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Identification and Expression of ATP Synthase F0 Subunit 6 in Tilapia Fish during Temperature Acclimation

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ABSTRACT

In the concept of global warming, the physiological mechanisms limiting and adjusting cold and heat tolerance have regained interest. Temperature is considered as one of the most important environmental stressor that can strongly affect physiological functions of fish. Therefore, to understand the role of metabolic regulation in environmental temperature stress tolerance, we have examined how different temperature acclimation (22°C and 32°C) for 7 days affects the mitochondrial properties exemplified by ATP Synthase F-type gene expression in tilapia fish *Oreochromis niloticus* as the cellular energy budget. The relative transcriptional activity of this gene using RT-PCR a semi-quantitative analysis indicated that ATP synthase F0 gene has remarkably stable expression in response to high temperature, in contrast this gene was up-regulated in case of low temperature exposure compared to ß-actin gene. ATP synthase F0 was identified and characterized**,** ATP Synthase F0 Subunit 6 cDNA with the gene bank accession no. GU991356 is composed of 1139 bps with three (1079 bp) open reading frame. The predicted gene is 353 amino acid with molecular weight of 39.75 kDa. ATP synthase F0 subunit 6 shares high identities with the same gene from other fishes and invertebrates.

Keywords: Tilapia ATP, gene expression, Temperature acclimation.

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INTRODUCTION

Mitochondrial bioenergetics plays a key role in environmental adaptation and stress tolerance because of the central role of mitochondria in providing ATP to cover energy expenses of an organism, which are required for survival, growth and reproduction. Environmental stress often results in a reduction of net energy balance due to a reduction in assimilation of energy and/or its conservation in the form of ATP and other high-energy phosphates (`supply-side effects'), increases in basal metabolic demands (`demand-side effects') or a combination of both [17,2]. The resulting energy deficit can have adverse effects on survival and performance of organisms and on the long-term persistence of their populations in the stressful environment.

Metabolic regulation also plays a key role in maintaining the optimal energy balance of the organism and matching energy demand with sufficient energy supply. Although mitochondrial proliferation is thought to be a response to chronic elevations in metabolic rate [14], a number of fish species also increase mitochondrial content in response to cold acclimation [7,10]. To date, case studies analyzing stress-induced changes of energy budgets have mostly focused on single environmental stressors [19], whereas effects of multiple stressors are not well understood. Because multiple stresses often have non-additive effects on physiology, analysis of environmentally relevant combinations of stressors is important to obtain a realistic picture of the impact of stress on animal bioenergetics in nature. .

Metabolic enzymes are enzymes which are involved in the production of adenosine triphospahte (ATP), therefore, their function is critical for the maintenance of cellular activities ATP synthase F0 subunit 6 is a component of the F0F1 ATP synthase complex, which is a key enzyme of ATP production providing cells with ATP that uses the transmembrane electrochemical proton gradient to drive synthesis of ATP and converts the energy stored in the form of the proton motive force to chemical energy in the form of ATP. This complex is composed of 2 major domains, F1 and F0. F1 is the catalytic portion of the enzyme. It lies on the matrix side of the inner mitochondrial membrane and is composed of 6 subunits of $\alpha 3\beta 3\gamma\delta\varepsilon$ [23] and an ATPase inhibitor protein [25]. F0 is the integral membrane domain of the complex and functions as a proton channel in ATP synthesis, subunit 6 is the product of the ATPase 6 gene. Little is known about the structure and functions of the subunit 6 protein except that it is an integral membrane protein and may be involved in energy coupling to the F1 sector.

Temperature (High / Low) is common stressors in fishes, and their importance is increasing due to global climate change, meantime little is known about potential response of fish to climate change-induced temperature changes. Fish provide a useful model for studying the factors that determine mitochondrial content in muscle fibers and physiological conditions. In poikilotherms, mitochondrial function is very sensitive to environmental temperature because of the direct effects of temperature on the rate of all biochemical and physiological reactions as well as its indirect effects on mitochondria through changes in their intracellular milieu [31, 14]. The aim of this study was to examine the effect of acclimation temperatures on cellular energy budget and mitochondrial capacity in Nile tilapia ATP Synthase F0 Subunit 6

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Gene expression in response to temperature challenge and exposure (22°C and 32 °C).

MATERIALS AND METHODS

Nile tilapia (*Oreochromis niloticus,* 50 fish) weighing between 120 and 200 g were collected from Giza province and reared in glass tanks supplied with tap water for a week's acclimation under natural photoperiod and fed with commercial pellets at a daily ration of 3% of their body weight. The water was aerated using submersible pumps to ensure that the water in each tank was constantly recirculated. After acclimation, they were divided into two groups where the desired experimental exposure temperatures (22°C and 32°C) were achieved for 7 days.

RNA extraction and reverse transcription-polymerase chain reaction

Tissues were homogenized in TRIZOL reagent using a glass–homogenizer, briefly, after 5 min room-temperature incubation (20°C), chloroform was added for phase separation. The upper aqueous phase was collected and the RNA was precipitated by mixing with isopropyl alcohol. The RNA pellet was washed once with 75% ethanol, and the RNA pellet was air-dried and finally re dissolved in RNase-free water. The *A*260/*A*280 ratios were between 1.6 and 1.8.

SMART cDNA synthesis and Semi- quantitative RT- PCR for muscle tissue expression of ATP Synthase F0 Subunit 6 Gene

Total RNA from muscles tissue was extracted from *O. niloticus* as described elsewhere [27]. Target fragments (30–50 ng) were treated with DNase, 2 µg RNA was reverse transcribed with M-MLV reverse transcriptase using hexanucleotides (Promega) and used as templates for RT-PCR. A housekeeping gene, ß-actin, was used as an internal control.

A degenerate oligonucleotide primer set was designed from a conserved part of published *Danio rerio, Acreichthys tomentosus, Squilla mantis*, mouse, and human ATP Synthase F0 Subunit 6 (ATPsfs6); forward: 5'- GCACTCCTACCCTCTTGAT -3'
and reverse: 5' CCCAGGCTTTACACTTTATG 3'. The reaction was carried out under the following conditions: reverse transcription step of 45°C for 45 minutes, 94°C for 2 minutes AMV RT inactivation and RNA/cDNA/primer denaturation and 40 cycles (94°C for 30 seconds, denaturation; 52°C for 1 minute, for annealing; 68°C for 2 minutes, for extension); 68°C for 7 minutes final extension and 4°C Soaking.

Following PCR amplification, the reaction products were run at 100 V on a 1.5% agarose gel with 0.5 µg/ml ethidium bromide. Amplification of ATPsfs6 a fragment of 339 bp in length. The total band volumes of amplified products were calculated by gel-documentation software (GELWORKS 1D; UVP). Quantification of single-band PCR products was performed by three-dimensional volume calculations of the digitized gel image prepared by using GelWorks 1D Advanced software (UVP) and expressed as the integrated optical density.

Cloning and sequencing

Products amplified by PCR or reverse transcription (RT-PCR) were ligated and cloned using the pGEM-T easy kit (Promega) according to protocol. Plasmid DNA was isolated from the cloned cells using the QIAprep Spin miniprep kit (QIAGEN) following the manufacture's protocol. Product was sequenced using dedoxy chain sequencer (ABI Applied Biosystems).

Sequence and phylogenetic analysis

Sequences were compared with the sequences in the database using the BLASTX program at the web server of the National biotechnology information. Protein prediction was performed using software at the ExPASY Molecular Biology Sever (http://expasy.pku.edu.cn) and SAPS program (Statistical Analysis of Protein Sequences). Sequences were aligned, employing the distance matrix; a distance-joining tree was constructed, Phylogenetic analysis was carried out for the deduced amino acid sequences of ATP Synthase F0 Subunit 6(ATPsfs6) using Clustal W version 1.83[30]. Sequence database alignments and comparisons were done with the BLAST family of programs (Blastx,) against database specifications of non-redundant protein, SWISS-PROT which were available from the BLAST website at the National Center for Biotechnology Information webserver, (http://www.ncbi.nlm.nih.gov/blast/) [1]. The following proteins were used in the alignment: Human ATPsfs6 (YP_003024031.1), House Mouse (*Mus musculus*, NP_904333.1) and some fish species*: Danio rerio* (NP_059336.1), *Capros aper* (YP_001974493.1), *Cyphocharax magdalenae* (AF412931.1), *Squilla mantis* (AAT69297.1). A signal peptide was predicted using Signal P 3.0 and analysis for transmembrane helices was applied using SOSUI dumbbell server and DAS (Transmembrane Prediction server, http://expasy.pku.edu.cn) was used to predict transmembrane regions.

RESULTS

Confirmation of PCR product specificity and sequence analysis

ATP Synthase F0 Subunit 6 primer pairs was shown to produce a prominent PCR product of approximately 339bp from cDNA template. Purification and sequencing of this product confirmed the reaction specificity for the intended target cDNA sequence. The complete sequence of the COX1 has been deposited in the GenBank Database (http://www.ncbi.nlm.nih.gov/Genbank/) and was assigned the GenBank nucleotide accession number GU991356. Blast queries of the sequenced nucleotides of ATPsfs6 demonstrated that the deduced amino acid sequence contains 1139 nucleotide bps with 1079 bps three open reading framed coding for a protein of 353 aa (Fig. 1). Through search of available sequence databases showed that the putative *O. niloticus* ATPsfs6 sequence is homologous to other known ATPsfs6 with varying levels of sequence identity and high similarity with *Squilla mantis*, *Capros aper*, *Danio rerio and* several vertebrates ATPsfs6.

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  1  VHIPGNSPNC PCSPTLNPLS KAHHTLIKPS HYTPRMIYPP YPTNFPALKL RGPVSHPPRL
 61  PYTLSYYLKH TWPSTLYLHP HNTTFSQHSL RCTPLTCYSH YWYTKPTYTC ARPPSARRHS
121  YPLDPCPNHY RNNPIYSAPR TWSSTNRKPH SWPPFNSTHR HCRLRSSSPY TYSGNSDRST
181  SAATTLISRG SPGTELEFVI MVIAVSCVKL LSAHNSTQHT SRKHKVSLGC LMSELTHINC
241  VALTARFPVG KPVVPAALMN RPTRGERRFA YWRSSASSLT DSLRSVVRLR RAVSAHSRRN
301  TVFYRNQGIT QERTVAKGQP KGPTVKGRVA GVFLKVRLPG EIPNPALQLK MGN
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Fig. (1): Deduced amino acid sequences of *O. niloticus* ATP Synthase F0 Subunit 6 (ATPsfs6).

Analysis of the predicted Protein structure (ATPsfs6) polypeptide

Analysis of the predicted aa sequence of the protein encoded by *O. niloticus* ATPsfs6 cDNA identified a polypeptide chain of 353 aa with a molecular weight 39.75 kilodaltons. A signal peptide was predicted using Signal P 3.0, this analysis confirmed that this sequence has a signal peptide probability=0.973, signal anchor probability: 0.025and the max cleavage site probability: 0.311 between pos. 64 and 65 and this protein is a non secretory protein.
DAS software revealed a curve for predicted location of the transmembrane segment. There were two cutoffs indicated on the plots: a "strict" one at 2.2 DAS score, and a "loose" one at 1.7. The hit at 2.2 is informative in terms of the number of matching segments, while a hit at 1.7 gives the actual location of the transmembrane segment. The segments reported in the "FT" records of the SwissProt database are marked at 1.0 DAS score (Fig. 2).

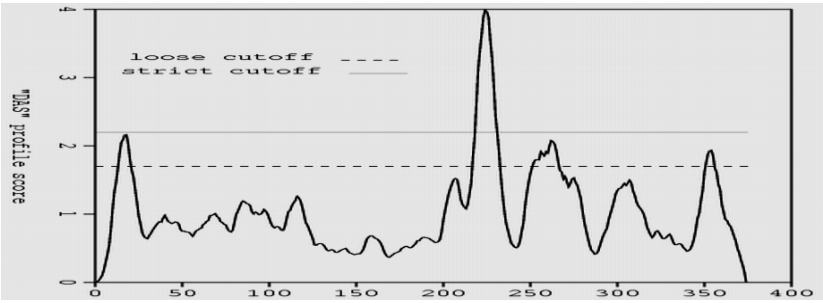

Fig. (2): DAS curve for predicted location of the transmembrane segment.

Alignment analysis and phylogenetic tree construction

Phylogenetic analysis

The *O. niloticus* ATPsfs6 aa sequence was aligned with sequences belonging to the ATPsfs6 from different vertebrates, including mammalian and fish species (Fig.3), Identical amino acids are indicated with an asterisk, similar amino acids are shaded. Dashes are gaps generated by alignment. From this alignment, indels were removed and a neighbor-joining tree was created using p-distance as an evolutionary model The percent identity between the *O. niloticus* and some fish species (*Danio rerio* and *Acreichthys tomentosus*) is approximately 85 % and with *Capros aper* is 71% in contrast, the percent identity between the *O. niloticus* and mammals is 77%. In this tree all vertebrate ATPsfs6 sequences formed of two separate clusters. The *O. niloticus* ATPsfs6 sequence clustered together with fish ATPsfs6 proteins (Fig. 4).

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Homo              --MNENLFASFIAPTILGLPAAVLIILFP-----PLLIPT-SKYLINNRL---------- 42
Mus               --MNENLFASFITPTMMGFPIVVAIIMFP-----SILFPS-SKRLINNRL---------- 42
giAcreichthys     --MTLSFFDQFMSPVFLGIPLIALALLLP-----WLLFPTPTSRWMNSRF---------- 43
Danio             --MMTSFFDQFASPYLLGIPLILVAMLLP-----WLLFPAPTSRWINNRL---------- 43
Capros            --MMLSFFDHFMSPVFLGVPLVALSLMLP-----WVIFPSPSSRYLHSRD---------- 43
Squilla           --MMSNLFSVFDPSTSLMNLQLNWLSTFI-----GLFIIPVTFWMLPNRL---------- 43
Oreochromis       VHIPGNSPNCPCSPTLNPLSKAHHTLIKPSHYTPRMIYPPYPTNFPALKLRGPVSHPPRL 60
                    :  .      ..                    ::  . .      :
Homo              ------ITTQQWLIKLTSKQMMTMHNT-KGRTWSLMLVSLIIFIATTNLLGLLP------ 89
Mus               ------HSFQHWLVKLIIKQMMLIHTP-KGRTWTLMIVSLIMFIGSTNLLGLLP------ 89
giAcreichthys     ------LTLQSWFINRFTSQLLLPISF-GGHKWALILTSLMLFLISLNMLGLLP------ 90
Danio             ------ITVQTWLTGRFTNQLMTPLNF-SGHKWALLFASLMVFLITINLLGLLP------ 90
Capros            ------LTIRKWTINLLTKQLISSLSP-AGHKWALMFISVLMFIVTMNILGLLP------ 90
Squilla           ------YFMWNSLLKTLHLEFKVLLGPGSSAGSTLLFVTLFSIIVFNNFMGLMP------ 91
Oreochromis       PYTLSYYLKHTWPSTLYLHPHNTTFSQHSLRCTPLTCYSHYWYTKPTYTCARPPSARRHS 120
                                              .*   :           .  *
Homo              HSFTPTTQLSMN--LAMAIPLWAGT-----------------------VIMGFRSKIK 122
Mus               HTFTPTTQLSMN--LSMAIPLWAGA-----------------------VITGFRHKLK 122
giAcreichthys     YTFTPTTQLSLN--MGLAVPLWLAT-----------------------VIIGLRNQPT 123
Danio             YTFTPTTQLSLN--MGFAVPLWLAT-----------------------VIIGMKNQPT 123
Capros            YAYTPTTQLSVS--LGLAVPLWLAT-----------------------VIIGFSNQPT 123
Squilla           YIFTSTSHLAMT--LSLSLPLWMGF-----------------------MLYGWINHTK 124
Oreochromis       YPLDPCPNHYRNNPIYSAPRTWSSTNRKPHSWPPFNSTHRHCRLRSSSPYTYSGNSDRST 180
                  :   . .:   .  :  :   * .                          *   : .
Homo              NALAHFLPQGTPTPLIPMLVIIETISLLIQPMALAVRLTAN----ITAGHLLMHLIGSAT 178
Mus               SSLAHFLPQGTPISLIPMLIIIETISLFIQPMALAVRLTAN----ITAGHLLMHLIGGAT 178
giAcreichthys     IALGHLLPEGTPTPLIPVLIIIETLSLFIRPLALGVRLTAN----LTAGHLLIQLIATAA 179
Danio             IALGHLLPEGTPIPLIPALIIIETISLFIRPLALGVRLTAN----LTAGHLLIQLIATAV 179
Capros            VAFGHFLPQGTPTLLIPILIMIETLSLFIRPMALGVRLAAN----LTAGHLMIQLLTTGT 179
Squilla           HMFAHLVPQGTPAFLMPFMVLIETLSNIMRPGTLAVRLAAN----MIAGHLLLTLLASTG 180
Oreochromis       SAATTLISRGSPGTELEFVIMVIAVSCVKLLSAHNSTQHTSRKHKVSLGCLMSELTHINC 240
                      ::..*:*   :  :::: ::* .    :      :.    :  * *:  *
Homo              LAMSTINLPSTLIIFTILI------------------LLTILEIAVALIQAYVFTLLVS 219
Mus               LVLMNISPPTATITFIILL------------------LLTILEFAVALIQAYVFTLLVS 219
giAcreichthys     YVLLPLMPAVAILTATLLF------------------LLTLLEVAVAMIQAYVFVLLLS 220
Danio             FVLLPMMPAVAILTASVLF------------------LLTLLEVAVAMIQAYVFILLLS 220
Capros            YFFMSAMPLVAPLMTALVI------------------TMTILEIAVALIQAYVFVLLLT 220
Squilla           ---PSLSTTILYLLLFSQI------------------LLLMLESAVAVIQSYVFAVLST 218
Oreochromis       VALTARFPVGKPVVPAALMNRPTRGERRFAYWRSSASSLTDSLRSVVRLRRAVSAHSRRN 300
                            :     :                     *. .* : ::       .
Homo              LYLHDNT------------------------------------------------ 226
Mus               LYLHDNT------------------------------------------------ 226
giAcreichthys     LYLQENV------------------------------------------------ 227
Danio             LYLQENI------------------------------------------------ 227
Capros            LYLQENI------------------------------------------------ 227
Squilla           LYAGEVN------------------------------------------------ 225
Oreochromis       TVFYRNQGITQERTVAKGQPKGPTVKGRVAGVFLKVRLPGEIPNPALQLKMGN 353
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Fig. (3): The alignment of *O. niloticus* ATPsfs6 deduced amino acid sequences of from Human ATPsfs6 (YP_003024031.1), *Mus musculus*, NP_904333.1) and some fish species*: Danio rerio* (NP_059336.1), *Capros aper* (YP_001974493.1), *Cyphocharax magdalenae* (AF412931.1), *Squilla mantis* (AAT69297.1). Identical amino acids are indicated with an asterisk, similar amino acids are shaded. Dashes are gaps generated by alignment.

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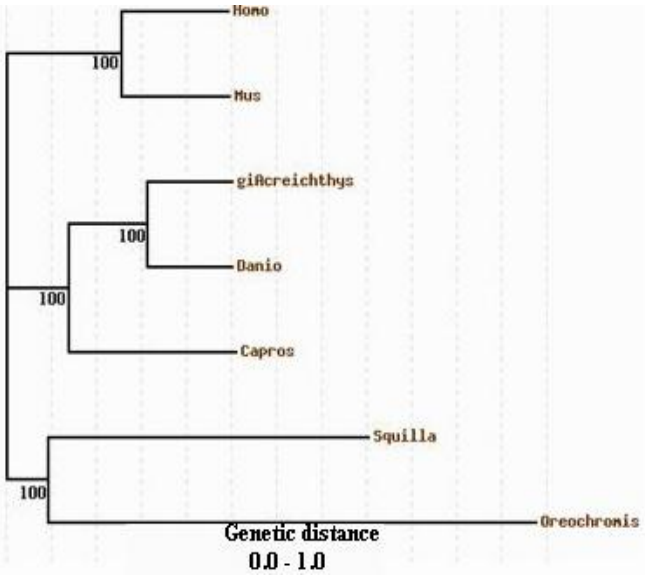

Fig. (4): Neighbour-joining tree of *O. niloticus* ATP Synthase F0 Subunit 6 from different vertebrates, Numbers at branch nodes represent bootstrap confidence levels of 1000 bootstrap replications.

ATP Synthase F0 Subunit 6 mRNA expression in *O. niloticus* tissue

Total RNA from *O. niloticus* muscles was isolated and analyzed for mRNA expression of ATP Synthase F0 Subunit 6mRNA by RT-PCR (Fig.5). A 339 bp fragment was amplified, sequenced and blasted on NCBI and confirmed that it is corresponded to the *O. niloticus* ATPsfs6 sequence. The ATPsfs6 transcripts were found to be ubiquitously expressed in all muscle samples tested. The obvious differences in level of expression was observed and revealed a higher expression level obtained in samples exposed 22°C compared to Beta-actine, which was used as a control, confirmed equal loading of RNA used as a template.

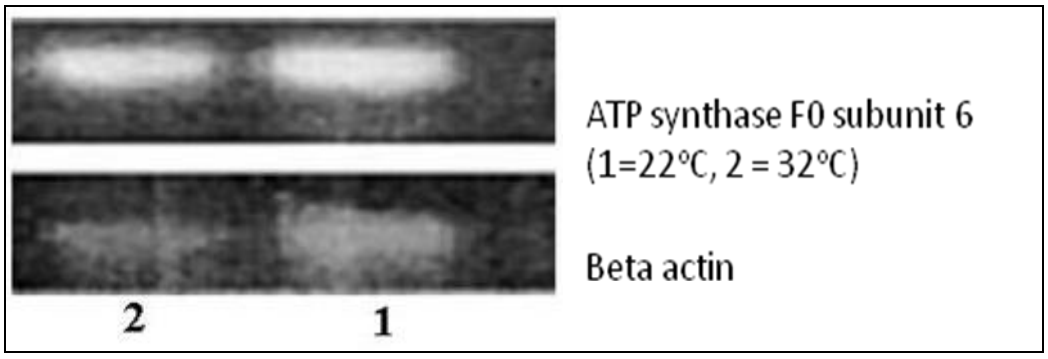

Fig.5: Tissue specific expression of ATP Synthase F0 Subunit 6 in *Oreochromis niloticus* revealed by RT-PCR.

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DISCUSSION

For most fish, body temperature is very close to that of the habitat. The diversity of thermal habitats exploited by fish as well as their capacity to adapt to thermal change makes them excellent organisms in which to examine the evolutionary and functional responses to temperature. An extensive literature links cold temperatures with enhanced oxidative capacities in fish tissues, particularly skeletal muscle. Closer examination of inter-species comparisons (i.e. the evolutionary perspective) indicates that the proportion of muscle fibers occupied by mitochondria increases at low temperatures [7, 10].

Muscle fibers from cold-acclimated goldfish were found to contain a larger number of smaller-diameter myofibrils and to have a higher surface density of sarcoplasmic reticulum than fibres in warm-acclimated fish [24]. With an increase in myofibrillar ATPase activity [16]. Although cold-induced mitochondrial proliferation in fish was first shown more than 30 years ago [17], its regulatory origins remain enigmatic. Muscle mitochondrial proliferation can be induced several ways, but each can be related directly (e.g. exercise, electrical stimulation) or indirectly (thyroid hormone) to elevated metabolic rates. Thus, mitochondrial proliferation is usually envisioned as a compensatory response to energetic shortfalls.

Cold temperatures reduce the metabolic rate of poikilotherms, yet, paradoxically, cold acclimation induces mitochondrial proliferation. There is no doubt that, in many species of fish, acclimation to low temperature causes a number of anatomical and biochemical adaptations that would enhance swimming performance. In cold-acclimated goldfish *Carassius auratus,* crucian carp *C. carassius* and striped bass *Morone saxatills,* there are an increases in the proportion of aerobic 'red' and 'pink' muscle fibers, in mitochondrial density (and hence in the activity of aerobic enzymes) and in capillary supply, and a decrease in diffusion path between the sarcoplasmic and mitochondrial compartments [17, 7). In addition to the above adaptations to low temperature, there are increases in myofibrillar ATPase activity in both 'red' and 'white' muscles in cyrinids [26].

Metabolic enzymes are enzymes which are involved in the production of adenosine triphospahte (ATP), therefore, their function is critical for the maintenance of cellular activities, most of which derive their energy from ATP hydrolysis [14]. There are three main pathways for the synthesis of ATP; aerobic ATP synthesis involves the complete oxidation of substrates. This process requires oxygen as the terminal proton and electron acceptor and utilises substrates such as glucose, glycogen, fatty acids or amino acids, the second pathway, fermentation, partially catabolises substrates in the absence of oxygen. In fish, the lactate is stored in the white muscle during burst work and is converted back to glycogen during recovery.

Anaerobic glycolysis produces ATP rapidly, but unlike aerobic ATP production, it can not be maintained long-term. A third pathway, phosphagenmobilisation, may be utilised. This pathway is also oxygen independent and is catalysed by only one enzyme - creatine phosphokinase [14]. Because it is catalysed by only one enzyme, this ATP-producing pathway is

the quickest and can supply immediate needs, but the supply of creatine phosphate within the cell is limited and is exhausted quickly [6].

In our study, we report the cloning, structural, characterization, and tissue expression of the Nile tilapia gene encoding ATP Synthase F0 Subunit 6 protein as the cells energy budget to assess the effect of low and high temperatures on tilapia. Degenerate PCR primer, based on a conserved region of mouse, human, and some fish ATPsfs6 sequences, was constructed for the *O. niloticus* equivalent of ATPsfs6. By repeated PCR a truncated sequence was obtained, which revealed that our product composed of 1139 bps with a 1079 bps three open reading frames. The predicted gene product encoding for 353 amino acid with molecular weight of 39.75 kDa. Some characters of the predicted protein showed that it has an actual location of the transmembrane segment marked as 1.0 DAS score. Comparing Nile tilapia ATPsfs6 the different ATPsfs6 sequences among several vertebrate species, revealed that percent identity between the *O. niloticus* and *some fish species* ranging from 71% to 85 %, in contrast, the percent identity between the *O. niloticus* and mammals was 77%. This suggests that the same isoform among these species has most likely been derived from the same ancestral gene and that a duplication of this gene might have occurred [11].

Reverse transcription-PCR (RT-PCR) provides a sensitive technique for both the detection and semi-quantification of specific mRNA transcripts [12]. In this study, the magnitude of difference between low (22°C) and high (32°C) acclimated Nile tilapia was in the same range as that observed to occur with exercise training in the same species [9]. The nature of the change in mitochondrial content with physiological stresses has many parallels with the differences observed across species [3]. The RT-PCR revealed significant differences in mRNA levels of ATP synthase F0 subunit 6 in either tissue with temperature acclimation variation, thus our recent study showed that decreased temperatures strongly enhance the mitochondrial ATP synthesis. [21,22] in their study indicated that changes in temperature are likely to impact the catalytic cycles of enzymes. Meantime, fish in thermally variable environments must be able to compensate for changes in temperature in order to maintain fully functional catalytic cycles of enzymes. At low temperatures, the rates of enzyme catalysed reactions are reduced [20]. This results from temperature-induced changes to enzyme structure and interactions with ligands, which ultimately affect the formation of the enzyme-substrate complex [5], this suggestion, support this study finding of increased ATP synthase F0subunit 6 gene expression in response to cold temperature. However, in cold adapted species, changes at the biochemical level (cold adaptation) are thought to offset these temperature-related effects and maintain aerobic performance [29, 10]. Cold adaptation involves a proliferation of mitochondria (seen as an increase in mitochondrial volume density) and an associated increase in the number of mitochondrial enzymes [28, 4]. Increased numbers of metabolic enzymes make it possible to increase the catalytic activity (kcat), and this partially or completely compensates for the rate depressing effects of low temperature [5, 27]. Compensatory increases of the aerobic capacity of fish swimming muscle are frequently observed in response to cold acclimation. Such thermal compensation occur both in fish that remain active in the cold and in fish that become dormant at cold temperatures. For cold-active fish, positive thermal compensation is best explained by conservation of the capacity for aerobic metabolic flux at low temperatures. The compensatory

responses of cold-active species can be used to suggest the temperature range over which the activities of glycolytic and tricarboxylic acid cycle enzymes in a muscle, i.e., the muscle's "metabolic profile," can suffice. For cold-inactive species that remain normoxic during winter dormancy, the compensatory metabolic modifications may facilitate lipid catabolism. Alternately, an increased aerobic capacity may be adaptive during the relatively cold periods that precede and follow winter dormancy.

CONCLUSION

This study demonstrated the relative transcriptional activity of ATP synthase F0 gene with different temperatures (22°C and 32°C) using RT-PCR a semi-quantitative analysis, it indicated that this gene has remarkably stable expression in response to high temperature, in contrast it was up-regulated in case of low temperature exposure compared to ß-actin gene. ATP synthase F0 cDNA was identified and characterized; it is composed of 1079 bps with a 895 bps open reading frame. The predicted gene product is 353amino acid with molecular weight of 39.75 kDa. , and Signal peptide probability equals 0.973. ATP synthase F0 subunit 6 shares high identities with the same gene from other fishes and invertebrates.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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