27:4680-4686.

Evans CT, Kurz LC, Remington SJ, Srere PA (1996). Active site mutants of pig citrate synthase: effects of mutations on the enzyme catalytic and structural properties. Biochem. 35:10661-10672.

Garcia JC, Strube M, Leingang K, Keller K, Mueckler MM (1992). Amino acid substituti ons at tryptophan 388 and tryptophan 412 of the HepG2 (Glut1) glucose transporter inhibit transport activity andtargeting to the plasma membrane in Xenopus oocytes. J. Biol. Chem. 267:7770-7776.

Hruz PW, Mueckler MM (1999). Cysteine-scanning mutagenesis of transmembrane segment 7 of the GLUT1 glucose transporter. J. Biol. Chem. 274:36176-36180.

Joost HG, Thorens B (2001). The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members (review). Mol. Memb. Biol. 18:247-256.

Kaestner KH, Christy RJ, McLenithan JC, Braiterman LT, Cornelius P, Pekala PH, Lane MD (1989). Sequence, tissue distribution, and differential expression of mRNA for a putative insulin-responsive glucose transporter in mouse 3T3-L1 adipocytes. Proc. Natl. Acad. Sci. U.S.A. 86:3150-3154.

Kasahara T, Kasahara M (1998). Tryptophan 388 in putative transmembrane segment 10 of the rat glucose transporter Glut1 is essential for glucose transport. J. Biol. Chem. 273:29113-29117.

Kurz LC, Nakra T, Stein R, Plugkhen W, Riley M, Hsu F, Drysdale GR (1998). Effects of changes in three catalytic residues on three relative stabilities of some of the intermediates and transition states in the citrate synthase reaction. Biochem.. 37: 9724-9737.

Mueckler M, Makepeace C (1997). Identification of an amino acid residue that lies between the exofacial vestibule and exofacial substrate-binding site of the Glut1 sugar permeation pathway. J. Biol. Chem. 272:30141-30146.

Mueckler M, Makepeace C (2002). Analysis of transmembrane segment 10 of the Glut1 glucose transporter by cysteine-canning mutagenesis and substituted cysteine accessibility. J. Biol. Chem. 277:3498-3503.

Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, Lodish HF (1985). Sequence and structure of a human glucose transporter. Science. 229:941-945

Mueckler M, Weng W, Kruse M (1994). Glutamine 161 of Glut1 glucose transporter is critical for transport activity and exofacial ligand binding. J. Biol. Chem. 269:20533-20538.

Mulholland AJ, Richards WG (1998). Modeling enzyme reaction intermediates and transition states citrate synthase. J. Phys. Chem. 102:6635-6646.

Olson AL, Pessin JE (1996). Structure, function, and regulation of the mammalian facilitative glucose transporter gene family". Annu. Rev. Nutr. 16:235-56.

Olsowski A, Monden I, Krause G, Keller K (2000). Cysteine scanning mutagenesis of helices 2 and 7 in GLUT1 identifies an exofacial cleft in both transmembrane segments. Biochem. 39:2469-2474.

Remington SJ (1992). Structure and mechanism of citrate synthase. Curr. Top. Cell. Reg. 33:209-229.

Scheepers A, Joost HG, Schurmann A (2004). The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. Parenter. Enteral. Nutr. 28(5):364-371.

Seatter MJ, De la Rue SA, Porter LM, Gould GW (1998). QLS motif in transmembrane helix VII of the glucose transporter family interacts with the C-1 position of D-glucose and is involved in substrate selection at the exofacial binding site. Biochem. 37:1322-1326.

Sharma GT, Nath A, Prasad S, Singhal S, Singh N, Gade NE, Dubey PK, Saikumar G (2012). Expression and characterization of constitutive heat shock protein 70.1 (HSPA-1A) gene in *in-vitro* produced and *in-vivo* derived buffalo (Bubalus bubalis) embryos. Reprod. Dom. Ani. DOI:10.1111/j.1439-0531.2012.02002.x.

Singh M, Brooks GC, Srere PA (1970). Subunit structure and chemical characteristics of pig heart citrate synthase. J. Biol. Chem. 245:4636-4640.

Srere PA (1975). The enzymology of the formation and breakdown of citrate. Adv. Enzymot. Relat. Areas. Mol. Biol. 47:57-101.

Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol. Biol. Evol. 24(8):1596-1599.

Wagstaff P, Kang HY, Mylott D, Robbins PJ, White MK (1995). Characterization of the Avian GLUTI Glucose Transporter: Differential Regulation of GLUT1 and GLUT3 in Chicken Embryo Fibroblasts. Mol. Bio. Cell. 6:1575-1589.

Weitzman PDJ, Danson MJ (1976). Citrate synthase. Curr. Top. Cell. Regul. 10:161-204.

Winger QA, Hill JR, Watson AJ, Westhusin ME (2000). Characterization of a bovine cDNA encoding citrate synthase, and presence of citrate synthase mRNA during bovine pre-attachment development. Mol. Reprod. Dev. 55:14-19.

Wood IS, Trayhurn P (2003). Glucose transporters (GLUTandSGLT): expanded families of sugar transport proteins. Br. J. Nutr. 89:3-9.

Zhao FQ, Keating AF (2007). Functional Properties and Genomics of Glucose Transporters. Curr. Genom. 8(2):113-128.