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PCR and cloning of recombinant growth hormone cDNA from Cholistani cow

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Abstract

Cholistani cow breed is a zebu (*Bos indicus*) or one humped breed of cattle being reared by the nomadic pastoralists of Cholistan desert, Pakistan. With an increasing human population and improving living standards, the demand for meat and milk is also increasing. To cope with such conditions in Pakistan, especially in Cholistan, recombinant technology is used. Importance of recombinant growth hormone rGH in milk and meat production bovine growth hormone bGH has been cloned from a number of species like buffalo, sheep, goat, sheep, and horse etc. The present study was carried out to clone bGH from Chlostani cow. For this purpose, total cellular RNA was extracted from the pituitary gland of freshly slaughtered animal, cDNAs were synthesized and amplified with the help of sequence specific primers. The amplified products were confirmed through restriction digestion and finally cloned in Thyamine Adenine T/A cloning vector followed by transformation in *E. coli* strain DH5α. Positive clones were confirmed by restriction digestion and sequence analysis confirmed clones were stored at -70˚C as glycerol stock. By using cDNA growth hormone, milk production in young cows can be enhanced. More quantity of milk will compensate the needs of growing population.

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Introduction

Cholistan is a typical rangeland which contributes significantly towards country supply line for milk and meat. The estimated number of livestock in the Cholistan areas is 1.6 million.

The total livestock population in Cholistan is estimated as 12, 95,462 out of which 567,510 heads are of cattle (Directorate General of Monitoring and Evaluation, Planning and Development, Govt. of Punjab, 2010). Cholistani cow breed, zebu (*Bos indicus*) is reared by the nomadic pastoralists of Cholistan desert, Pakistan. The evolution and genetic adaptation the Zebu cattle acquired genes of thermo-tolerance making them better able to regulate body temperatures than the *Bos Taurus* or non-humped breeds of temperate region (Farooq *et al.,* 2010).

By increasing human population and rising standards, the demand for meat and milk is also increasing. Recombinant DNA technology has provided solutions to problem like this. The techniques available in genetic engineering have provided the boost to dairy industry by the production of large quantities of recombinant bovine somatotropin (rbST) (Etherton and Bauman, 1988). Bovine growth hormone (bGH) or bovine somatotropin (rbST), is a hormone produced by pituitary gland of animal.

The bovine pituitary gland naturally secretes bST into the bloodstream, which acts on receptors in the liver to produce Insulin-like growth hormone which enhances milk production in body and improves the efficiency of milk synthesis factor (Bauman, 1992). bGH has dramatic physiological actions in the cow, it stimulates a significant increase in mammary gland development (Hauser *et al.,* 1990).

Endocrine growth control in Cholistani cow involves complex interactions of several hormones and growth factors, acting in both an endocrine and a paracrine or autocrine manner. Although bGH blood levels depend on physiological states and other regulatory factors, there is evidence of an association of genetic characteristics with GH plasma levels. Increased GH blood levels have been reported in dairy cattle selected for elevated milk yield (Mauro *et al.,* 2002). bGH not only improves the efficiency of milk production (per unit of food consumed) but also improves the production (body weight) and quantity (muscle: fat ratio) of meat. The advent of recombinant DNA technology allowed the production of relatively large quantities of synthetic rbST (Ahmad and Sarwar, 2002).

The purpose of rbST is to enable animals like cattle and camel to produce milk and meat upto their natural potential. It works by altering gene expression of glucose transporters in the animal's mammary gland, skeletal muscle and mental fat. The use of bGH has increased the chances of bacterial infections in cows by 25 percent. The gene facilitates the repartitioning of glucose to the mammary gland, which in turn produces more milk (Paul, 1998). Keeping all in view, the present work was conducted to produce a clone of recombinant growth hormone cDNA from Cholistani cow.

Materials and methods

RNA extraction and cDNA synthesis

Pituitary was collected from freshly slaughtered Cholistani cow and carried in ice to Biochemistry laboratory, University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur. Total cellular RNA was extracted from pituitary by using the method described by (Chomezynski & Sacci, 1987) and cDNAs were synthesized by using RvertAid™, First Strand cDNA Synthesis Kit (Fermentas #K 1622) along with two different primers: random hexamer and sequence specific.

Polymerase Chain Reaction PCR

PCR conditions were optimized for bST genes by using changing primers, $MgCl_2$, template concentrations and annealing temperatures. In optimized PCR reaction 1 µl of 100 pM each sequence specific primer, 1.2 µl of 25mM $MgCl_2$, 0.4 µl of 10mM dNTPs, 0.2ul of Taq DNA polymerase and 2.5

µl of cDNAs in total vollume of 20 µl were used. PCR programme included, denaturation at 94˚C for 5 minutes in first cycle, then for other cycles, denaturation at 94˚C for 30 seconds, primer annealing at 58˚C for 45 seconds followed by extension at 72˚C for 90 seconds for 35 cycles. The final extension was done at 72 ˚C for 25 minutes. The amplified products were run on 1% agarose gel and then visualized in UV Tran illuminator.

Ligation and Transformation

After confirmation of amplified product through restriction digestion, it was ligated in T/A vector and finally transformed into competent cells. Both primers were added equally with following constituents Vector pTZ57R/T 1.5ul, 5X Ligattion Buffer 3ul, PCR product 4ul, Deionized water 5.5ul, DNA Ligase 1ul. Clones were cultured by using the nutrient agar and broth media. Culture was grown in the form of colonies in agar plates. The cells were plated on ampicillin positive agar plates and kept in incubator at 37°C for 24 hours.

Plasmid extraction

The protocol for plasmid extraction was adapted from BIO BASIC INC.BS71918 EZ-10 Spin Column Endotoxin Free Plasmid Preps Kit. The overnight culture (1.5ml) was added to 1.5ml Eppendorf tube and centrifuged at 12,000rpm for 1 minute. Pellet was formed in the tube after the centrifugation, supernatant was discarded completely.

Solution I (100ul) was added into the pellet and mixed gently and kept at room temperature (RT) for 2 minutes and vortexed for few seconds. Solution II (200ul) was added to the mixture and mixed gently by inverting the tube 4-6 times and kept at RT for 1 minute. Then 350µl of Solution III was added and mixed gently. The mixture in the tube was incubated at RT for 2 minutes and centrifuged for 10 minutes at 12,000 rpm. The supernatant was transferred into EZ-10 spin column and centrifuged at 12,000 rpm for 1 minute. After centrifugation, the flow-through was discarded in the tube. Column was washed by adding 750ul wash buffer and centrifuged at 10,000 rpm for 1 minute. Washing procedure was repeated. The mixture was centrifuged at 10,000 rpm for 2-3 times to remove any residue.

The column was transferred to a sterilized 1.5ml Eppendorf tube and 50µl of Elution Buffer was added into the central part of column. The column was kept at RT for 2 minutes and centrifuged at 10,000 rpm for 2 minutes. The purified DNA was stored at -20°C. The size and quality of DNA was checked through agarose gel electrophoresis.

Restriction digestion

After obtaining positive results, the digestion of DNA was done with restriction enzymes. The restriction enzymes Bam H1 and Xba1 were used for restriction digestion studies and each reaction mixture was incubated at 37˚C for 3-5 hours.

Results

Polymerase chain reaction

PCR technique was used to amplify cDNA of rbST. All cDNAs including cDNAs of developed n7 random hexamer primer, sequence specific primers and control cDNA were confirmed by PCR. The random hexamer base cDNA and sequence specific primer based cDNA has produced 592bp amplified product which is correct (Fig 1).

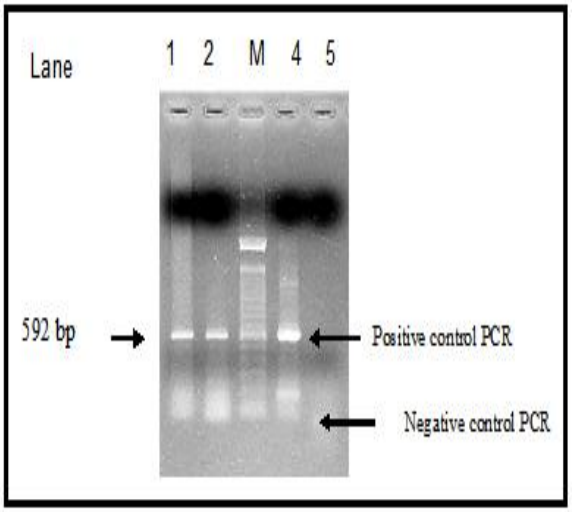

Fig. 1. Optimization of PCR, Lane 1: cDNA by sequence specific primer of rbST, Lane 2: 100bp DNA Ladder (Cat # 0323, Thermo scientific), Lane 3: cDNA by Random Hexamer primer of rbST, Lane 4: Control cDNA.

Confirmation of Amplified product

Amplified cDNA was digested with restriction enzyme DpnI which cuts the DNA at 2 sites i.e. at 206bp and 407bp as expected .The purpose of restriction was to confirm the amplified product. The fragments of correct sizes were obtained as shown in (Fig 2).

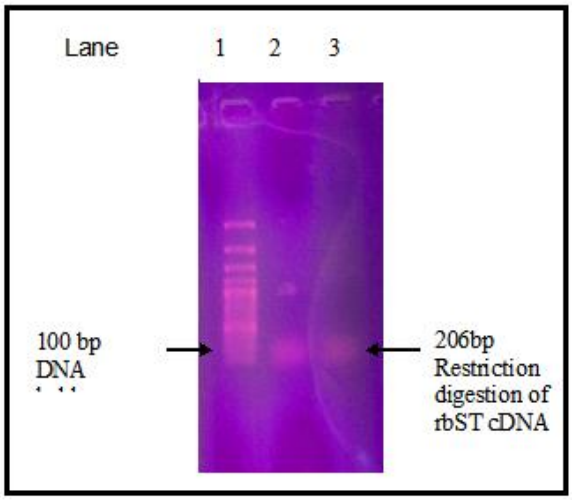

Fig. 2. Restriction of random hexamer of amplified product. Lane1: 100 bp DNA Ladder (Cat # 0323, Thermo scientific). Lane 2 : Restriction digestion of rbST cDNA by DpnI. Lane 3: Restriction digestion of bGH based cDNA by DpnI.

T/A Cloning

All cDNAs were ligated into vector Ptz57R/T followed by the transformation into DH5α cells (Fig 3). A colony on ampicillin positive agar plates facilitates the selection of positive clones.

Discussion

The present study was designed with an objective to clone cow growth hormone from local breed Cholistani. The similar kind of work was done by other researchers on other live stocks species (Venugopal, 2002). Growth hormone gene has been cloned from various organisms like cattle, bovine, equine, porcine, ovine, caprine and even from aquatic animals (Butt *et al.,* 2014). The cloning, expression and purification of cDNA for horse growth hormone and expressed in *E. coli* cells was performed by a group of researchers (Stewart and Tuffnell, 1991). The cDNA cloning of growth hormone from giant panda was performed by (Liao *et al.,* 2003). Another practice regarding cloning of equine growth hormone just like cow growth hormone was performed from Brushtail possum (Saunders *et al.,* 1998).

The amplification of rbST cDNA was done with different primers including sequence specific, random hexamer primers. The PCR results are consistent with the results of (Zamani *et al.,* 2015) where 576 bp product was obtained from Buffalo growth hormone cDNA with sequence specific primers. Restriction digestion of PCR has further confirmed the originality of molecule. The amplified products were ligated into pTZ57R/T vector and positive clones were confirmed by restriction digestion. Similar, confirmations were ended by (Munaretto *et al.,* 2009) using Nde1 and Bamh1 restriction enzymes on cGH.

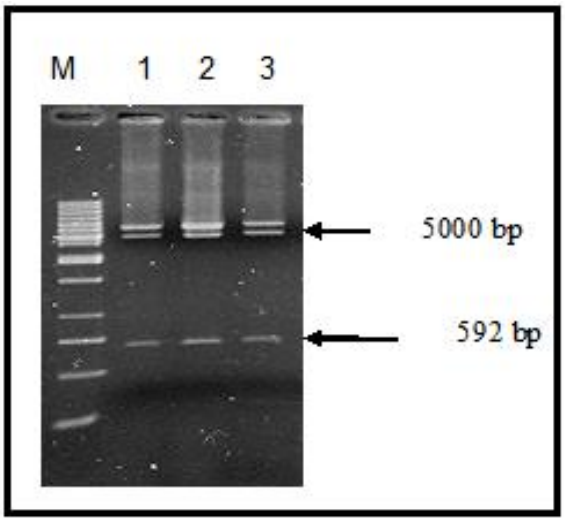

Fig. 3. Confirmation of T/A clones. M: 1 kb DNA markers (SM0311, Fermentas). Lane 2-4: restricted product of clones.

The cDNA of cGH will be a sub-clone in mammalian expression vectors, sequences and finally be used as DNA vaccine(s) as shown in work conducted by (Hussain *et al.,* 2014).

Conclusion

cDNA of cholistani cow was successfully cloned and confirmed through restriction enzymes. Those constructs will be used in making final constructs in prokaryotic and eukaryotic vectors.

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