Caspian J. Environ. Sci. 2017, Vol. 15 No. 4 pp. 309~319

CJES
Caspian Journal of Environmental Sciences

[Research]

Analysis of vitellogenin gene structure in Caspian roach, *Rutilus caspicus* (Pisces: Cyprinidae) during exposure to Atrazine

Oveysi M.[1], Jamili SH.*[2], Behdani M.[3], Mashinchian Moradi A.[1], Sharifpoor E.[2]

1. Department of Marine Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran
3. Biotechnology Research Center, Pasteur Institue of Iran, Tehran, Iran

**Corresponding author's email: shahlajamili45@yahoo.com*

(Received: June 28. 2017 Accepted: Nov. 22. 2017)

ABSTRACT

Chemical contamination of aquatic environments to EDCs has become a major focus of environmental toxicology research. The exposure of fishes to estrogenic EDCs in aquatic environments is most frequently assessed by analyzing vitellogenin (Vg) (the egg yolk precursor protein) expression. Therefore, characterization of Vg gene is of high priority for EDCs bio-monitoring. So, we prepared liver tissue samples of Caspian roach, *Rutilus caspicus* for RNA extraction. Following the cDNA synthesis, specifically-designed primers were employed to amplify the Vg gene and ultimately sequence it. The evolutionary analyses of the sequence were performed using MEGA7 software. The obtained results indicated that the designed primers successfully amplified the partial cDNA sequence. Our results indicated that this sequence most probably belongs to the Vg1 form of the gene. Moreover, it was demonstrated that Caspian roach and *Petroleuciscus esfahani* share a common ancestor. Noteworthy, the study of Vg gene would be helpful to understand the molecular mechanisms of development and would be used to establish a bio-monitoring tool for detection of exposure to different EDCs.

Key words: Vitellogenin gene, Caspian roach, *Rutilus caspicus*, Endocrine disrupting compounds

INTRODUCTION

Chemical contamination of the aquatic environments (with chemical compounds produced over the last century) and the consequences of this contaminations have become a major focus of environmental toxicology research and have captured public attention (DeLorenzo *et al*. 2001; Mills *et al.* 2005). Endocrine disrupters or endocrine disrupting chemicals (EDCs) are a diverse class of chemical stressors including estrogenic EDCs (environmental estrogens). EDCs are indicated to have potential to affect the vertebrate neuroendocrine system. This system plays vital biological roles in regulating processes like development, growth, metabolism and reproduction. EDCs exert their functions through mimicking the action of endogenous estradiol-17β (E2) (Matozzo *et al.* 2008). Amongst, Atrazine (2-chloro-4-ethy-lamino-6-isopropylamino-s-triazine) (ATZ) is an endocrine disrupting chemical which has become one of the most commonly used pesticides worldwide (Solomon *et al*. 2008). Although ATZ use is banned in the EU (2004/248/EC), it continued to be used in the rest of the world. Changing kidney morphology (Fischer-Scherl *et al.* 1991) affecting swimming behavior (Saglio & Trijasse 1998) and altering hormonal pathways in various taxa, including fish (Moore & Waring 1998; Spanò *et al.* 2004; Thibaut & Porte 2004) are among the previously - reported adverse effects of ATZ use. Due to detrimental role of

environmentally persistent man-made chemicals in endocrine disruption, developing a highly - specific molecular indicator capable of diagnosing the exposure of aquatic organisms to estrogenic compounds like ATZ is of great significance.

Vitellogenin (Vg) or the egg yolk precursor protein is the most - frequently used molecular indicator to assess the exposure of fishes to estrogenic EDCs in aquatic environments (Hiramatsu *et al.* 2005; Hiramatsu *et al.* 2006). Liver is the original site of synthesis for this large protein (250-600 kDa depending on the fish species). Vg synthesis is under the tight estrogen control and follows by transportation via the circulatory system to the ovaries (Prakash *et al*. 2007). So, Vg is a process in which matured female (teleost oviparous) fishes use Vg as a nutritional source for growing oocyte and developing embryo (Romano *et al*. 2004). Therefore, Vg could be detected in adult vitellogenic females while it is absent in male and immature females (Nath 1999). Although male and immature females are capable of Vg expression, they do not have significant amounts of Vg due to lack of sufficient circulating estrogens to stimulate higher expression levels (Palumbo *et al.* 2006). However, administration of estrogen and estrogen-mimicking contaminants could induce Vg expression in male and immature females.

Wide spread use of fish in previous field studies of ECDs and aquatic health, pivotal roles of ECDs in inducing vitellogenesis as a specific physiological response of fishes for estrogen or estrogenic chemicals, dose-dependency of inducing Vg synthesis by estrogen and absence of Vg expression in males or immature fish makes the Vg to be deemed as a functional biomarker for ECDs screening (Hiramatsu *et al.* 2006). Given the extent of agricultural activities in the coastal area of the Caspian Sea (Nouri *et al.* 2008), a functional biomarker would bring about valuable insights in bio-monitoring of EDCs. Caspian roach, *Rutilus caspicus* (Yakovlev, 1870) is of high commercial value which its commercial culture is currently established. The sequence of Vg gene for Caspian roach is yet to be studied. In the present study, we aim to isolate and characterize partial sequence of Vg gene for Caspian roach. So that, the cDNA of immature Caspian roach was synthesized from liver tissue and then was used for construction of sequencing vector and eventually sequencing. As a complementary approach, bioinformatics tools are widely used to understand the mechanism and structure of various proteins (Mohammadpour *et al.* 2015; Khalili *et al.* 2016; Mohammadpour *et al.* 2016; Khalili *et al.* 2017; Khalili *et al.* 2017; Jahangiri *et al.* 2017) vaccine design (Khalili *et al*. 2014; Khalili *et al*. 2015; Sefid *et al.* 2015) and phylogenetic analyses (Wang *et al.* 2000; Lee *et al.* 2008). Phylogenetic analysis of the resulted sequence indicated that the amplified Vg gene is evolutionary related to other members of Cypriniformes order and Cyprinidae family, while *Rutilus rutilus* (roach minnow) is the most evolutionary close one. Moreover, *Petroleuciscus esfahani* (small cyprinid fish) and *Phoxinus oxycephalus* (Chinese minnow) belong to the same clade. The obtained results could eventually be used in endocrine disruptor bioassays, reproductive studies in developing aquaculture, determine the maturity status of economically-important species and farm management.

MATERIALS AND METHODS

Animals

Two hundred Caspian roaches (mean weight: 51.9gr; mean height: 13.8cm) were obtained from Gorgan Fisheries Research Center (from Gharasoo station , Bandar Torkaman, Golestan Province) and maintained using standard procedure in tank, while the tank conditions including pH (7.45) dissolved oxygen (7mg.l^{-1}), temperature (20-22°C) and photoperiod (12h light: 12h darkness) were maintained at optimal levels. The fishes were kept in the tank for 12 days to adapt to the tank conditions. The identity of the species was confirmed by morphological characteristics. The fishes were exposed to 5 mg.l^{-1} of the ATZ and prepared for tissue sampling after 30 days.

RNA extraction

Liver tissue was dissected from the obtained fishes. One milligram of liver tissue was flash-frozen in liquid nitrogen and powdered with a mortar and pestle. Total RNA was extracted from the powdered tissue using TRISOL reagent (Invitro gene, Life Technologies, USA) exactly according to manufacturer's instructions. The RNA concentration and purity was quantified by spectrophotometer, and the RNA integrity was verified by 1% agarose gel containing 0.5 $\mu g.ml^{-1}$ ethidium bromide. Only samples with high purity and free from degradation were used to synthesize complementary DNA. In order to attain DNA-free total RNA, 1µg of total RNA was treated with DNaseI according to manufacturer's instructions (Life Technologies) and re-quantified (Alderman *et al.* 2016).

cDNA synthesis

Using the High Capacity cDNA Synthesis Kit (Life Technologies Cat. No. 4368814, uses the random primer scheme for initiating cDNA synthesis) 500ng of DNA-free total RNA was reverse transcribed to cDNA in 20µl reactions, according to the manufacturer's instructions. All cDNA reactions were diluted 5-fold with molecular-grade water and stored at 20 °C.

Primer design and Vg DNA amplification

The NCBI data base at https://ww_w.ncbi.nlm.nih.gov/nuccore/EU930850 was searched to find a closest nucleotide sequence for *R. caspicus*.

Then, the Gene Runner software was employed to design a specific primer pair to amplify partial sequence of Vg gene from the synthesized cDNA.

The sequence for *EcoRV* restriction site was added to the 5' end of both primers for following cloning process.

The RT-PCR reaction cycles were set according to Fig. 1. The RT-PCR results were analyzed by 1% agarose gel containing 0.5 $\mu g.ml^{-1}$ ethidium bromide.

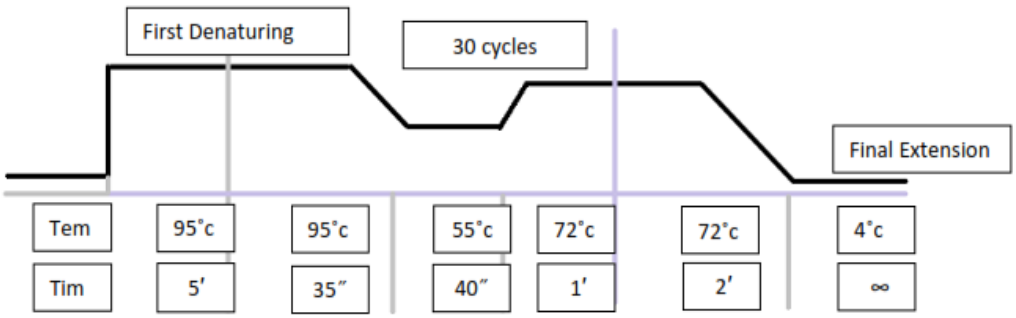

Fig. 1. PCR reaction. The conditions for PCR reaction including the temperatures and the number of cycles for each step are presented.

Vg gene cloning into pUC57 vector and sequencing

The pUC57 plasmid was selected to clone the amplified gene. Therefore, primarily this plasmid was amplified and extracted using AccuPrep® Nano-Plus Plasmid Mini Extraction Kit from BIONEER Company (Korea). The amplified gene was digested with *EcoRV* restriction enzyme, while the pUC57 plasmid was digested with the same enzyme. Following digestions, the gene was ligated into the digested pUC57 plasmid using T4 DNA ligase to get pUC57-310bp vector. The plasmid was transformed into *Escherichia coli* DH5α using standard $CaCl_2$ method. To confirm the cloning and transformation process, multiple colonies were prepared, then colony PCR amplifications were performed using previously designed specific primers. One of the confirmed colonies was sequenced to

confirm the cloned sequence using the same primers. The confirmed colony was used to amplify the pUC57-310bp plasmid and sequence using the designed specific primers. To confirm the identity of the cloned partial Vg gene, the sequencing result was compared with the Vg gene under the gene bank ID of EU930850.

Phylogenetic analysis of the Vg gene

To find the homologous sequences for the sequenced partial Vg gene, a BLAST search was performed using the NCBI nucleotide BLAST tool at https://blast.ncbi.nlm-.ni_ h.gov/Blast.cgi. The resulting sequences were obtained and used for the multiple sequence alignment using the using Molecular Evolutionary Genetics Analysis Version 7.0. Software (MEGA7) (Kumar *et al.* 2016).

The phylogenetic tree was constructed using the neighbor-joining algorithm by MEGA7 software after Muscle alignment with 1000 bootstrap trials. Bootstrap analysis with 1000 replicates was applied to assign confidence levels to the nodes in the trees.

RESULTS

RNA extraction and cDNA synthesis

The tissue samples were successfully dissected and prepared for RNA extraction.

The existing 800 and 1500 bp nucleotide bands (compared to the DNA ladder) indicates the high quality of the extracted RNA regarding its integrity (Fig. 2). Moreover, obtained 2 value for the OD260/280 ratio indicates the purity of the extracted RNA. The high quality total RNAs were used for cDNA synthesis.

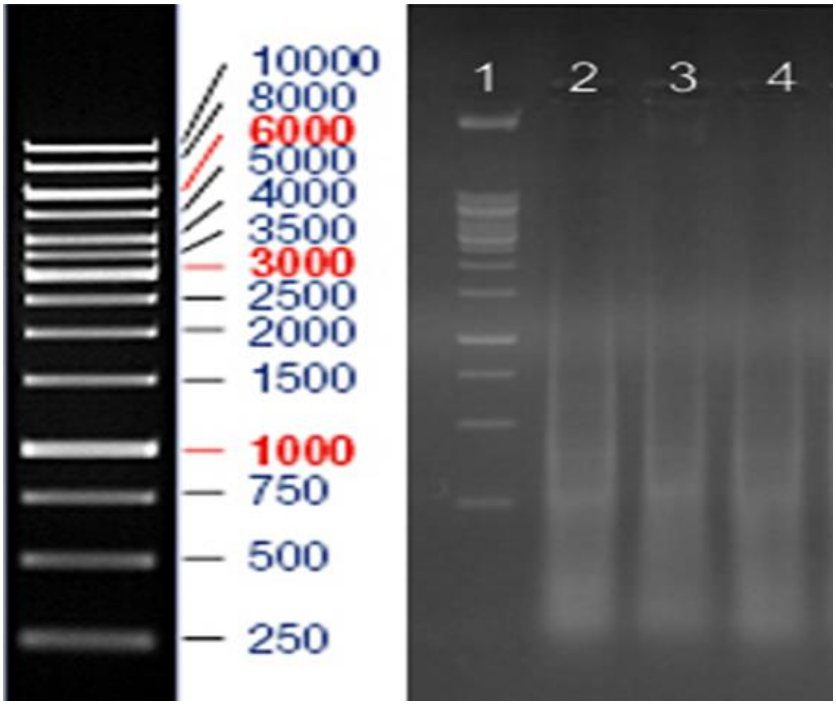

Fig. 2. RNA extraction results. The panel on the left is the map for the employed ladder. The panel on the right includes: (1) ladder, (2) sample 1, (3) sample 2 and (4) sample 3.

Amplifying the Vg gene

A pair of primers was designed using a close Vg sequence (under the gene bank ID of EU930850). The primer sequences were GAAGCTATACCGATGGTTACTGG for the forward primer and AGCTATACTT_ GGTCTAGCTTCAGC for the reverse one. RT-PCR results using the designed primers indicated the successful amplification of an amplicon at the expected molecular weight (310 bp) (Fig. 3).

Gene cloning and sequencing

The sequencing results indicated that a sequence 310 pb in length (Fig. 4) is successfully

cloned into the pUC57 plasmid. The comparison of this sequence to Vg gene indicates over 95% identity. This result confirms the robustness of the whole process of primer design, gene amplification and its cloning.

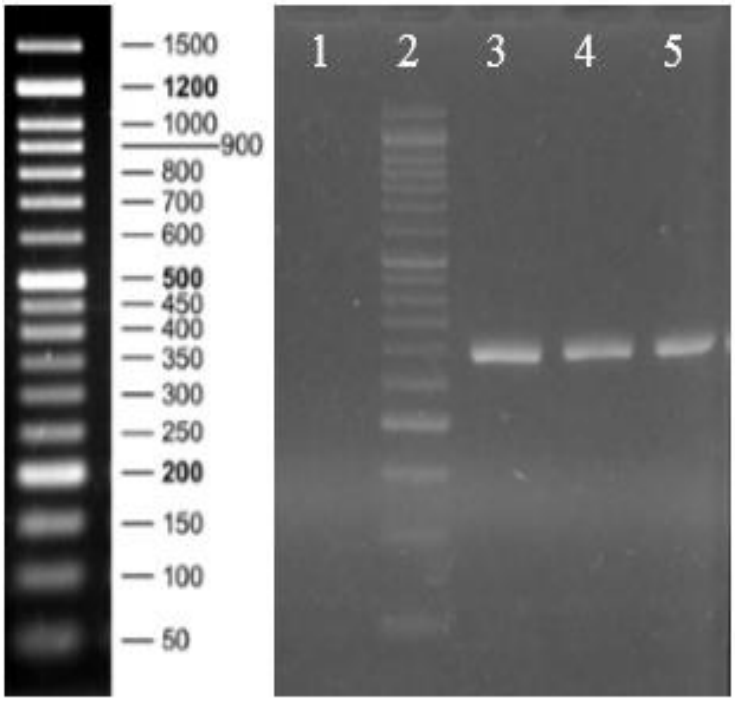

Fig. 3. RT-PCR results. The results of RT-PCR reactions are presented. (1) negative control, (2) ladder, (3) sample 1, (4) sample 2 and (4) sample 3.

5'GAAGCTATACCGATGGTTACTGGACCTAAACCACGTGAACTGCTGAAGAG
TGCCCTTAAAGCTTTGCAGGAAGGAGTCGCCTTCCAGTATGCCAAACCCCTG
CTGGCATCTGAAGTGCGTCGTATCCTGCCAACAGCAGTTGGTTTGCCCATGG
AGCTCAGATTGTACACTGCTGCTGTCGCTGCTGCAAGACTCAATGTTAAGGC
CACCATTACACCTCCTCTCCCCGAAGAAATTGAGACTATGACTCTTGAGCAA
CTCAAGAAGACTGATATTCAACTCCAGGCTGAAGCTAGACCAAGTATAGCT3'

Fig. 4. The result of the gene sequencing. The 5' and 3' end of the sequence are presented in red.

Phylogenetic analysis

The BLAST search results revealed that the obtained 310 bp sequence has the highest similarity (100 coverage and 96% identity) to the Caspian roach vitellogenin which does not come as surprise due to the *R. caspicus* origin of our gene sample. The BLAST search resulted in 27 vitellogenin sequences, all from the Cypriniformes order and Cyprinidae family which are freshwater fishes. Multiple sequence alignment results indicated high sequence similarity between the obtained sequences. Phylogenetic analysis indicated that *R. rutilus* (roach minnow) is the most evolutionary close species to the sequenced Vg gene, while the *Petroleuciscus esfahani* (small cyprinid fish) and *Phoxinus oxycephalus* (Chinese minnow) belong to the same clade.

On the other hand, these species share a common ancestor with *Pimephales promelas* (fathead minnow), indicating their evolutional proximity. On the other hand, *Tanichthys*

albonubes is the outgroup which seems to be the most evolutionary unrelated species. Fig. 5 shows the phylogenetic tree for the analyzed sequences.

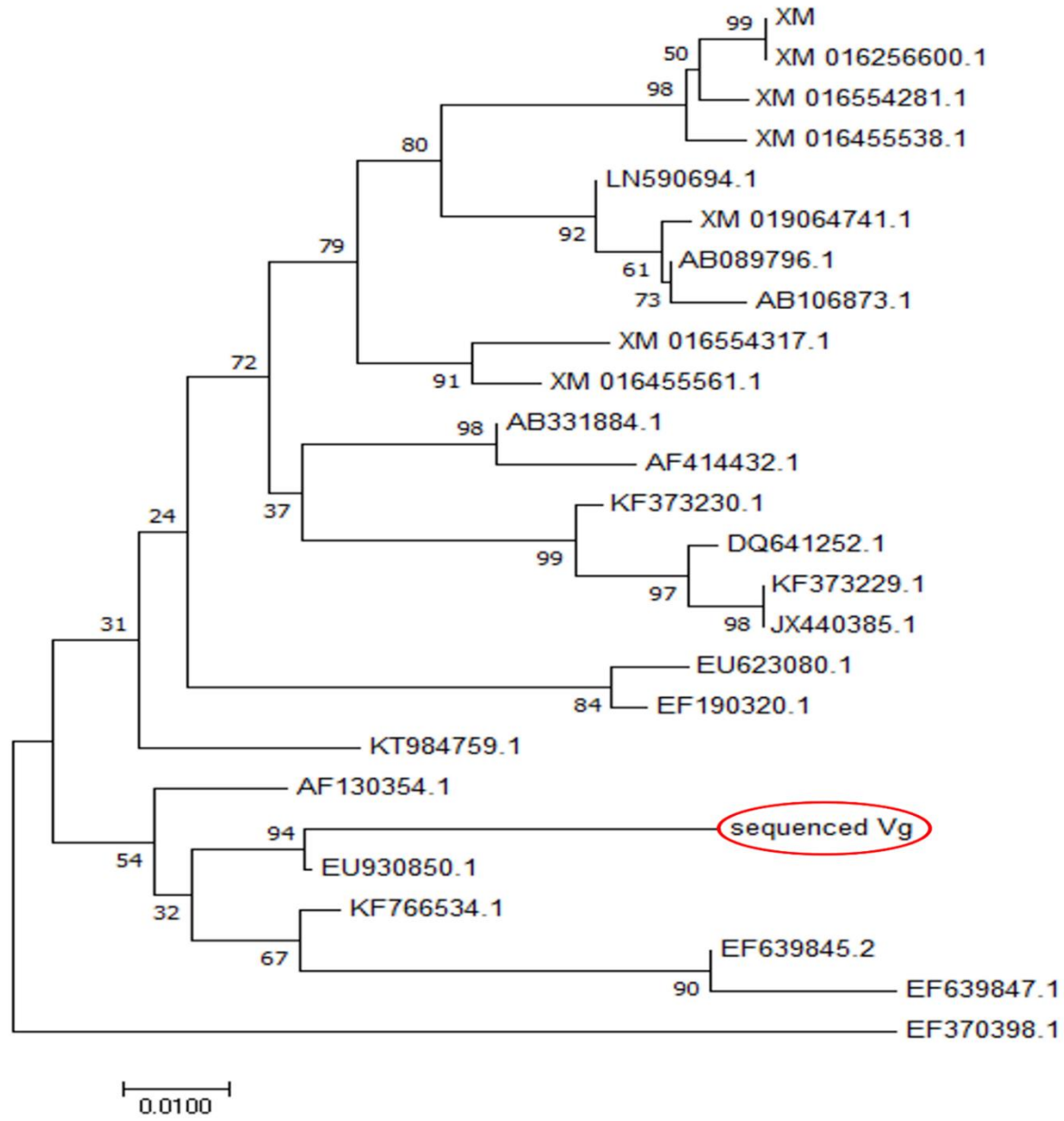

Fig. 5. Phylogenetic tree for Vg gene related sequences. Phylogenetic analysis was conducted by using MEGA7. Numbers next to branch points are the percentage of replicate trees in which the associated taxa clustered together. For the sequences, Gen Bank accession numbers are presented in the figure. The sequenced Vg is in red circle. Scale bar indicates nucleotide substitutions per site. The EF370398 is the out group, while the EU930850 is the most evolutionary close species to the sequenced Vg gene.

DISCUSSION

The Vg expression has extensively been used as a biomarker of estrogenic disruption following the EDCs exposure (Blanchet-Letrouvé *et al.* 2013). Gene transcriptional responses could be contemplated as the primary interactions sites between the chemical contaminants and biota, therefore these processes could present essential clues regarding the effects of chemical exposure on organismal health (Moens *et al.* 2007). In the present study, we have investigated the Vg gene in Caspian roach to characterize its partial cDNA sequence. To best of our knowledge, it's the first study to analyze

the Vg sequence of this species which is a commercially - important fish. Our results indicated that a Vg partial cDNA has successfully been obtained and sequenced from liver tissue samples. Moreover, we have indicated that the obtained Vg sequence is closely related to other members of Cyprinidae family namely *Rutilus rutilus* (direct submission), *Petroleuciscus esfahani (*Gilannejad *et al.* 2016), *Phoxinus oxycephalus* (direct submission) and *Pimephales promelas* (Korte *et al.* 2000).

Gene characterization should be started with a proper RNA extraction step. The RNA samples which are contaminated with impurities may strongly compromise the experimental results of the following applications (Fleige & Pfaffl 2006; Taylor *et al.* 2010). To ensure that the RNA samples meet minimal acceptance criteria to be employed for downstream workflow, their purity and integrity should be assessed as nonrelated properties. Varying and incorrect quantification results as well as inhibition of the RT and PCR reaction could be direct consequences of impure RNA samples. Therefore, assessing the purity and integrity of RNA samples seems to be an inevitable requirement for DNA characterization efforts. Our results have indicated that the obtained RNA samples are devoid of contaminations (protein or DNA) and meet the criteria for both purity and integrity.

Given a high quality RNA sample, cDNA synthesis could be performed efficiently. Specific and efficient amplification of specific target gene (from synthesized cDNA) requires both credible primer design and careful choice of target sequence. Designed primer pair should be tested for specificity and should target unique sites which do not contain any stable secondary structures (Abd-Elsalam 2003). In addition, PCR products should be studied on an agarose gel or polyacrylamide gel to ensure that the product is of the correct size. Observed 310 bp DNA band on the agarose gel has confirmed the accuracy of our primer design and PCR amplification. The sequencing results have further confirmed partial Vg cDNA amplification. The research on the reproductive physiology of fishes has strongly influenced by the structural and functional multiplicity of Vgs as a relatively new paradigm.

Although, only a single type of Vg has been identified till mid-1990s, more than 2 Vg transcripts or translated products are now characterized in at least 17 teleost species (Hiramatsu *et al.* 2002; Hiramatsu *et al.* 2005). This multiplicity has made the naming and classification of this gene to become somewhat confusing. So that, Hiramatsu *et al.* (2002) have devised a novel classification scheme for multiple teleost Vgs which divided the Vgs to A, B and C type Vgs. A and B types are the "complete" Vgs which possess a complete yolk protein domain structure, while the C type lacks a Pv domain or possesses a greatly shortened Pv domain (Hiramatsu *et al.* 2005; Hiramatsu *et al.* 2006). Several studies have demonstrated that different Vgs could have some discrete functions. However, different physiological functions of the distinct Vgs have mostly remain to be elucidated (Hiramatsu *et al.* 2006). Our results are in line with the diversity of this gene among the species. Our BLAST search resulted in only 27 sequences which all were members of Cyprinidae family. This means that the sequence of Vg gene should be highly diverse among different species that there was not any significant similarity between our sequence and the species from other biological orders. However, there was a high sequence identity between the members of the Cyprinidae which could be construed as some kind of sequence conservation among the Vg genes of this family.

On the other hand, our results indicated that the Caspian roach and *Petroleuciscus esfahani* share a common ancestor. This could be rationalized by the fact that both species have a close geographical habitat which belongs to the same country. Gilannejad *et al.* have indicated that the identity of their characterized Vg gene is higher for vtg1 form (Gilannejad *et al.* 2016). Over 94% identity and 100 coverage between our sequence and the Vg of *Petroleuciscus*

esfahani indicate that our sequence most probably belongs to the Vg1 form of the gene. Moreover, our phylogenetic analyses are in line with the study performed by the Gilannejad *et al.* (2016).

Our results indicate similar evolutionary relations among the *P. esfahani, Phoxinus oxycephalus* and *P. promelas*. However, it seems that Caspian roach Vg has more recently been evolved.

In conclusion, noteworthy, finding a proper bio-indicator to screen the aquatic environment for any exposure to EDCs is of great significance. Since various anthropogenic activities could be potential sources of EDC contamination, assessing Vg expression in male and immature females would drastically help for EDC bio-monitoring.

Hence, characterization of Vg gene within a native and commercially available species should be the first step. We have successfully characterized a partial cDNA sequence of Caspian roach Vg and investigate its evolutionary properties.

The study of Vg gene would be helpful to understand the molecular mechanisms of development and would be used to establish a bio-monitoring tool for tracing different EDCs.

ACKNOWLEDGMENTS

The authors would like to thank Gene Transfer Pioneers Group, Shahid Beheshti University of Medical Sciences, Pharmaceutical Technology Development Center, Tehran, Iran for supplying materials.

REFERENCES

Alderman, SL, Lin, F & p Farrell, AP 2016, Effects of diluted bitumen exposure on juvenile sockeye salmon: from cells to performance. *Environmental Toxicology and Chemistry*, 36: 354-360.

Abd-Elsalam, KA 2003, Bioinformatic tools and guideline for PCR primer design. *African Journal of Biotechnology*, 2: 91-95.

Blanchet-Letrouvé, I, Lafont, A-G & Poirier, L 2013, Vg mRNA induction in an endangered fish species (*Anguilla anguilla*) from the Loire estuary (In French). *Ecotoxicology and Environmental Safety*, 97: 103-113.

DeLorenzo, ME, Scott, GI & Ross, PE 2001, Toxicity of pesticides to aquatic microorganisms: A review. *Environmental Toxicology and Chemistry*, 20: 84-98.

Fischer-Scherl, T, Veeser, A & Hoffmann, RW 1991, Morphological effects of acute and chronic atrazine exposure in rainbow trout (*Oncorhynchus mykiss*). *Archives of Environmental Contamination and Toxicology*, 20: 454-461.

Fleige, S & faffl, MW 2006, RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular Aspects of Medicine*, 27: 126-139.

Gilannejad, N, Dorafshan, S & Heyrati, FP 2016, Vitellogenin expression in wild cyprinid *Petroleuciscus esfahani* as a biomarker of endocrine disruption along the Zayandeh Roud River, Iran. *Chemosphere,* 144: 1342-1350.

Hiramatsu, N, Cheek, AO & Sullivan, CV 2005, Vitellogenesis and endocrine disruption. *Biochemistry and Molecular Biology of Fishes*, 6: 431-471.

Hiramatsu, N, Matsubara, T & Fujita, T 2006, Multiple piscine vitellogenins: biomarkers of fish exposure to estrogenic endocrine disruptors in aquatic environments. *Marine Biology*, 149: 35- 47.

Hiramatsu, N, Matsubara, T & Weber, GM 2002, Vitellogenesis in aquatic animals. *Fisheries Science*, 68: 694-699.

Jahangiri, A, Rasooli, I & Owlia, P 2017, In silico design of an immunogen against Acinetobacter baumannii based on a novel model for native structure of outer membrane protein A. *Microbial Pathogenesis.* US *National library of* Medicine, National Institutes of Health, 105:201-210.

Khalili, S, Jahangiri, A & Borna, H 2014, Computational vaccinology and epitope vaccine design by immunoinformatics. *Acta Microbiologica et Immunologica Hungarica*, 61: 285-307.

Khalili, S, Jahangiri, A & Hashemi, ZS 2017, Structural pierce into molecular

mechanism underlying *Clostridium perfringens* Epsilon toxin function. *Toxicon*, 127:90-99.

Khalili, S, Rahbar, MR & Dezfulian,MH 2015, In silico analyses of Wilms′ tumor protein to designing a novel multi-epitope DNA vaccine against cancer. *Journal of Theoretical Biology*, 379: 66-78.

Khalili, S, Rasaee, M & Bamdad, T 2017, 3D structure of DKK1 indicates its involvement in both canonical and non-canonical Wnt pathways. *Molecular Biology*, 51: 155-166.

Khalili, S, Mohammadpour, H & Barough, MS 2016, ILP-2 modeling and virtual screening of an FDA-approved library: a possible anticancer therapy. *Turkish Journal of Medical Sciences,* 46: 1135-1143.

Korte, JJ, Kahl, MD & Jensen, KM 2000, Fathead minnow vitellogenin: Complementary DNA sequence and messenger RNA and protein expression after 17β-estradiol treatment. *Environmental Toxicology and Chemistry*, 19: 972-981.

Kumar, S, Stecher, G & Tmura, K 2016, MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, msw054.

Lee, K-W, Hwang, D-S & Rhee, J-S 2008, Molecular cloning, phylogenetic analysis and developmental expression of a vitellogenin (Vg) gene from the intertidal copepod *Tigriopus japonicus*. *Comparative Biochemistry and Physiology* Part B: *Biochemistry and Molecular Biology*, 150: 395-402.

Matozzo, V, Gagné, F & Marin, MG 2008, Vitellogenin as a biomarker of exposure to estrogenic compounds in aquatic invertebrates: a review, *Environment International*, 34: 531-545.

Mills, LJ & Chichester, C 2005, Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? *Science of The Total Environmen*t, 343, 1-34.

Moens, LN, Smolders, R & Van der Ven, K 2007, Effluent impact assessment using microarray-based analysis in common carp: a systems toxicology approach. *Chemosphere*, 67: 2293-2304.

Mohammadpour, H, Khalili, S & Hashemi, ZS 2015, Kremen is beyond a subsidiary co-receptor of Wnt signaling: an in silico validation. *Turkish Journal of Biology*, 39: 501-510.

Mohammadpour, H, Pourfathollah, AA & Zrif, MN 2016, Key role of Dkk3 protein in inhibition of cancer cell proliferation: an in silico identification. *Journal of Theoretical Biology*, 393: 98-104.

Moore, A & Waring, CP 1998, Mechanistic Effects of a triazine pesticide on reproductive endocrine function in mature male atlantic salmon (*Salmo salar* L.) parr. *Pesticide Biochemistry and Physiology*, 62: 41-50.

Nath, P 1999, Some aspects of teleost vitellogenesis. Ichthyology: recent research advances, Science Publishers Inc, Enfield.

Nouri, J, Karbassi, AR & Mirkia, S 2008, Environmental management of coastal regions in the Caspian Sea. *International Journal of Environmental Science & Technology*, 5: 43-52.

Palumbo, A, Linares-Casenave, J & Jewell, W 2006, Induction and partial characterization of California halibut (*Paralichthys californicus*) vitellogenin.

Prakash, O, Goswami, S & Sehgal, N 2007, Establishment of ELISA for murrel vitellogenin and choriogenin, as biomarkers of potential endocrine disruption. *Comparative Biochemistry and Physiology Part C*: *Toxicology & Pharmacology*, 146: 540-551.

Romano, M, Rosanova, P & Anteo, C 2004, Vertebrate yolk proteins: a review. *Molecular Reproduction and Development*, 69: 109-116.

Saglio, P & Trijasse, S 1998, Behavioral responses to atrazine and diuron in goldfish. *Archives of Environmental Contamination and Toxicology*, 35: 484-491.

Solomon, KR, Carr, JA & Du Preez, LH 2008, Effects of Atrazine on fish, amphibians, and aquatic reptiles: A critical review. *Critical Reviews in Toxicology*, 38: 721-772.

Spanò, L, Tyler, CR & van Aerle, R 2004, Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*). *Aquatic Toxicology*, 66: 369-379.

Sefid, F, Rasooli, I & Jahangiri, A 2015, Functional exposed amino acids of BauA as potential immunogen against *Acinetobacter baumannii*. *Acta biotheoretica*, 63: 129-149.

Taylor, S, Wakem, M & Dijkman, G 2010, A practical approach to RT-qPCR—publishing data that conform to the MIQE guidelines. *Methods*, 50: S1-S5.

Thibaut, R & Porte, C 2004, Effects of endocrine disrupters on sex steroid synthesis and metabolism pathways in fish. *The Journal of Steroid Biochemistry and Molecular Biology*, 92: 485-494.

Wang, H, Yan, T & Tan, JT 2000, A zebrafish vitellogenin gene (vg3) encodes a novel vitellogenin without a phosvitin domain and may represent a primitive vertebrate vitellogenin gene. *Gene*, 256: 303-310.

آنالیز ساختار ژن ویتلوژنین در ماهی کلمه خزری *Rutilus caspicus* در معرض آترازین

اویسی م.[۱]، جمیلی ش.*[۲]، بهدانی م.[۳]، ماشینچیان مرادی ع.[۱]، شریف پور ع.[۲]

۱- دانشگاه آزاد اسلامی، واحد علوم و تحقیقات، گروه بیولوژی دریا، تهران، ایران
۲- موسسه تحقیقات علوم شیلاتی کشور، سازمان تحقیقات آموزش و ترویج کشاورزی، تهران، ایران
۳- مرکز تحقیقات بیوتکنولوژی، انستیتو پاستور ایران، تهران، ایران

(تاریخ دریافت: ۹۶/۰۴/۰۷ تاریخ پذیرش: ۹۶/۰۹/۰۱)

چکیده

آلودگی ترکیبات شیمیایی در محیط‌های آبی توسط مخرب‌های اندوکرینی (EDC) به کانون مورد توجه در تحقیقات سم‌شناسی محیط زیست تبدیل شده است. مواجهه ماهیان با مخرب‌های اندوکرینی استروژنی در محیط‌های آبی اغلب با آنالیز بیان ژن ویتلوژنین (پروتئین زرده تخم) سنجیده می‌شود. بنابراین، شناسایی ساختار ژن ویتلوژنین دارای اولویت زیادی در کنترل زیستی مخرب‌های اندوکرینی است. در این مطالعه، ما نمونه‌های بافت کبد ماهی کلمه خزری را برای استخراج فراهم کردیم. پس از سنتز cDNA با طراحی پرایمرهای مخصوص، ژن ویتلوژنین و در نهایت تعیین توالی آن انجام شد. آنالیزهای توالی مورد نظر با استفاده از نرم افزار MEGA7 انجام داده شد. نتایج به دست آمده نشان داد که پرایمرهای طراحی شده در مشخص کردن بخشی از توالی cDNA موفق بوده است. نتایج ما مشخص کرد که این توالی متعلق به فرم ۱ ویتلوژنین است. علاوه بر این، با این آزمایش ها اثبات شد که دو گونه ماهی ک مرکز تحقیقات بیوتکنولوژی، لمه خزری ***Rutilus caspicus*** و ***Petroleuciscus esfahani*** دارای جد مشترک هستند. به این مطلب باید توجه کرد که مطالعه ژن ویتلوژنین در درک پیشرفت مکانیسم‌های مولکولی کمک خواهد کرد و به عنوان ابزار کنترل زیستی برای آشکارسازی مواجهه با ترکیبات مخرب هورمونی مختلف استفاده خواهد شد.

* مؤلف مسئول