Arabinose Promoter Based Expression of Biologically Active Recombinant Human Growth Hormone in E. coli: Strategies for Over Expression and Simple Purification Methods

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Abstract

An arabinose promoter based expression system in E. coli for the production and purification of recombinant human growth hormone (rhGH) was designed and implemented. The shake flask studies indicated appreciable amounts of rhGH expressed in modified pBAD24 vector (pBAD24M) in comparison to the original pBAD24 vector. While the levels of rhGH reached merely 75 mg l⁻¹ with pBAD24 in a bioreactor, it reached ~1860 mg l⁻¹ with pBAD24M vector under similar conditions as judged by densitometry of proteins resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The rhGH protein was successfully purified from inclusion bodies after urea denaturation by two simple ion-exchange chromatography steps with an overall recovery of 40% amounting to ~750 mg l⁻¹ of purified hGH which is the highest reported yield of purified rhGH to date. Such a purified bacterial derived rhGH was characterized by N-terminal sequence, CD spectrum studies, mass fingerprint analysis and analysis on Agilent 2100 bioanalyzer. The bioactivity of the purified rhGH was comparable with the commercially available hGH (somatotropin).

Keywords: Arabinose promoter; Human growth hormone; Inclusion bodies; Receptor activity assay; Recombinant proteins

Introduction

Human growth hormone (hGH) is a heterogeneous protein hormone consisting of several isoforms and the GH gene cluster on chromosome 17q contains 2 GH genes (GH1 or GH-N and GH2 or GH-V) in addition to 2 to 3 genes encoding the related immunity (Jorgensen, 1987) and is used in the treatment of hypopituitary dwarfism (Hindmarsh and Brook, 1997). Various expression systems that have been tried for hGH expression include Bacillus subtilis (Franchi et al., 1991), mammalian cells like CHO (Dallia et al. 2003), baculovirus system (Geng et al., 2002) and yeast such as Pichia pastoris (Ascacio-Martinez and Barrera-Saldana, 2004; Deshpande et al., 2009).

Although each system has its own advantages and successes, the use of E. coli for the production of rhGH is documented in several reports with higher success rates. Since the gene is not glycosylated, it was first expressed in E. coli (Goeddel et al., 1979). The interesting aspect of the E. coli system with respect to hGH is that the rhGH has been tested for expression in E. coli periplasmic space (Chang et al., 1987; Ghorpade and Garg, 1993) and in the cytoplasm as inclusion bodies (Mukhija et al., 1995; Schoner et al., 1992).

The strong inducible promoter systems (eg lac, T7, trpA, tac, lambda pl, etc) commonly used in recombinant E. coli are advantageous for over producing recombinant proteins at high cell density in fed-batch fermentations. Of these, the arabinose promoters are well studied systems since they are of low cost, have tight regulation and result in high level expression of the gene upon induction (Banerjee et al., 2009). Because the ara system can be induced by arabinose and is repressed by both catabolite repression in the presence of glucose or by competitive binding of the anti-inducer fucose, these plasmids have very low background levels of expression. In addition, gene expression can be turned on and off rapidly by changing the sugars in the medium (Siegele and Hu, 1997).

Our goal was to develop a high yielding rhGH1 (hereafter referred as rhGH) clone in E. coli K12 system under the arabinose promoter. This was mainly chosen since E. coli K12 is a biosafety level I organism, is a recA minus strain, capable to maintain high plasmid copy number and is free of endogenous integrated bacteriophages. We also tried working on simple purification protocols for purification of bacterially derived rhGH so that the protocol is scalable and cost-effective. This paper also focuses on characterization of such a purified rhGH preparation.

Materials and Methods

Bacterial strain, plasmids and growth conditions

E. coli strain DH5α was used for transformation, propagation and expression studies. Both the plasmid pBAD24 (gift from

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Dr. Yasuda, Institute of Genetics, Japan) and the modified pBAD24 vector, pBAD24M (Banerjee et al., 2009) with optimized Shine-Dalgarno (SD) sequence and T7g10 enhancer elements from λ phage were used for cloning and expression studies. The restriction enzymes were purchased from Bangalore Geneci, Bangalore India while L + arabinose was procured from Hi Media, Mumbai, India. The polyclonal anti-hGH antibody was from Abexome Life Sciences, Bangalore, India and all other fine chemicals and oligos were procured from Sigma, USA. Synthetic hGH gene was custom synthesized from GenScript, USA without codon-optimization for E. coli host. Both rhGH receptor and commercial rhGH were procured from R & D Systems, USA.

Cloning and expression of hGH in pBAD24 and pBAD24M vectors

hGH gene was PCR amplified using a synthetic hGH gene as a template to yield a PCR product of 585 bp. The EcoRI/HindIII digested PCR product was ligated to pBAD24 vector for 16 hours at room temperature. Additionally, another aliquot of the PCR product was ligated with NdeI/HindIII and ligated to pBAD24M vector (Banerjee et al. 2009) at similar sites. After transformation of DH5α cells with both the ligation mixes independently, the recombinants were selected by colony PCR using hGH gene specific primers. Recombinant plasmids carrying hGH gene were further confirmed by restriction analysis and DNA sequencing.

E. coli DH5α cells harboring either pBAD24-hGH or pBAD24M-hGH recombinants were grown in LB with ampicillin at 100 μg ml−1 and induced with 13 mM arabinose for 4 hours at 37°C. At the end of induction, the induced cells were harvested and lysed with glass beads to separate the soluble and the insoluble fractions. Both the fractions were analyzed on SDS-PAGE and visualized by Coomassie blue staining.

Determination of hGH yield by densitometry

Scanning densitometry has been reported as a reliable method for the quantitation of protein bands on Coomassie blue-stained polyacrylamide gels (Vincent et al., 1997). Hence, the protocol described by Dallia et al. (2003) was followed for this parameter. The hGH protein bands on the Coomassie blue-stained gel were determined by comparing the hGH monomer band scores with standard amount of BSA run on the same gel.

Bioreactor studies

The seed inoculum was prepared by inoculating E. coli DH5α cells carrying either pBAD24-hGH or pBAD24M-hGH plasmid, in LB medium and growing it for 16 hours at 37°C with agitation at 200 rpm.

Fermentation was carried out in a 5L bioreactor (Sartorius, Germany) to achieve high levels of rhGH expression. The fermentation was carried in 2.5 L medium in a batch mode. The medium comprised of yeast extract (3.6%), tryptone (1.8%), (NH₄)₂SO₄ (0.13%), NaH₂PO₄ (0.3%), Na₂HPO₄ (0.4%), KCl (0.067%), MgSO₄ (0.05%), glycerol (1.5%) and ampicillin (100 μg ml⁻¹).

The fermentation medium was inoculated with overnight seed culture at 10% v/v. Aeration was kept at 1vvm (volume of air / volume of fermentation medium / minute) and the dissolved oxygen level was maintained at 37°C by PID controller of the instrument. While the agitation was set between 300 to 800 rpm, the dissolved oxygen level was maintained at 30% by automatic addition of pure oxygen to air, which was controlled by mass flow controller of the instrument. The fermentation was carried out at pH 7.0 that was maintained by the automatic addition of either 30% H₃PO₄ or 12% NH₄OH and foaming controlled by addition of 0.05% antifoam US 1510 (Dow Corning, USA). The growing culture was induced at OD₅₀₀ of 10 to 15 by the addition of 13 mM L+ arabinose. The biomass achieved at the end of five hours post-induction was measured by estimating wet cell weight (WCW) per liter of a fermentation medium. For estimating WCW, 10 ml of the fermentation broth was taken in pre-weighed centrifuge tube (C) in triplicates and spun at 7000 g for 10 minutes. The supernatant was discarded and the tubes were wiped dry and weight of each tube was measured (T). The difference between T and C constituted the WCW.

For isolation of the hGH from the fermentation broth, the harvested broth was centrifuged (Kubota, Japan) at 8000 rpm for 10 minutes and the induced cell pellet - 140 g wet cells were suspended in 10 mM Tris buffer pH 8.0 and volume of the cell suspension was made to 1 L. Cell disruption was carried out at 900 to 1000 bars on a high pressure homogenizer (Niro Soavi, Italy). The cell disruption process was carried out for three passages. The obtained cell lysate was centrifuged at 10,000 rpm for 15 minutes to separate soluble and insoluble fractions. Each fraction was analyzed on SDS-PAGE followed by Coomassie R250 staining method. The total protein was estimated by BCA method (Thermo Scientific, USA) while the densitometer scanning was performed for estimating the amount of hGH achieved along with calculations of percentage purity of the inclusion bodies.

Purification and analysis of purified rhGH

Since only the pBAD24M-hGH clone expressed significant amounts of hGH, this clone was used for purification of rhGH.

Prior to processing, every gram of wet weight of the inclusion bodies was washed with 40 volumes of wash buffer 1 (50 mM Tris.Cl, pH 8, 1% Triton X 100 and 20 mM EDTA), wash buffer 2 (50 mM Tris.Cl, pH 8, 2M urea and 20 mM DTT) and wash buffer 3 (Milli Q water). The inclusion bodies (8.5 g wet wt) obtained from a liter of fermentation broth was solubilized in 340 mL of 8 M urea, pH 12.5 for 30 minutes at room temperature. The solubilized inclusion bodies were then refolded in 10 volumes of 50 mM Tris.Cl, pH 8.8 by fast dilution method. The refolded protein (3400 ml) was buffer exchanged with 20 mM sodium acetate pH 4.5 containing 2.5% sucrose, 0.2% Tween-80 and 0.5 mM EDTA (equilibration buffer). After buffer exchange, the protein was clarified by centrifugation at 13000 rpm for 15 minutes and the resultant supernatant (4080 ml) was loaded on an anion exchanger (Q Sepharose FF, GE Healthcare, Sweden) column at the flow rate of 8 ml min⁻¹. The column was then washed with the equilibration buffer till the OD₂₈₀ reached zero and the elution of the bound proteins was carried out by using a linear gradient of NaCl solution (0.0-1.0 M) in the same buffer in an AKTA explorer protein purification system (GE
Healthcare, Sweden). The flow through fraction (4780 ml) containing hGH protein was later loaded on a cation exchanger (SP Sepharose FF, GE HealthCare, Sweden) column. The bound proteins were eluted by a linear gradient of 0.0-1.0 M NaCl in 20 mM sodium acetate pH 4.5 containing 2.5% sucrose, 0.2% Tween 80 and 0.5 mM EDTA in a volume of 1870 ml. The purity of hGH was checked by subjecting the protein to SDS-PAGE followed by silver staining.

Characterization of purified rhGH

Peptide mass fingerprinting (PMF) for the purified hGH preparation was done using Bruker Daltonics MALDI-TOF/TOF (JISC, Bangalore, India) and the peptide masses obtained by MALDI-TOF/TOF were analyzed by MASCOT software (www.matrixscience.com).

The N-terminal sequencing of the purified rhGH protein was carried out at Australian Proteome Analysis Facility for determining its authenticity.

The circular dichroism (CD) spectroscopy of the purified rhGH preparation (0.5mg/mL) was taken in a Jasco J-715 recording spectropolarimeter using a 2-mm cylindrical quartz cell. The data was represented as molar ellipticity (mdeg) against wavelength (nm) and secondary structural elements were determined by using an online tool k2d (http://www.emb-heidelberg.de/~andrade/k2d) at IISC, Bangalore, India.

Purified rhGH was also analyzed on Agilent 2100 Bioanalyzer which is a new generation Capillary Electrophoresis (CE) instrument with a microfluidics-based platform available commercially. Due to a constant mass-to-charge ratio and presence of sieving polymer matrix in the chip of bioanalyzer, the molecules are separated based on size. The samples were prepared following the manufacturer’s protocol and the Protein 80 kit was used for the analysis of proteins, ranging between 5 and 80 kDa. The data was represented in an electropherogram by plotting fluorescence intensity units (FU) versus retention time in seconds. Bacterially derived rhGH and commercially available rhGH were compared using this instrument as per the manufacturer’s protocol.

Biological activity of hGH

hGH activity was determined by measuring the binding of hGH to hGH receptor in an in vitro assay as reported by Schellenberger et al. (2009). Briefly, hGH receptor fused to human Fc (R&D Systems, USA) was coated at 25 ng per well onto a Nunc 96-well plate in triplicates. The wells were blocked with 3% BSA followed by incubation for 1 h with various concentrations of hGH. After extensive washing, the plates were incubated with 1:750 diluted rabbit anti-hGH antibody at 37°C for 1 h followed by incubation with goat anti-rabbit IgG-HRP. 3,3’,5,5’-Tetramethylbenzidine (TMB, Sigma) was used as chromogenic substrate and absorbance was read at 370 nm after 30 min.

Results

Selection of pBAD24-hGH and pBAD24M-hGH recombinants

Colony PCR for recombinants of pBAD24-hGH and pBAD24M-hGH showed several clones of pBAD24-hGH and pBAD24M-hGH yielding right size PCR product with hGH gene specific primers (data not shown) confirming the insertion of hGH gene into the respective vectors. The expression studies (Figure 1a, lane 2) carried out in shake flasks indicate that pBAD24M-hGH clone expressed very high levels of rhGH while the levels obtained from pBAD24-hGH clones was insignificant (Figure 1a, lanes 1). The identity of the protein was established using polyclonal anti-hGH antibody (Figure 1b, lane 1 and 2). These results support our observations of over expression of proteins from modified pBAD vectors (Sampali et al., 2009).

rhGH expression in a bioreactor

The growth profile of the fermentation of both pBAD24-hGH and pBADM-hGH constructs under identical conditions is shown in Figure 2. Although the growth pattern were similar in both the cases, the expression levels of rhGH from the pBAD24M-hGH construct was nearly 25 folds higher as compared to that achieved with pBAD24-hGH supporting the shake flask data (Figure 3). While the harvest OD was almost close for both the constructs, the yield of hGH was only 75 mg/mL for pBAD24-hGH construct while it was 1862 mg/mL for pBAD24M-hGH.

![Figure 1: rhGH expression in shake flask and western blot.](image)

**Figure 1a:** Expression of rhGH in shake flasks, using pBAD24-hGH (Lanes 1) and pBAD24M-hGH clones (Lanes 2). M: Molecular weight marker (14.3-97.4 kDa).

**Figure 1b:** Western blot of rhGH derived from both the vectors.

**Lane1:** rhGH expression derived from pBAD24-hGH clone. **Lane 2:** rhGH expression derived from pBAD24M-hGH clone. M: protein molecular weight marker showing 14 and 20kDa bands. Note the dramatic increase in the hGH signal from pBAD24M-hGH construct.

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When the inclusion bodies were analyzed by densitometry, the results indicated that the pBAD24-hGH derived inclusion bodies comprised of only 1.8% of the hGH while the pBAD24M-hGH derived ones had 59% of the total proteins as evident from Figure 3.

Purification of bacterial hGH

The refolded bacterial hGH on anion exchanger Q Sepharose FF resulted in removal of majority of the impurities of the starting material and yielded nearly 66% pure hGH in the flow through. This step also removed the host DNA contaminants that are bound to the column due to their negative charge at pH 8.0 (data not shown). The flow through containing the hGH protein was later loaded on a cation exchange column (SP Sepharose FF) and the bound proteins were eluted using NaCl gradient (0-1 M NaCl in the equilibration buffer). As depicted in Figure 4, lane 2, SP Sepharose FF eluted fractions (0.4 M NaCl) was >95% pure as judged by silver staining with ~40% recovery (Table 2).

Characterization of pure rhGH

Characterization of purified rhGH using N-terminal sequencing revealed the sequence to be MFPTIP that matched with the native human growth hormone N-terminal sequence.

In addition, the protein identity was also confirmed by MASCOT analysis of peptide masses obtained by MALDI-TOF/TOF (Figure 5a) which showed 86% peptide coverage identity to human somatotropin.

The CD data indicate that the alpha helical content was 85%.

The sample biomass, wet weight (g/L), total protein in inclusion body (mg/L), inclusion body purity (%), and hGH concentration (mg/L) for pBAD24-hGH and pBAD24M-hGH are listed in Table 1.

Table 1: Analysis of yield of rHGH from pBAD24-hGH and pBAD24M-hGH construct at the time of harvest.

| Sample       | Bio mass Wet wt (g/L) | Total protein in Inclusion body (mg/L) | Inclusion body Purity (%) | hGH concentration (mg/L) |
|--------------|-----------------------|----------------------------------------|---------------------------|--------------------------|
| pBAD24-hGH   | 70                    | 4214                                   | 1.80                      | 75                       |
| pBAD24M-hGH  | 76                    | 3156                                   | 59.00                     | 1862                     |

*Protein estimation by BCA method

*by densitometry analysis as detailed out in the materials and methods section

Figure 2: Growth profile of E.coli DH5α cells transformed with either pBAD24-hGH or pBAD24M-hGH clones. The arrow indicates the point of induction at OD$_{600}$ of 10 with 13 mM L+ arabinose. Note the similar growth profile of both the fermentation batches.

Figure 3: SDS-PAGE profile of inclusion bodies and lysed supernatants of cells transformed with two hGH expression constructs. Lanes 2 and 3 represent IB and lysed supernatant of cells transformed with pBAD24 vector respectively whereas lanes 4 and 5 depict IB and lysed supernatant of cells transformed with pBAD24M vector respectively. Arrow indicates rhGH expression. M: Molecular weight marker.

Figure 4: SDS-PAGE profile on purification of rhGH. Lane1: Protein molecular weight marker (14.3-97.4 kDa). Lane 2: SP FF Sepharose elute at 0.4 M NaCl. Arrow depicts the purified rhGH (22 kDa).
Figure 5: Protein characterization of rhGH.

Figure 5a: MALDI-TOF data for rhGH protein. The peptide masses obtained by MALDI-TOF were analyzed by MASCOT software and showed 86% similarity to human somatotropin.

Figure 5b: Circular dichroism (CD) spectrum of rhGH showing alpha helix structure.

Figure 6: Electropherogram of commercial rhGH and purified rhGH as visualized in Agilent 2100 Bioanalyzer. Gel electrophoresis of individual proteins was carried out independently and the peak profile was overlapped for analysis. Peak corresponding to commercial rhGH (somatotropin) is labeled as 1 whereas peak representing bacterially derived rhGH is labeled as 2 on the line plot where X axis represents retention time in seconds (which represents molecular wt in kDa) and Y axis represents fluorescence units (FU). Peak 3 represents the peak of bovine serum albumin (BSA) used as a stabilizer in marketed somatotropin.
Burns. Hence, a continuous and a stable supply of hGH would become gradually more important and developing a cost-effective treatment of chronic renal insufficiency, bone fractures and the CD spectra data.

Disulfide bonds and has proper secondary structure as judged by hGH (Uchida, 1991), it is possible to speculate that the hGH modifications have been reported to exhibit less biological activity compared to monomeric forms (Baumann, 2009) and oligomeric GH forms have been reported to have diminished bioactivity (Lehman et al., 2004).

Table 2: Purification table of rhGH from IB [8.5 g wet wt l⁻¹ of fermentation broth] expressed from pBAD24M-hGH construct.

| Step No. | Fraction         | rhGH content (mg) | Volume (ml) | % Recovery |
|----------|------------------|-------------------|-------------|------------|
| 1.       | Inclusion body   | 1862*             | 620         | -          |
| 2.       | Refolded protein | 1800*             | 3400        | 100        |
| 3.       | Q Sepharose load | 1350*             | 4050        | 74         |
| 4.       | Q Sepharose F.T./SP | 1200*          | 4780        | 66         |
| 5.       | SP Sepharose eluates | 750*            | 1870        | 41         |

*%rhGH by densitometry  
*increase in volume due to buffer exchange

Table 2: Purification table of rhGH from IB [8.5 g wet wt l⁻¹ of fermentation broth] expressed from pBAD24M-hGH construct.

Figure 7: hGH receptor binding assay showing bioactivity of hGH. Filled bars represent commercial hGH preparation while empty bars represent in-house purified rhGH. Values are mean ± SEM. For control, a non-specific protein like casein was used.

With minima at 209 nm (Figure 5b) of the purified rhGH preparation. It was interesting to see that the rhGH purified preparation showed a single peak on Agilent 2100 Bioanalyzer with a retention time matching exactly with the commercially available somatotropin preparation (Figure 6). The biological activity data of the purified rhGH described in this study showed equal activity in comparison to the commercially available somatotropin (Figure 7). The assay was found to be specific to hGH since another recombinant protein (pure Parathyroid hormone, PTH) failed to exhibit any binding to the hGH receptor under similar experimental conditions. In addition, the hGH receptor binding activity was also found to be concentration dependent. Since the hGH variants with their cysteine modifications have been reported to exhibit less biological activity and less hGH receptor binding activity than that of intact hGH (Uchida, 1991), it is possible to speculate that the hGH purified following the protocol described here assembles correct disulfide bonds and has proper secondary structure as judged by the CD spectra data.

Discussion

Use of human growth hormone is expected to expand in the future, for the management of pituitary dwarfism, for the treatment of chronic renal insufficiency, bone fractures and burns. Hence, a continuous and a stable supply of hGH would become gradually more important and developing a cost-effective process for the large scale manufacturing of a pharmaceutically acceptable high-grade human growth hormone becomes an indispensable need.

There is only a single report (Ghasemi et al., 2004) on hGH expression in E. coli using arabinose promoter. However, this report highlights hGH expression in the periplasmic space of E. coli. As observed by us earlier for proteins like granulocyte colony stimulating factor (GCSF) and green fluorescent protein (GFP), the modified pBAD vector actually yielded very high levels of rhGH expression in comparison to the regularly used pBAD24 vector. Such increase in yield is due to efficient Shine-Dalgarno sequence and translational enhancer elements incorporated in the pBAD24M vector as discussed elsewhere (Sampali et al., 2009). Although Jensen and Carlsen (1990) have reported 2 mg l⁻¹ of the fermentation broth, the report is restricted only to the fermentation with no emphasis on the purification yield.

There are many reports on purification of hGH, however many of them use conventional methods, including ammonium sulfate precipitation, gel filtration chromatography and ion-exchange chromatography. Majority of the papers highlight more than five steps of purification which indirectly would result in poor yields and high production costs. Also, affinity chromatography has been used for purification of this protein which is an impressive alternative (Shin et al., 1998). Hydrophobic interaction chromatography has been also suggested for isolation of rhGH from the supernatant of transformed monkey kidney cell cultures. Lefort and Ferrara (1986), de Oliveira et al. (1999) describes seven steps of purification of recombinant hGH from E. coli to achieve a clinical grade material. Such a strategy included precipitation and five chromatography steps to give a recovery of 40%. Although these authors used an E. coli K12 strain for expression, the promoter was of lambda pL requiring subjection of the bacterial cells to 42°C for induction. This would not be feasible in large scale and hence the use of E. coli K12 strains in the current study with arabinose is a good alternative. Since majority of the rhGH expression reported so far has been a periplasmic protein, the degradation of the expressed rhGH is unavoidable since several proteases are known to reside in the E. coli periplasmic space throwing a challenge to the downstream operations.

Jones and co-workers (1979) adopts six steps of purification of hGH while Ribela et al. (2000) report the use of a single size exclusion chromatography for such a process. Although, Niimi et al. (1987) described a six step purification process of Met-hGH, their process yield was only 19%.

Choi et al. (1998) also detail out purification of E. coli derived rhGH from inclusion bodies using 10 M urea followed by refolding and purification by ion-exchange and gel filtration achieving a yield of only 50 mg l⁻¹ of the E. coli culture. Zomorrodipour et al. (2004) also report a yield of 53 mg l⁻¹ of hGH from the bacterial system while Mihijsa et al. (1995) report merely 50 mg l⁻¹ from bacterial inclusion bodies. Our results of achieving nearly 750 mg l⁻¹ of purified hGH using the arabinose promoter system from bacterial inclusion bodies is the highest report to date.

Oligomeric GH forms have been reported to have diminished bioactivity compared to monomeric forms (Baumann, 2009) and...
hence expression of the right form of hGH in any expression system is critical. The data presented in this article about observation of single form of monomeric rhGH with no dimer indicate the potential of this expression system for large scale manufacturing of rhGH. This data is also represented in the form of a single peak on an Agilent 2100 bioanalyzer with the retention time matching with the commercial growth hormone somatotropin indicating the usefulness of this equipment for assessing the purity of any recombinant protein preparation as suggested elsewhere (Deshpande et al., 2009; Somani et al., 2009).

A small difference in the amino acid extension was found to lead to a change in the specific hGH expression rate (Dalbøge et al., 1990) and this has been achieved with twenty different hGH genes with the sequence Met-Xxx-Glu-Glu-hGH where Xxx denotes each of the 20 different amino acids highlighting the continued efforts of researchers to enhance hGH expression levels using different strategies.

In conclusion, we have demonstrated the comparative biochemical characterization of bacterial derived rhGH and standard rhGH by examining their N-terminal sequence, MALDI-TOF/TOF, CD spectra and Agilent 2100 bioanalyzer. The observation that the purified rhGH obtained in this study shows equal bioactivity in comparison to the commercially available hGH preparation makes the described process very simple and cost-effective for large scale manufacturing.

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