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SHORT COMMUNICATION

Characterization and Partial Sequencing of Calcium Regulating Hormone Stanniocalcin Gene in Freshwater Fish, *Labeo rohita* (Hamilton, 1822)

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Abstract A 421 bp fragment of stanniocalcin gene was amplified in rohu (*Labeo rohita*). The partially characterized stanniocalcin gene of *Labeo rohita* is 96% homologous to *Sinocyclocheilus rhinocerous* stanniocalcin-like mRNA and 95% to *Cyprinus carpio* stanniocalcin-like mRNA, respectively. The sequence of partially characterized Stanniocalcin was submitted to GenBank (Accession number MF376164) and at present, it is the first report on Indian major carp, rohu (*Labeo rohita*), which is most popularly cultured fish in aquaculture system in India.

Keywords *Labeo rohita* · Corpuscles of Stannius · Characterization · Stanniocalcin gene · Calcium regulating

Stanniocalcin (STC) is a homodimeric glycoprotein hormone involved in calcium and phosphate regulation, a major hypocalcemic factor found in both teleost fish and mammals. Stanniocalcin hormone is synthesized and secreted by small kidney-associated endocrine glands referred to as the corpuscles of Stannius (CS) and also found in some specific regions of the distal renal tubules of basal teleost (Order Osteoglossiformes). Two related mammalian stanniocalcin genes, STC1 and STC2, were found to be expressed in various tissues as paracrine regulators [1]. Although stanniocalcin-1 (STC-1) was originally described in fish, it is now known to be present throughout the animal kingdom in both vertebrates and invertebrates. The morphology of these STC cells was embryologically derived from the pronephric and/or mesonephric ducts and also similar to renal ‘chloride’ (mitochondrial-rich) cells. The number of CS varies from a pair to several hundred in various species [2] from freshwater to marine water. The activation of CS and the release of STC hormone are higher in marine species than that of freshwater species. The changes in their activation are due to the external ionic concentration, and the higher activation of STC hormones is found to be expressed in various regions like the kidney, intestine, gill, and bones. Characterization, expression, and sequencing of STC had been done in several species such as zebrafish [3], salmonids [4], tilapia [5], freshwater and seawater salmon [6], and male and female freshwater teleost *Mastacembelus armatus* [7].

Hang and Balment [8] studied the expression of euryhaline European flounder (*Platichthys flesus*) STC in various tissues and organs. They also concluded that the STC mRNA expression levels in seawater-adapted fish CS were about threefold higher than in freshwater-adapted fish CS. At the same time, Shin et al. [9] have cloned and characterized a full-length cDNA of STC-1 from the turbot (*Scophthalmus maximus*) CS and examined its expression pattern in various tissues like CS, brain, kidney, liver, heart, muscle, and gonad. After 3 years, Shin and Sohn [10] had cloned and characterized a full-length STC2 cDNA from Japanese flounder (*Paralichyhus olivaceus*) ovary and analyzed the expression pattern of STC2. They found that STC2 encoded 286 amino acids and highly identical to STC2 gene of pufferfish, zebrafish, and humans (57.7–89.0%), and this gene also expressed almost in a wide variety of tissues. By considering the above research

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work, the present study is an attempt to characterize and partially sequence the STC gene of freshwater fish, rohu (*Labeo rohita*).

Healthy *Labeo rohita* fish were collected (50 ± 2 g), and the tissue such as kidney along with the corpuscle of Stannius (CS) gland is taken for RNA isolation by the TRIzol method [11] in aseptic condition. The precipitated RNA was dissolved in 20–50 µl of nuclease-free water. The quality, purity, and concentration of RNA were checked by Nanodrop spectrophotometer. The sample that showed an OD ratio (OD260/OD280) in the range of 1.9–2.1 was assessed to be of good quality. The integrity of the isolated was checked by running it on a 2% agarose gel. The extracted RNA was treated with DNAse I (RNase free) to remove the genomic DNA contamination. Then, the template RNA is converted into cDNA by the following procedure. A volume of 12.5 µl mixture was made by adding 2 µg template RNA, oligo (dT) 0.5 µg, and remaining with DEPC water. It was gently mixed and briefly centrifuged. After that it was incubated at 65 °C for 5 min and chilled on ice. After that the following components were added in the mentioned amounts, 5 × reaction mixtures 4 µl, RibolockRNase inhibitor 0.5 µl, 10 mM dNTP mix 2 µl, and Revert Aid H minus M-MuLV reverse transcriptase 1 µl, so that the total volume becomes 20 µl. It was mixed gently and centrifuged briefly. It was again incubated at 42 °C for 60 min, and the reaction was terminated by heating at 70 °C for 10 min.

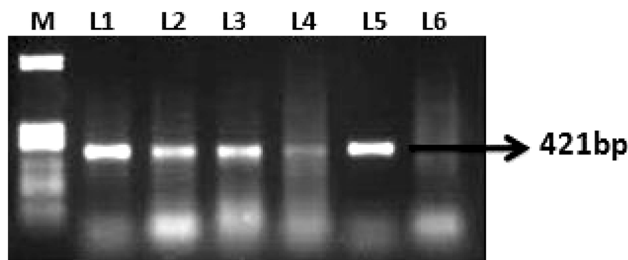

Fig. 1 PCR product of *Labeo rohita* STC analyzed in 1% Agarose gel, where M—1 kb Marker. (Ladder), L1—Lane 1, L2—Lane 2, L3—Lane 3, L4—Lane 4, L5—Lane 5

A set of STC primers were designed by using Gene Runner v.3.01 software and Multiple Sequence Alignment Tool based on selecting gene from conserved regions of closely related fish such as chum salmon (S80134), coho salmon (S59519.1), and zebrafish (NM_200539) to obtain a genomic sequence of 5′ GATGTCGCCCGCTGCCTGAA 3′ as the forward primer and 5′ CGGCTCGCACCACCT-CAACAATG 3′ as a reverse primer to amplify a fragment of 421 bp (Figs. 1, 2, 3) in *Labeo rohita*.

The primer stock was reconstituted by nuclease-free water, and the working solution was then prepared by a further tenfold dilution of stock solution. The reaction mixture and PCR programme were optimized to achieve the satisfactory level of amplification in a final volume of 25 µl containing 12.5 µl of Master Mix, 1 µl of forward and reverse primer each, 1 µl of the cDNA, and then finally nuclease-free water was added to make the volume up to 25 µl. Samples were amplified for 35 cycles with an initial denaturation at 94 °C for 5 min, cyclic denaturation 94 °C for 30 s, cyclic annealing at 57.5 °C for 40 s, cyclic extension at 72 °C for 30 s followed by a final extension at 72 °C for 7 min and hold at 4 °C. PCR product was resolved in 1% agarose gel stained with ethidium bromide in 0.5X TAE buffer and visualized under UV light, and images were captured by the geldoc system. The amplicon of STC gene was eluted, cloned, ligated, transformed, and sequenced. The obtained sequence was then aligned and analyzed using BLAST (Basic Logical Alignment Search Tool) software. It gives the homology with other available sequences of different species from GenBank (www.ncbi.nlm.nih.gov).

The sequencing results revealed that the amplicon of *Labeo rohita* was 421 bp (Fig. 1), which was submitted to GenBank (Accession no. MF376164.1). Alignment reports indicated that the genomic length of 421 bp was similar to that of ray-finned fish (cichlid), Accession no: XM_016525895.1and common carp, Accession no: XM_019078049.1. The respective protein coding for the STC gene is also found in GenBank: AUG90930.1. This partial characterization of STC gene can be further taken into consideration for full sequencing using advanced molecular techniques in future studies. This is a first report

Stanniocalcin, partial [*Labeo rohita*];*Name:* Stanniocalcin; *RefSeq Selected Product:*AUG90930.1, 140 amino acids; GenBank: AUG90930.1;DBSOURCE accession MF376164.1;GenPept Identical Proteins Graphics; >AUG90930.1 stanniocalcin, partial [*Labeo rohita*]

1 DVARCLNGAL QVGCATFACL ENSTCDTDGM HEICNAFLHT AAVFNTEGKT FVKESIKCIA
61 NGITSKVFQT IKRCSTFQKM IAEVQEECYK KLDICEVARS NPEAIGDVVQ VPSHFPNRYY
121STLLQSLMEC DDDIVEVVRA

Fig. 2 Protein sequence of stanniocalcin

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  1 gatgtcgccc gctgcctgaa cggagctctc caggtgggct gcgcaacttt cgcctgtctg
 61 gagaattcga cctgcgacac tgacgggatg catgagatct gcaacgcttt cctccacact
121 gctgcagttt ttaatacaga gggtaagacg tttgtgaaag agagcatcaa gtgcatcgcc
181 aacggtatca cctctaaggt cttccagacc atcaaacgct gttccacctt ccagaagatg
241 attgctgaag tgcaggagga atgctacaag aagcttgaca tctgcgaagt ggccagatcc
301 aaccctgagg ccattggaga cgtggtccag gtccccagcc acttcccaaa caggtactac
361 agcacacttc tgcagagctt gatggagtgc gacgacgaca ttgttgaggt ggtgcgagcc
421 g
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Fig. 3 Gene sequence of stanniocalcin

on the gene, characterized and partially sequenced in rohu (*Labeo rohita*). The knowledge will help the science to understand the calcium regulation in this most popular aquaculture fish, cultured in India.

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Availability of data and material Raw data is available with the Authors and can be made available on the request.

Code availability Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The research undertaken complies with the current animal welfare laws in India, and the use of animals in this study was in accordance with the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment & Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. The study was undertaken with the approval of statutory authorities of the Central Institute of Fisheries Education, Mumbai, India (University under Sec. 3 of University Grants Commission Act and ISO 9001:2008 certified).

Consent to participate U Sivagurunathan carried out the field, laboratory, and molecular work; Prem Prakash Srivastava participated in the experimental design, facilitated in arranging reagents, analytical tools, molecular analysis for experiment, and wrote the manuscript.

Consent for publication Both the authors provided important remarks on the manuscript and gave final consent for publication.

Guidelines and regulations This is to state that all methods were carried out in accordance with relevant guidelines and regulations and all the experimental protocols were approved by ICAR-Central Institute of Fisheries Education (Deemed to be University), Mumbai, India.

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