Advances in Bioresearch
Adv. Biores., Vol 8 (2) March 2017: 122-126

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.2.122126

Advances in Bioresearch

ORIGINAL ARTICLE

Cloning of Lori Bakhtiari Growth Hormone gene and its expression in *E. coli*

Mehdi Dayani Nia[1], Vahab Babapour[2], Abbas Doosti[*3]
[1.] Student of Physiology,Islamic Azad University, Science and Research Branch, Tehran, Iran
[2.] Department of Physiology, Islamic Azad University, Science and Research Branch, Tehran, Iran
[3.] Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran
* Corresponding author: Abbas Doosti, E-mail: biolongco@yahoo.com

ABSTRACT
One of the most common native breed in the southwest of Iran is the Lori Bakhtiari sheep breed. Also Lori Bakhtiari breed has the largest fat-tail among all breeds in Iran. Growth hormone (GH) is a 191 aa peptide hormone that stimulates cell reproduction and growth in different type of animals. The aim of this study was to clone and sequence the Lori Bakhtiari sheep growth hormone gene in E. coli. The cDNA that encodes bovine growth hormone was isolated from total mRNA of the pituitary gland and amplified by RT-PCR using GH specific PCR primers (Ovis-GH-F and Ovis-GH-R). cDNA fragment of GH gene (690 bp) was cloned by T/A cloning technique in TOPO Vector and this construct (TOPO-GH) was transformed into E. coli. Presence of the target gene in TOPO vector had confirmed using PCR,XhoI/NotI restriction enzymes and sequencing. The results of present study showed that GH cDNA was successfully cloned in E. coli. Sequencing confirmed that GH cDNA was cloned; BLAST search showed that the sequence of growth hormone cDNA of the Lori Bakhtiari sheep from Iran has 100% homology with other records existing in GenBank .
Keywords*: Lori Bakhtiari sheep, Growth Hormone (GH), Cloning, Gene expression, E. coli.*

Received 30/11/2016 Revised 02/01/2017 Accepted 19/02/2017

How to cite this article:
M Dayani Nia, V Babapour, A Doosti . Cloning of Lori Bakhtiari Growth Hormone gene and its expression in *E. coli*. Adv. Biores., Vol 8 [2] March 2017: 122-126.

INTRODUCTION

Escherichia coli (*E. coli*) known as the main prokaryote organism for many years in the genetic examination of microorganism. On the basis of the knowledge accumulated on *E. coli* it has become the most utilizable host organism for expression of recombinant products with the best results in cultures [1] **.** In many condition, the protein is synthesized and remains in the cytoplasm from which it should be isolated and purified. However, there are certain disadvantages to the cytoplasmic production of proteins; high-level expression often leads to insoluble protein aggregates which may be difficult to solubilize ;a refolding step is often required to acquire the native structure and to form accurate disulfide bonds; the protein of concern often contains an N-terminal methionine [2].Preferment in recombinant DNA technology has made exist the proteins expression in host cells, such as *E. coli*[3].

Growth hormone (GH);the 191 amino acid with the molecular mass of near22 kilodalton [4], isa polypeptide secreted by the anterior pituitary gland and is rolled in arrangement of production that related to growth and milk, two main productive traits in farm animals. Recently the corresponding GH genes of the rat, human ,bovine and ovine were successfully sequenced and outfitted to be highly conserved in mentioned species[5].GH has been shown to be synthesized locally, in regional lymph nodes by lymphoid cells [6]. It is released into the blood circulation where it share in a wide range of biological functions containing protein synthesis, cell proliferation, immune regulation, lactation, and proteins metabolism, carbohydrates and lipids[7].GH influence the partitioning of nutrients among tissues in sheep and cattle, raising bone growth and milk production, and decreasing fatness. Plasma concentrations of GH are increased in sheep selected for low levels of fatness [8] and in cattle genetically selected for high milk production [9], evidencing that GH is genetically associated with these traits. The role of GH in sheep

has been examined by hormone injections, but longer-term studies have been simplify by the production of sheep carrying an additional copy of the ovine GH gene with a downregulated metallothionine promoter [10]. Sheep that carry the GH transgene had decreased SC fat up to the age of 18 month and grew more rapidly [11]. A diversity of other functions in mammals are influenced by GH, including the metabolism of water and sodium [12], female reproduction and milk production [13], increased soft tissue growth in the outrace and impaired cardiac function [14].The LoriBakhtiari sheep breed is one of the most prominent native sheep breeds in southwestern parts of Iran (the Zagros Mountains), with more than 1.7 million heads population [15].Majority of the sheep population is managed by a nomadic system, utilizing natural pastures as the main origin of nutrition. Also the largest fat-tail among all breeds in Iran belong to LoriBakhtiari breed [16].

The aim of this study was to cloning and sequencing of Lori Bakhtiari bovine growth hormone gene and comparing it to the results reported by other investigators from other parts of the world.

MATERIALS AND METHODS

Sampling

The sheep brain was removed rapidly from the neurocranium to expose the pituitary. The pituitary gland was immediately excised and stored at -80°C until used.

RNA extraction and cDNA synthesis

Total RNA was isolated from the pituitary gland tissue using a Qiagen RNA extraction kit (Qiagen,Ltd., Crawley, UK). RNA was reverse transcribed tocDNA with a first strand cDNA synthesis kit (Fermentas, Germany) according to the manufacturer's protocols.

Amplification of ovine GH cDNA

Ovine GH cDNA was amplified using *Taq* DNA polymerase (Roche applied science) and the primers specific for the coding region of ovine GH cDNA .Primers were designed according to the published sequence for growth hormone cDNA of the ovine(accession number: X15976) in GenBank. Primer sequences were the following: the forward primer was Ovis-GH-F: 5'–TGGCTCGAGCTATGATGGCTGCAGG–3'and the reverse primer was Ovis-GH-R: 5'–ATAGCGGCCGCACAGGGGAGGGGTAACA–3'. Restriction enzyme sites of *Xho*I and *Not*I (underlined nucleotides in the above sequences) were integrated into the 5'end of primers Ovis-GH-F and Ovis-GH-R, respectively. In order to amplify ovine growth hormone cDNA, PCR was performed in a 50µl total volume containing 1µg of template cDNA, 1µM of each primer (Ovis-GH-F and Ovis-GH-R), 2mM $MgCl_2$, 200 µM dNTP, 5µM of 10X PCR buffer and 1unit of *Taq* DNA polymerase. The 30-cycle amplification was performed in a thermal cycler system (Master Cycler Gradient, Eppendorf, Germany) with the following program: 94°Cfor 60 s, 58°C for 60 s, 72°C for 40 s. A final 5 mine tension was performed at 72°C. The PCR product was analyzed by electrophoresis in 1% agarose gel in 1X TBE buffer and visualized by ethidium bromide staining on UV transilluminator. The agarose gel slice containing the relevant ovine GH cDNA fragment was excised and purified by gel extraction kit (Bioneer, Koria) according to the manufacturer's recommendation.

Cloning and preparation of ovine TOPO-GH

Bovine growth hormone cDNA (as prepared above) was cloned using T/A cloning technique. T/A cloning is one of the most popular methods of cloning the amplified PCR product using *Taq* and other polymerases. Ovine GH cDNA fragment ligated to the T-Vector using TOPO T/A cloning kit (pCR8/GW/TOPO, Invitrogen) according to the manufacturer's instructions to obtain TOPO-GH. *Xho*I/*Not*I restriction analysis and PCR technique were used to confirm gene cloning. Finally, the recombinant plasmid (TOPO-GH) was sequenced by specific primers.

RESULTS

Total RNA was extracted from sheep pituitary tissue and the cDNA was successfully prepared. Desirable fragment with 690 bp length was obtained on a 1% agarose gel under UV light. An example of agarose gel containing amplified GH gene is obtained in Figure 1, which was cloned with T/A cloning technique in a T/A vector (pCR 8/GW/TOPO Vector). Chemical competent cells of *E. coli*were transformed with TOPO-GH recombinant plasmid. Plasmid purification and *Xho*I/*Not*I restriction endonuclease digestion of TOPO-GH recombinant plasmid, confirmed the correction of GH cDNA cloning.

To confirm of the cloning, extracted plasmid from transformed cells were digested by *Xho*Iand *Not* I and two distinct bands were detected. Figure2 shows recombinant plasmids after digestion. A 3956bp large fragment is related to the TOPO vector and the 690 bp fragment is the GH cDNA band. The sequence of the GH cDNA was compared with GH nucleotide sequences of other records in the GenBank using basic local alignment search tool (BLAST) software.

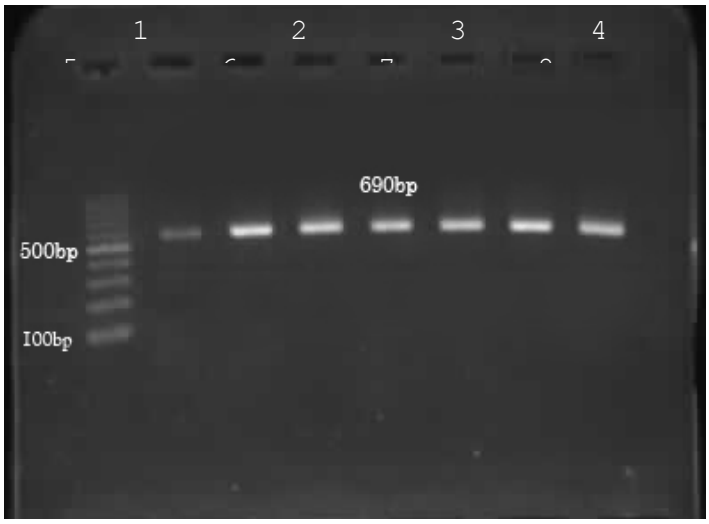

Figure 1: Gel electrophoresis for detection of *GH* gene in samples. Lane 1 is 100 bp DNA ladder (Ferments, Germany); lane 2-7 are positive samples, lane 8 is positive control.

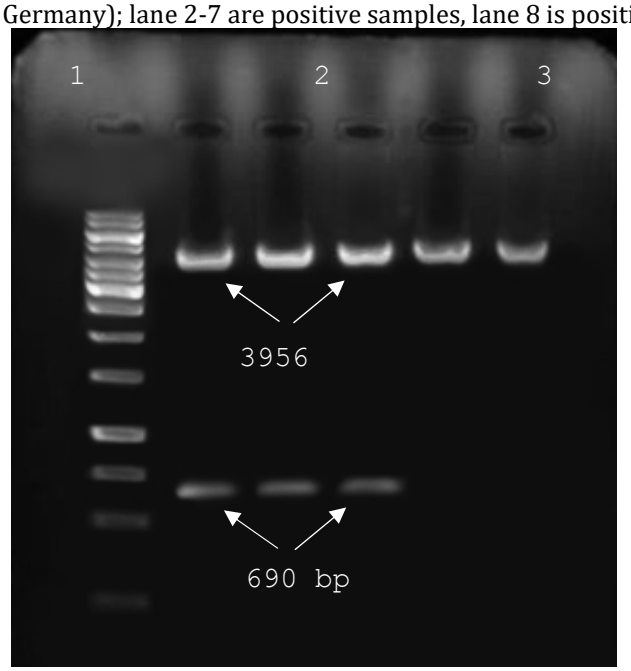

Figure 2: Analysis of TOPO-GH recombinant vector using EcoRI restriction endonuclease enzyme (Line 1 is 100 kb DNA ladder (Fermentas, Germany), lines 2, 3 and 4 are Topo-GH, and line 5 and 6are Topo vector without GH).

DISCUSSION

Sheep is one of the most important group of ruminants in Iran, specially in rural region. Sheep population is almost 57% of the avilable animal units in this country.As a matter of fact ovine milk and dairy products had long traditions in human nutrition in all period of times [17].In this study to produce a recombinant expression vector, we have cloned ovine GH from a pituitary cDNA library from Lori Bakhtiari sheep in *E. coli* microorganism. Expression of recombinant protein in *E. coli* allows its rapid and economical production in large amounts [18].

GH is a polypeptide of basic importance for growth regulation in vertebrates and, together with prolactin and somatolactin, organizing a family of pituitary hormones with similar structure and function that appears to have originated from a common ancestral gene before the evolution of animals [19]. GH plays an critical role in metabolism of lipids, proteins and carbohydrates. Further to improvement of hypopituitary dwarfs, it is applied in the treatment of variety of ailments. Therefore, production of GH has been in the center of attentions in various heterologous gene expression systems [20]. The long-term effects of GH on somatic growth and regeneration require changes in gene expression that are regulated by sequence-specific transcription factors activated through GH-stimulated signal transduction pathways

[21]. As several signaling pathways are motivated by GH, it is not surprising that a variety of transcription factors serve as mediators of GH action under different conditions[19].
Recombinant DNA technology is another major DNA-based tool that has gained popular attention in the past decade. This technology allows scientists to find individual genes, cut them out, and insert them into the genome of another organism. Recombinant DNA technology has applications in health and nutrition. In medicine, it is used to create pharmaceutical products such as human insulin [22]. In agriculture, it is used to impart favorable characteristics to plants to increase their yield and improve nutritional content. This technology has allowed the detailed characterisation of mRNAs for pituitary GHs from a number of species, such as rat [23], ox [24], pig [25], sheep [26] and man [27], and work has been carried out on the expression in *E. coil* and other microorganisms of some of these hormones, particularly human [28], ovine [24] and bovine GH [29].
The popularity of enteric bacterium *E. coli*as expression host is mainly due to its high growth rates on inexpensive substrates [30], and the ability to express high levels of heterologous proteins [31].In comparison with the other expression systems, *E. coli*is considered to be one of the most widely used prokaryotic hosts for the industrial production of recombinant GH, since this protein does not require post-translational modifications like glycosylation [32].Recent work by Talmadge et al., [33] has shown that the presence and precise location of the signal peptide plays a crucial role in the transport of proteins into the bacterial periplasmic space, and that both bacterial and eaukaryotic signals are recognized and processed correctly by bacteria [34].

CONCLUSION

Molecular technology is used for the production of many therapeutic proteins, such as antibodies, cytokines, blood products, growth factors, recombinant enzymes, hormones and veterinary and human vaccines. This study describes the successful cloning in E. coli of the GH cDNA. The TOPO-GHplasmid that was generated in this study is ready for sub-cloning and production of recombinant growth hormone protein in future studies.

ACKNOWLEDGMENT

The authors would like to thank all the staff members of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch and also Islamic Azad University of Science and Research Branch in Iran for their sincere support.

REFERENCES

1. Bech Jensen, E.&Carken, S. (1990). Production of Recombinant Human Growth Hormone in *Escherichia coli*; Expression of Different Precursors and Physiological Effects of Glucose, Acetate, and Salts. Biotechnol. Bioeng., 36(1):1-11.
2. Becker, G.W.& Hsiung, H.M. (1986). Expression, secretion and folding of human growth hormone in Escherichia coli. FEBS. Lett., 204(1):145-150.
3. Khodabandeh, M., Yakhchali, B., Rahimi, M., Vaseli, N., Moghaddasi Jahromi, Z., Zomorodipour, A.R., Dizaji, A.A.K.H., Ansari, S.& Sanati, M.H. (2003). Purification of large quantities of biologically active recombinant human growth hormone. Iran. J. Biotechnol., 1(4):207-212.
4. Zomorrodipour,A., Yakhchali,B., Khodabandeh,M., Deezagi,A., Hosseini Mazinani,S.M., Valian Borujeni,S., Ahmadzadeh Raji,M., Rahimi,M., Ahmadi Danesh,H.& Sanati,M.H. (2004). The Over-Expression of Biologically Active Human Growth Hormone in a T5-Based System in Escherichia coli, Studying Temperature Effect. Journal of Sciences, Islamic Republic of Iran., 15(1):27-32.
5. Orian, J.M., O'Mahoney,J.V.&Brandon,M.R. (1988). Cloning and sequencing of the ovine growth hormone gene. Nucleic. Acids. Res., 16(18):9046.
6. Sommese, L., Donnarumma, G., de l'Ero, C., Marcatili, A., Vitiello, M.& Cipollaro, D. (1996).Growth hormone modulates IL-alpha and IFN-gamma release by murine splenocytes activated by LPS or porins of Salmonella typhimurium. J. Med. Microbiol., 45(1):40-47.
7. Ding, H., Shan, J., Zhang, B., Ma, H.B., Zhou, L., Jin, R., Tan, Y.F.& He, L.X. (2005). Combined human growth hormone and lactulose for prevention and treatment of multiple organ dysfunction in patients with severe chronic hepatitis B. World. J. Gastroenterol.,11(19):2981-2983.
8. Francis, S.M., Veenvliet,B.A., Littlejohn,R.P., Stuart,S.K.& Suttie,J.M.(1995). Growth hormone (GH) secretory patterns in genetically lean and fat sheep. Proc. N. Z. Soc.Anim. Prod., 55:272-274.
9. Bonczek, R.R., Young,C.W., Wheaton,J.E.& Miller,K.P.(1988). Responses of somatotropin, insulin, prolactin, and thyroxine to selection for milk yield in Holsteins. J. Dairy. Sci., 71(9):2470-2479.
10. Ward, K.A.& Brown,B.W.(1998). The production of transgenic domestic livestock: Successes, failures and the need for nuclear transfer. Reprod. Fertil. Dev., 10(7-8):659-665.
11. Adams, N.R., Briegel,J.R. & Ward,K.A.(2002). The impact of a transgene for ovine growth hormone on the performance of two breeds of sheep. J. Anim. Sci., 80(9):2325-2333.

12. Johannsson, G., Sverrisdottir,Y.B., Ellegard,L., Lundberg,P.A.& Herlitz,H.(2002). GH increases extracellular volume by stimulating sodium reabsorption in the distal nephron and preventing pressure naturiesis. J. Clin. Endocrinol. Metab., 87(4):1743-1749.
13. Hull, K.L.& Harvey,S.(2001). Growth hormone: Roles in female reproduction. J. Endocrinol. 168(1):1-23.
14. Bollano, E., Omerovic,E., Bohlooly,M., Kujacic,V., Madhu,B., Isaksson,O., Soussi,B., Schulze,W., Fu,M.L.X., Matejka,G., Waagstein,F.& Isgaard,J.(2000). Impairment of cardiac function and bioenergetics in adult transgenic mice over expressing the bovine growth hormone. Endocrinology., 141(6):2229-2235.
15. Vatankhah, M. (2013). Genetic analysis of ewe body weight in Lori-Bakhtiari sheep using random regression models. Journal of Livestock Science and Technologies., 1(1):44-49.
16. Vatankhah, M.& Zamani, F. (2007). Phenotypic and genetic characteristics of longevity in Lori-Bakhtiari sheep. Biotechnol. Anim. Husb., 23(5-6):323-329.
17. National Cartographic Center.(1999). National atlas of Iran animal husbandry. Islamic Republic of Iran, Plan and Budget Organization, Tehran. Iran. 52pp.
18. Adams, N.R. & Briegel, J.R. (2005). Multiple effects of an additional growth hormone gene in adult sheep. J. Anim. Sci., 83(8):1868-1874.
19. Meier, K.M., Castano, C., Laurino, J., Levy, J.A.& Marins,L.F. (2006). cDNA cloning and phylogenetic analysis of growth hormone genes from the mullet Mugil platanus (Mugilomorpha, Mugilidae) and the halfbeak Hemiramphus brasiliensis (Atherinomorpha, Hemiramphidae). Atlântica, Rio Grande., 28(2):97-102.
20. Hindmarsh, P.C.&Brook, C.G. (1987). Effect of growth hormone on short normal children. Br. Med. J.Clin. Res. Ed. 295(6598):573-577.
21. Woelfle, J., Chia, D.J., Massart-Schlesinger, M.B., Moyano, P.& Rotwein,P.(2005). Molecular physiology, pathology, and regulation of the growth hormone/insulin-like growth factor-I system. Pediatr. Nephrol., 20(3):295-302.
22. Dyck, M.K., Lacroix, D., Pothier, F.& Sirar,M.A. (2003). Making recombinant proteins in animals different systems, different applications. Trends. Biotechnol., 21(9):394-399.
23. Tritos, N.A.&Mantzoros, C.S. (1998). Recombinant human growth hormone: old and novel uses. Am. J. Med., 105(1):44-57.
24. Kim, M.J., Park, H.S., Seo, K.H., Yang, H.J., Kim, S.K.& Choi, J.H. (2013). Complete solubilization and purification of recombinant human growth hormone produced in Escherichia coli. PLoS One., 8(2):e56168.
25. Vasquez-Bahena, J., Vega-Estrada, J., Santiago-Herna ´ndez, J., Ortega-Lo ´pez, J., Flores-Cotera, L., Montes-Horcasitas, M.&Hidalgo-Lara,M.E. (2006). Expression and improved production of the soluble extracellular invertase from Zymomonas mobilis in Escherichia coli. Enzym. Microb. Tech., 40(1):61-66.
26. Hahm, M.S.&Chung, B.H. (2001). Secretory expression of human growth hormone in Saccharomyces cerevisiae using three different leader sequences. Biotechnol. Bioproc. Eng., 6(4):306-309.
27. Ozdamar, T.H., Sentürk, B., Yilmaz, O.D., Calik, G., Celik, E.& Calik, P. (2009). Expression system for recombinant human growth hormone production from Bacillus subtilis. Biotechnol. Prog., 25(1):75-84.
28. Roehr, B. (2002). The many faces of human growth hormone. BETA Bulletin of Experimental Treatments for AIDS: A Publication of the San Francisco AIDS Foundation., 15(4):12-16.
29. Gelato, M., McNurlan, M.& Freedland, E. (2007). Role of recombinant human growth hormone in HIV-associated wasting and cachexia: pathophysiology and rationale for treatment. Clin. Ther., 29(11):2269-2288.
30. Baneyx, F.& Mujacic, M. (2004). Recombinant protein folding and misfolding in Escherichia coli. Nat. Biotechnol., 22(11):1399-1408.
31. Rai, M.& Padh, H. (2001). Expression systems for production of heterologous proteins. Curr. Sci. Bangalore., 80(9):1121-1128.
32. Ramanan, R.N., Tik, W.B., Memari, H.R., Azaman, S.N.A., Ling, T.C.& Tey, B.T. (2010). Effect of promoter strength and signal sequence on the periplasmic expression of human interferon- a2b in Escherichia coli. Afr. J. Biotechnol., 9(3):285-292.
33. Talmadge, K.& Gilbert, W. (1982). Cellular location affects protein stability in Escherichia coli. Proc. Natl. Acad. Sci. USA., 79(6): 1830-1833.
34. Talmadge,K., Kaufman, J.&Gilbert, W.(1980). Bacteria mature preproinsulin to proinsulin. Proc. Natl.Acad. Sci.USA., 77(7):3988-3992.