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Original article

GENERATION OF pcDNA 3.1^{+}-GH AS A RECOMBINANT EXPRESSION VECTOR OF OSTRICH GROWTH HORMONE cDNA IN *SACCHAROMYCES CEREVISIAE*

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Summary

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Growth hormone is essential hormone for vertebrates like the ostrich (*Struthio camelus*) for growth stimulation, carbohydrate metabolism, protein assimilation etc. Growth hormone is secreted by the pituitary gland and expressed in many cells and tissues. The purpose of this study was generation of pcDNA 3.1^{+}-GH recombinant expression vector in order to sub-clone ostrich growth hormone cDNA into *Escherichia coli*. In brief, total RNA was extracted from the pituitary gland tissue and cDNA sample was synthesised. The cDNA was amplified by PCR and revealed a 672 bp fragment on 2% agarose gel electrophoresis. Then, the ostrich growth hormone cDNA was extracted from the gel and was cloned into pCR8/GW/TOPO vector by T/A cloning technique to produce pCR8/GW/TOPO-GH. After obtaining the sequence of cDNA of the ostrich in Iran, it was submitted in GenBank (Accession number: JN559394). Finally, the GH cDNA was sub-cloned using pcDNA 3.1^{+} into *Saccharomyces cerevisiae* and pcDNA 3.1^{+}-GH recombinant expression vector was generated. The results of present study were showed that ostrich growth hormone cDNA was successfully sub-cloned into *Saccharomyces cerevisiae*. Therefore, the pcDNA 3.1^{+}-GH recombinant expression vector generated in this study could be useful to express the ostrich growth hormone in yeast cells as a simple and affordable way to produce this hormone at a large scale.

Key words: growth hormone, ostrich, pcDNA 3.1^{+}-GH, *Saccharomyces cerevisiae,* sub-cloning

INTRODUCTION

The ostrich is one of the flightless bird species native to Africa (class Aves, order Struthioniformes, family Struthionidae, genus *Struthio)* and the biggest living bird and mono-gastric vegetarian. Its body size and weight are 1.8–2.0 m and 200 kg, respectively (Bona-Gallo *et al.*, 1983; Peng *et al.*, 2010). They have features of cursorial animals, effective locomotors. The birds generally live in dry African

areas and hot Saudi desert (Smith *et al.*, 2006). Food plays an important role in ostrich production but price of feeding is the biggest outlay in ostrich farming. The ostrich's diet consists chiefly of plant matter, green grass, berries, seeds, and small insects. The feed conversion is efficient in the first 210 days of the life (Aganga *et al.*, 2003). Ostrich oil is a source for diverse commercial products as body oils, soaps and moisturizing creams. This oil is similar to human skin lipid and very worthy (Aghdam Shahryar & Lotfi 2012).

Growth hormone (GH) known as somatotropin or somatropin, is a peptide hormone involved in cell duplication and growth in animals and humans. This protein consists of single-chain polypeptide with 191 amino acids that is produced, stored and secreted by somatotropic cells (Doosti *et al.*, 2012). GH is secreted from pituitary somatotrophs into the bloodstream and then activated. It is present in many extra-pituitary tissues, so *GH* gene expression in neural, immune, alimentary, respiratory tissues, muscular, skeletal, and cardiovascular systems is important (Harvey, 2010). The hormone is used as a prescription drug in medicine to treat children's growth disorders and adult growth hormone lack. GH is a necessary protein required for the development of the ostrich, carbohydrate breakdown, growth stimulation, lipid and electrolyte metabolism, and protein assimilation (Sekine *et al.*, 1985; Komano *et al.*, 1999). The *GH* gene in mammalian and avian species consists of five exons and four introns (Komano *et al.*, 1999). Different parameters could affect on levels of growth hormone secretion and successful growth and reproductive performance of the ostrich like genetic background and environmental condition. (Cooper, 2001; Hassan *et al.*, 2004). Therefore, the present study was performed for sub-cloning synthesised cDNA from ostrich growth hormone mRNA into *Saccharomyces cerevisiae* by generation of pcDNA 3.1$^+$-GH recombinant expression vector.

MATERIALS AND METHODS

Pituitary gland isolation

A 3-year-old healthy male ostrich at sexual maturity was euthanised by carbon dioxide inhalation according to American Veterinary Medical Association (AVMA) guidelines (2013 edition). The bird was dissected, the brain removed from the skull and then the pituitary gland was immediately excised and stored frozen at –70 ^{o}C until used.

RNA extraction and cDNA synthesis

Total RNA was isolated from the pituitary gland tissue using RNX-Plus buffer (Cinnagen, Iran) according to the manufacturer's instructions. The concentration of extracted RNA was measured by NanoDrop ND-1000 (PeqLab) spectrophotometer according to the method described by Sambrook & Russell (2001). Then, 1 µg of RNA sample was reverse-transcribed to synthesised cDNA using the PrimeScript^{TM} RT Reagent Kit (Takara Bio Inc, RR037A) for 15 min at 37 ^{o}C to the reverse transcription, and the reverse transcriptase enzyme was inactivated at 85 ^{o}C for 5 s.

Gene amplification

The extracted ostrich GH cDNA was amplified by polymerase chain reaction (PCR) using designed specific oligonucleotide primers (Ost-GH-F: 5'-TCCTC GAGACAGAAATGGCTCC-3' and Ost-GH-R: 5'-CCATGGAGATGGTGCATTG CTT-3' containing *Xho*I and *Nco*I restric-

tion sites, respectively) according to the published sequence for ostrich growth hormone gene in DNA data bank (accession number AB028191). PCR reaction was performed in a final volume of 50 µL in 0.5 mL micro-tubes containing 2 mM $MgCl_2$, 200 µM dNTP, 5 µM of 10× PCR buffer, 1 unit of *Taq* DNA polymerase (all Roche Applied Science, Germany), 1 µg of template cDNA, and 1 µM of each primer (Ost-GH-F and Ost-GH-R). The temperature programme was performed in Gradient Palm Cycler (Corbett Research, Australia) involving an initial denaturation at 94 °C for 5 min; followed by 32 cycles at 94 °C for 1 min, annealing at 58 °C for 1 min, elongation at 72 °C for 1 min, and a final elongation at 72 °C for 5 min. The amplified cDNA of GH was loaded in 2% agarose gel electrophoresis and after staining by ethidium bromide, gel photograph was obtained under UVIdoc gel documentation systems (Uvitec, UK).

Purification and cloning of cDNA products

The amplified cDNA was purified from the gel using QIAquick Gel Extraction Kit (Qiagen, Inc.) according to manufacturer's recommendation. The extracted cDNA of ostrich growth hormone were T/A cloned into pCR8/GW/TOPO vector (Invitrogen) according to the manufacturer's protocol at 4 °C overnight. Then, the recombinant pCR8/GW/TOPO-GH vectors were transferred to a competent *E. coli* TOP10F´ using ice-cold calcium chloride solution (0.1 M) and cells were then subjected to heat shock at 42 °C for 90 s. After that the competent cells were cultured in LB agar plate (Merck Co., Germany) at 37 °C overnight. The media was supplemented with ampicillin (100 µg/mL), Xgal (20 mg/mL) and IPTG (0.1 M) for screening of recombinant pCR8/ GW/TOPO-GH vectors. The white colonies were selected to preparation of matrix and colonies were cultured again at 37 °C in LB broth media containing ampicillin (the selective antibiotic) overnight. The recombinant plasmids were extracted from bacterial cells using Plasmid Mini Extraction Kit (BIONEER, South Korea) using manufacturer's instructions. PCR and restriction enzymes (*Xho*I and *Nco*I) analysis were used for confirmation of cloning. Finally, the recombinant plasmid (pCR8/ GW/TOPO-GH) was sequenced by Sanger sequencing method (Macrogen, Korea) and the sequence of ostrich GH cDNA was submitted to GenBank.

Sub-cloning of GH gene into expression vector

The pcDNA 3.1^+ expression vector (Invitrogen, San Diego, CA) and pCR8/GW/TOPO-GH containing inserted cDNA (672 bp fragment) of ostrich GH were digested by *Xho*I and *Nco*I restriction enzymes. Then, removed gene was ligated by T4 ligase enzyme into linearised pcDNA 3.1^+ and the pcDNA 3.1^+-GH recombinant expression vector was generated and subcloned into *Saccharomyces cerevisiae*.

RESULTS

The PCR products of amplified *GH* gene on 2% agarose gel revealed a 672 bp fragment. Growth hormone cDNA of ostrich were T/A cloned into pCR8/GW/TOPO vector (2817 bp) and pCR8/GW/TOPO-GH was produced successfully.

For confirmation of gene cloning pCR8/GW/TOPO-GH recombinant vector was digested by *Xho*I and *Nco*I restriction enzymes (Fig. 1). The sequence of the GH cDNA (cDNA of the ostrich in Iran) was submitted successfully to the GenBank (Accession number: JN559394). For subcloning of GH cDNA, the pCR8/GW/

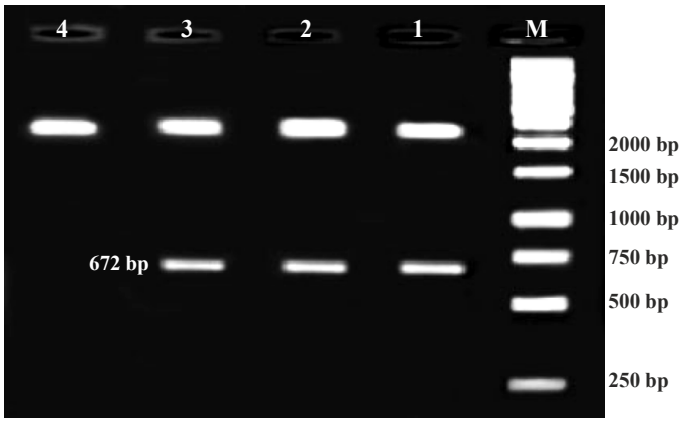

Fig. 1. Digestion of pCR8/GW/TOPO-GH vector using *Xho*I and *Nco*I restriction enzymes. Lane M: 1 kb DNA marker (Fermentas, Germany); lanes 1, 2 and 3: pCR8/GW/TOPO-GH; lane 4: pCR8/GW/TOPO vector without inserted gene.

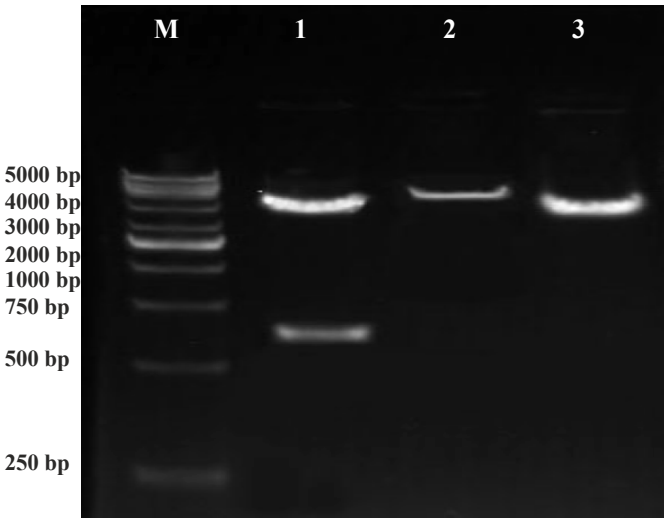

Fig. 2. Analysis of pcDNA 3.1$^+$-GH using *Xho*I and *Nco*I restriction enzymes. Lane M: 1 kb DNA ladder (Fermentas, Germany); lane 1: digested pcDNA 3.1$^+$-GH to pcDNA 3.1$^+$ vector (5428 bp) and inserted gene (672 bp); lane 2 is undigested pcDNA 3.1$^+$-GH recombinant vector (6100 bp); lane 3: pcDNA 3.1$^+$ without inserted *GH* gene.

TOPO-GH and pcDNA 3.1$^+$ expression vector were digested and linearised with *Xho*I and *Nco*I restriction enzymes and finally pcDNA 3.1$^+$-GH recombinant expression vector in *Saccharomyces cerevisiae* were successfully generated.

The results of enzyme digestion of pcDNA 3.1$^+$-GH expression vector on 2% agarose gel electrophoresis revealed 6100 and 5428 bp for pcDNA 3.1$^+$-GH and pcDNA 3.1$^+$ vector (without inserted gene), respectively (Fig. 2).

DISCUSSION

In recent decades recombinant DNA technology and genetic engineering allows researchers to find individual genes, amplify and insert them alone or together into the genome of another organisms (Dyck *et al.,* 2003; Ma *et al.,* 2005). For example, the cDNA that encodes ostrich growth hormone can be isolated from an ostrich pituitary cDNA library. In the present study, the sequence of ostrich GH cDNA in this research showed 100% similarity with other reported sequence in other records (such as accession number AB028191 in GenBank). Finally, the pcDNA 3.1^{+}-GH recombinant expression vector was generated successfully and subcloned into *Saccharomyces cerevisiae*. The amino acid sequence homology between ostrich GH and that of ducks, chickens and turkeys is very high (94–90%), while *GH* gene sequence homology between ostrich and mice, rates, bovines, goats and humans are relatively low (72–71%) (Komano *et al.*, 1999). The authors cloned and sequenced the cDNA of ostrich growth hormone and showed that the length of this cDNA was 866 bp consisting of 651 bp coding area and encoding a polypeptide with 217 amino acid residues.

In 2012, ostrich GH cDNA was successfully cloned into *E. coli.* Sequencing results and BLAST search showed that the sequence of GH cDNA of the ostrich in Iran had 100% homology with other records existing in GenBank (Doosti *et al.*, 2012). Rezaei & Zarkesh-Esfahani (2012) optimised the production of recombinant human growth hormone (rhGH) in *Escherichia coli* and high levels of rhGH were produced using prokaryotic protein production system. They showed this system as simple and cost effective means for large scale production of rhGH. In the current study, our recombinant expression vector was useful for production of ostrich GH in eukaryotic protein production system. Furthermore, rhGH was produced at a high level in *E.coli* Bl-21 (Shin *et al.,* 1998). We, however cloned ostrich GH in *E. coli* TOP10F′ and then subcloned in pcDNA 3.1^{+} and pcDNA 3.1^{+}-GH recombinant expression vector was created. In another study, Anathy *et al.* (2001) have successfully cloned, sequenced and expressed the cDNA encoding growth hormone of Indian catfish (*Heteropneustes fossilis*) in bacterial and zebrafish systems using appropriate expression vectors. The cDNA that encodes insulin-like growth factor 1 in Iranian cattle was cloned in *E. coli* and sequenced for the first time in Iran using methods resembling those used in the present work (Doosti *et al.*, 2013).

In conclusion, the generated pcDNA 3.1^{+}-GH recombinant expression vector in this study could produce high levels of ostrich GH in eukaryotic host as a good source of this hormone for further investigations and reduce the production costs. Also, our study can potentially provide insight into industrial supplement such as hormone synthesis.

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