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Original Research

Cloning and expression of recombinant human platelet-derived growth factor-BB in *Pichia Pink*

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Abstract: The PDGF-BB plays a key role in several pathogenesis diseases and it is believed to be an important mediator for wound healing. The recombinant human PDGF-BB is safe and effective to stimulate the healing of chronic, full thickness and lower extremity diabetic neurotrophic ulcers. In the present study, we attempted to produce a PDGF-BB growth factor and also, evaluate its functionality in cell proliferation in yeast host *Pichia pink*. *Pichia pink* yeast was used as a host for evaluation of the rhPDGF-BB expression. The coding sequence of PDGF-BB protein was synthesized after optimization and packed into the pGEM. Recombinant proteins were produced and purified. The construct of pPinkα-HC-*pdgf* was confirmed by sequence, the PDGF-BB protein was expressed and purified with using a nickel affinity chromatography column and then characterized by SDS-PAGE electrophoresis. The biological activity of PDGF-BB was estimated with using human fibroblast cell line. The measurement of protein concentration was determined by Bradford and human PDGF-BB ELISA kit. Purified rhPDGF-BB showed similar biological activity (as the standard PDGF-BB) and suggested that the recombinant protein has a successful protein expression (as well as considerable biological activity in P. *pink* host). The exact amount of recombinant PDGF-BB concentrations were measured by specific ELISA test which it was about 30 µg/ml. Our study suggested that efficiency of biological activity of PDGF-BB protein may be related to its conformational similarity with standard type and also, it practically may be important in wound healing and tissue regeneration.

Key words: PDGF-BB, *Pichia Pink,* pPinkα-HC, Recombinant protein.

Introduction

Platelet-derived growth factor has multiple influences on cells which were derived from a mesenchymal origin and it has potency for a pharmaceutical application which it has become a point of focus in many studies (1). The PDGFs comprise a family of homo or heterodimeric growth factors including PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD (2). These growth factors exert their functions through binding to three different kinds of transmembrane tyrosine kinase receptors which are homo- or heterodimers of α- and/or β-polypeptide chains (2, 3). The PDGF was first growth factor which it was shown to be a chemoattractant for cells migration into the healing skin wound such as; neutrophils, monocytes and fibroblasts. It also enhances the production of extracellular matrix. In addition, PDGF stimulates fibroblasts proliferation and contract collagen matrices to them and also induces myofibroblast phenotype in these cells (4). Therefore, it has been suggested that it plays a crucial role in wound healing. Indeed, experimental series and clinical studies have demonstrated a beneficial effect of PDGF for treatment in wound healing disorders (5). Furthermore, PDGF was first growth factor which it has been approved for human ulcers treatment (6, 7).

Although it is considered to be a therapeutic product which recombinant human PDGF-BB has been applied for therapeutic uses. Human trials have only been performed by PFGF-BB and it was concluded that the once-daily topical application of rhPDGF-BB was safe and effective in stimulating the healing of chronic, full-thickness, lower-extremity diabetic neurotrophic ulcers (8). PDGF-BB are structurally arranged in an anti-parallel fashion and joined by two disulfide bonds which result in folding of the PDGF-B monomers in the BB dimer which it causes to form three loops at the end of the molecule (9). These loops have been proposed to be responsible for the binding of PDGF to its receptors (10).

The rhPDGF-BB homodimer has been produced in a variety of heterologous systems, including mammalian cells, insect cells, bacteria, fungi and yeast. In every case, the recombinant protein is purified, either as a monomer followed by dimerization or as a complete molecule that is synthesized inside the cell and secreted into the growth medium. The low expression level of rhPDGF-BB in *Saccharomyces cerevisiae* is a key issue that leads to high production costs (11). On the other hand, there are multiple promising advantages which regard to the production of recombinant proteins in *Pichia pink* yeast, which it includes rapid growth rate, high levels of recombinant protein (g/L) production, appropriated for laboratory and industrial purposes, elimination of endotoxin and bacteriophage contamination, easy genetic manipulation of well-characterized yeast expressing vectors, lower production cost than mammalian cell culture, simplicity of transformation and selection process and compared to bacteria. The lack of

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determined human pathogenicity is applied in protein expression with high disulfide in the spectrum of lytic viruses. Also, there are key advantages of *P. pink* yeast such as removal of methionine from the amino terminal of the protein, ability to secrete target protein insoluble in the culture medium, the capability to produce complex human protein, the availability of an unusual tightly regulated promoter with methanol-regulated alcohol oxidase I (AOX1) gene. The preference of *Pichia pastoris* is grown in a respiratory mode which it has a reduced fermentation benefit activity with producing excretion such as ethanol or acetic acid which allows achieving an interestingly high level of cell densities in medium (12-14).

On the basis of data are reported by previous studies**,** we investigated that *Pichia pink* host cell can be a suitable system for expression of a rhPDGF-BB protein with curative properties.

Materials and Methods

Bacterial strains, plasmids and media conditions

Escherichia coli strain DH5α was used for the propagation of recombinant plasmid and *Pichia pink* strain was used for hPDGF-BB protein expression. The ppinkα-hc expression vector was provided by Invitrogen company. *E. coli* transformations were selected on Luria-Bertani plates and supplemented with 80μg/ml of ampicillin.

Construction of pPinkα-HC_ *pdgf* plasmid and *E. coli* transformation

The *pdgf -bb* gene (GenBank accession no.NM_033016.2) was optimized according to codon usage bias of *P. pink* and synthesized (by Shine Gene Co.) in the pGEM_ *pdgf* vector. Synthesized sequence *Xho*I and *Kpn*I were digested and inserted into similarly digested pPinkα-HC to generate the pPinkα-HC-*pdgf-bb* plasmid. Using a micro-pulsar (Bio-Rad) at 1200, *E. coli* was transformed in a 0.1 cm electroporation cuvette. Preparation of electrocompetent cells and electroporation were carried out as described in Bio-Rad's Micro Pulsar electro proration apparatus operating instructions and applications guide (Bio-Rad, #165-2100). The cells were plated on LB agar ; containing 40 μg / ml ampicillin to obtain positively transformed colonies. Transformants were confirmed by PCR technique using primers designed for pPinkα-HC (Table 1). Then, double-digested was performed by restriction enzymes and DNA sequencing was performed with (090617KR-029, MACROGEN, Seoul, Republic of Korea) using primers in the vectors. Plasmid pPinkα-HC-*pdgf-bb* isolation was carried out with using GeneJE^{Ttm} Plasmid Miniprep Kit (ferment as, # K0502) following the manufacturer's protocol. Amplification was made in a total volume of 20μl of reaction mixture containing 0.3μl of genomic DNA(0.5μg/ml) , 2μl of Taq polymerase buffer (10×), 0.8μl $MgCl_2$ (25mM), 0.4 μl dNTP Mix(10 m M each), 0.4μl of each primer (25 pmol) and 1μl Taq polymerase PCR mix. A total of 30 cycles was performed as follow; the first denaturation initiated at 95 °C for 15 s, then all 30 cycles followed by the same thermal pattern of 94 °C for 50 seconds, 59 °C for 50 second and 72 °C for 1 min. The final extension occurred with using72 °C for 3 min. The amplicons were separated by electrophoresis on a 1% agarose gel, then it was analyzed by ethidium bromide staining and UV-transilluminator visualization.

Table 1. Nucleotide Primers Used for pPinkα-HC

Target Gene	Primers
F	5′ACTGCTGTTTTATTCGCAGCATCC3′
R	5′GGCGTGAATGTAAGCGTGACATAAC3′

Pichia pink competent cell preparation and transformation

10 ml YPD was inoculated with a single colony of *P. pink* and incubated at 30 °C under 300 rpm for 1 day. Then, 2 ml of 10 ml YPD was added to 100 ml fresh YPD to reach the first optical density of 0.2 at OD_{600} and then it was incubated at 30 °C under 300 rpm to reach the final optical density of 1.5 at OD_{600}. Cells were harvested by centrifugation under 1500 ×g for 5 min at 4 °C. The cells were resuspended in 250 ml Distilled water and harvested next to centrifugation under1500 ×g for 5 min at 4 °C. The process was repeated for 50 ml distilled water as mentioned above and also it was the same for 10 ml cold 1.0 M sorbitol too. After centrifugation for 5 min, cells were harvested and then, it was done suspension 300 μl of 1.0 M sorbitol. Cells were aliquoted into 1.5 mL tubes. 300 μg pPinkα-HC_ *pdgf* plasmid was linearized with using *Afl*II and used to transform P. pink by electroporation (Bio-Rad Genepulser; Bio-Rad, Hercules, CA, USA). 1 ml of cold 1 M sorbitol was immediately added to the cuvette after pulsing. Cells were cultured on selective medium after incubation for 2h at 30°C [SD, 18.6% sorbitol, 5% glucose and 2% agar]. Transformations were stretched on minimal glucose (MD) medium for screening purpose (1.34% YNB, 2% glucose and 2% agar).

PDGF expression

At first, colonies were inoculated into 250 ml flasks supplemented with 25 ml Buffered Minimal Glycerol complex medium (BMGY) [1% (w/v) yeast extract, 2% (w/v) peptone, 100 mM phosphate buffer pH 6.0, 1.34% (w/v) yeast nitrogen base, 0.4 μg/ml biotin and 1% (w/v) glycerol] and then, incubated at 30°C while shaking at 300 rpm for 72h. Then, the cells were inoculated in 500 ml Buffered Minimal Methanol complex medium (BMMY) [1% (w/v) yeast extract, 2% (w/v) peptone, 100 mM phosphate buffer pH 6.0, 1.34% (w/v) yeast nitrogen base, 0.4 μg/ml biotin and 0.5% (v/v) methanol] and incubated at 30°C under 300 rpm for 16 h to reach an OD_{600} of 0.5-0.6. The cells were resuspended in 200 ml BMMY 1:5 and incubated again at 30°C under 300 rpm for 72 h. Induction was carried out by adding 50% pure ethanol after 24h and proceed with incubation at the same condition for an overnight to reach the optimum induction time and then, followed by cells harvesting. In order to achieve intracellular recombinant protein, the cells harvesting was broken with using Breaking buffer.

Purification of recombinant protein with using nickel affinity column

Induced cells were harvested. Supernatant and whole cell were studied to purify the protein content. The cells were resuspended in 5 ml Yeast Buster reagent and THP

solution per mg of cell dry weight, the process followed by incubation at room temperature for 20 min under 50 rpm. Then, the supernatant was collected at 4 ºC under 1600g. The denaturing condition was applied to purify the recombinant protein for IMAC (Immobilized Metal Affinity Chromatography) column with using Ni-NTA agarose (Qiagen, USA) (15). The prepared column was washed with double distilled water and then, washed with 1.5 ml Mes buffer (20 mM). 2 ml protein solution was added to the column after completing elution of Mes buffer,and it followed by 2 ml washing buffer. The elusion buffers number 1(1 ml of 100 mM imidazole) and 2 (1 ml of 250 mM imidazole) were added in ordered to elute the recombinant protein attached to the nickel column. Finally, 500 µl Mes buffer (20 mM) was used. The purified protein content was analyzed SDS-PAGE electrophoresis on 12% polyacrylamide to verify PDGF expression and then, followed by Western blotting.

Western blot analysis

Proteins in the culture supernatants were subjected to a 15% polyacrylamide SDS–PAGE with using Bio-Rad Mini Protean II System. The separated protein bands on polyacrylamide gel were electroblotted onto a polyvinylidene fluoride (PVDF) membrane with using a transfer buffer (39 mM glycine, 48mM Tris-base, 0.037% SDS, and 20% methanol). The membrane was blocked with 3% BSA for one hour at 37°C. Then, it was incubated in a 1: 250 dilution of mice anti-His-tag (Roche) in the PBST (PBS contains 0.05% Tween 20) while gently shaking were performed for 2 h at 37 ◦C. the process was followed by membrane incubation in 1:100 dilution of secondary antibody anti-mouse conjugated to HRP. Slides were incubated with DAB (3,39-diaminobenzidine tetrahydrochloride) after three times washing with PBST and was immediately washed with tap water after the color development.

Biological Activity

The biological activity of the purified rh PDGF-BB was examined with using mouse fibroblast 3T3 clone A31 cells and received from institute pasture, Iran branch. The cells were cultured in DMEM medium supplemented with FBS 10%, 100µ/ml penicillin and 100 µg/ml streptomycin at 37 ºC in the presence of 5% CO_2. The medium was used to replace every other day. To assesses the biological activity of rh PDGF-BB, 1×10^4 cell/well were transferred to 96-well plates containing; DMEM and 10% FBS. The grown cells were supplemented with 0.1-100 ng/ml rh PDGF-BB protein after three days and then, cultured for another 3 days. Then, the medium replaced with a 100µl solution of 0.5 mg/ml MTT. After four hours, the solution was removed and 100µl isopropanol was added and incubated for 5h at 37 ºC. The amount of dissolved material was evaluated in isopropanol by ELISA reader at 490nm. The well with more cells showed more OD value. MTT color in the mitochondria of living cells was modified from yellow to purple.

Measurement of protein concentration was determined by Bradford in micrograms per milliliter. For this purpose, it needs a standard diagram which graph was drawn with using a standard protein BSA. The exact amount of recombinant PDGF-BB protein concentration was done by ELISA with a kit Human PDGF-BB, Mini ABTS ELISA Development Kit (Peprotech company). Range diagnostic kits was 1pg / ml to 10000pg / ml. Bradford was shown that protein concentration was about 40 µ/ml. ELISA kit was read at a 650 nm. According to the manufacturer's instructions, the Anti-PDGF-BB was on the floor and then, Human PDGF-BB protein was coated with different concentrations of standard kit (picograms per liter) and then, it was added to Avidin -HRP poly and finally, it was added to ABTS substrate (2,2 - azino-bis 3- ethylbenzothiazoline-6- sulphonic- acid) and the absorbance was read at a 650 nm.

Results

Construction of Recombinant Plasmid

The polymerase chain reaction products were amplified from pGEM-PDGF and cloned into digested pPinkα-HC vectors. The pPinkα-HC-*pdgf* was fragmented,as a result of two *KpnI* and *Xho*I restriction enzymes activity (Figure. 1A) and isolation of a 714 bp fragment was related to the *pdgf* gene with using PCR (Figure. 1B). The PCR product was also sequenced for further confirmation of correct cloning.

Transformation of Pichia Pink

The pPinkα-HC-*pdgf* expressing plasmid was linearized by *Afl*II and introduced into the *P. pink* strain (Figure. 2A). The white colonies were grown on selective medium with supporting the *pdgf* gene integration into AOX1 locus which was on the chromosome of the transformed *P. pink* (Figure. 3) and also, it was additionally confirmed by PCR technique (Figure. 2B).

Expression of Recombinant Platelet-Derived Growth Factor Protein

The colonies were confirmed as a result of genomic PCR and selected to express the target gene. The expression level was studied in cell fractions with using SDS-PAGE analysis. The SDS-PAGE analysis of cell fractions showed a new protein band about 35 kDa in a column which was related to the intracellular fraction

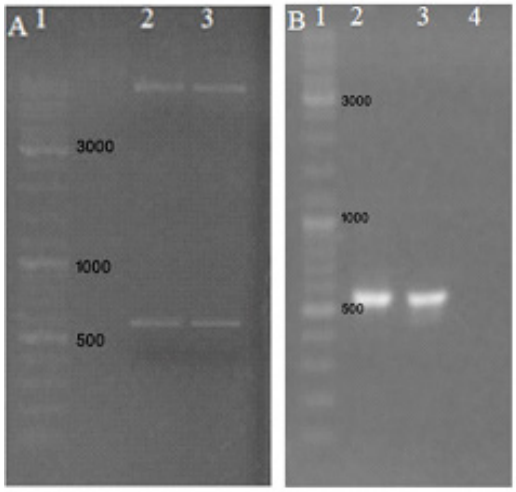

Figure 1. Cloning confirmation with using PCR and Double digest techniques. A) lane 1, Gene ruler sm0333. Lane 2, 3, Released *pdgf* fragment from the pPinkαHC_PDGF plasmid using *Kpn*I and *Xho*I. B) lane 1, Gene ruler sm0333. Lane 2, 3, PCR products from the pPinkαHC_PDGF plasmid. Lane 4, Negative control.

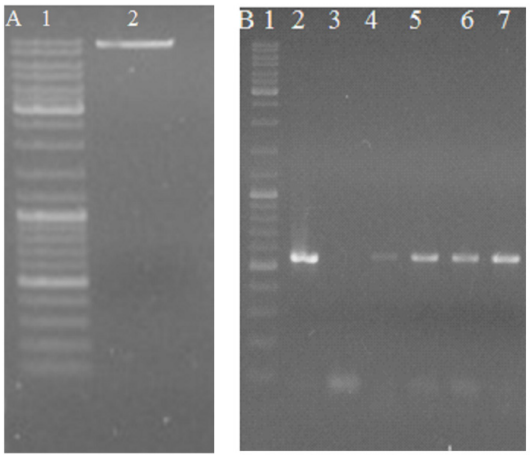

Figure 2. A) lane 1, Gene ruler SM0333, Lane 2, Linearized. The pPinkαHC_pdgf by *Bsp*tI. B) Identification of the recombinant pPinkαHC_pdgf containing the *pdgf* gene after transformation into *P. pink*: Lane 1, Gene ruler SM0333. Lane 2, 4, 5, 6, 7, PCR product.

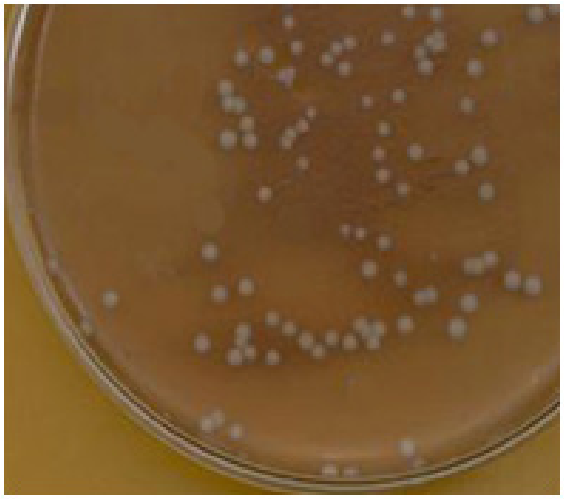

Figure 3. White colonies of *P. pink* on selective medium after transformation.

(Figure. 4).

Purification of the Recombinant Protein and Western Blot Analysis

The 35 kDa PDGF-BB protein was purified by chromatography of concentrated yeast supernatant with using nickel-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen Inc., Valencia, CA, USA) (Figure. 5) and confirmed by appearing a proper band in Western blotting (Figure. 6). These results showed that the PDGF-BB was correctly translated into the transformed *P. pink*.

Biological Activity

The biological activity of the purified recombinant protein was studied by measuring the proliferation of 3T3 cell with using the rapid colorimetric method. The rhPDGF-BB produced from *P. pink* is a homodimer in its native and biologically active form.

Recombinant protein concentration was measured in µg / ml with standard protein BSA. BSA concentration curve charts were shown of PDGF-BB protein production in 40 µg / ml. The exact amount of PDGF-BB concentrations of recombinant protein was calculated by a specific ELISA test. The amount had been compared with the standard curve Human-PDGF-BB protein

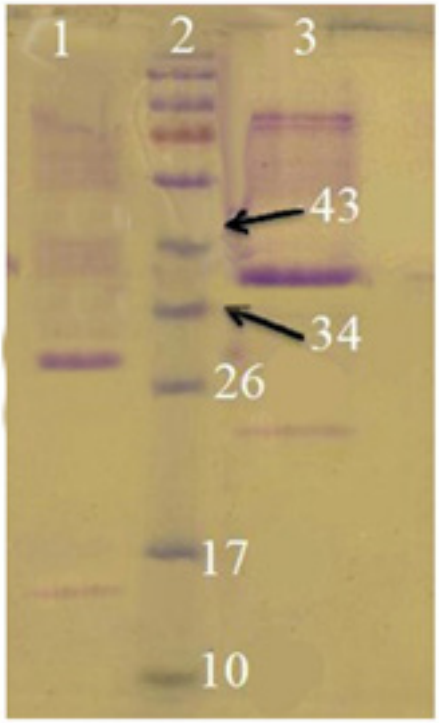

Figure 4. Protein detection was expressed by pPinkα-HC_pdgf using 12% SDS-PAGE. Lane 1: Recombinant glycoproteins in the supernatant, Lane 2: Protein size marker (cat No#sm0671), Lane 3: Recombinant glycoprotein intracellular fraction.

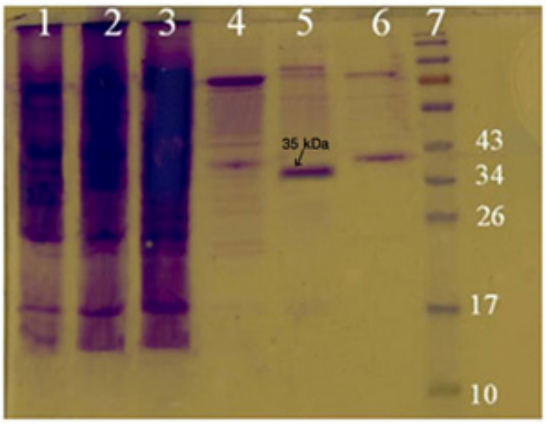

Figure 5. Purification analysis of recombinant PDGF protein with SDS-12% PAGE gel. Lane 1, flow-through. Lane 2, 3, 4, wash columns, Lanes 5, purified protein after elution with 250mM imidazole. Lane 6, Washing with 20mM MES buffer, Lane 7, Protein marker (#sm0671).

which was used in the kit and calculated 30 µg / ml.

Discussion

PDGF-BB with a weight of about 25-35 KD is two polypeptide chains linked by disulfide bonds. The PDGF-BB has a functional group containing 17 amino acids (RVRRPPKGKHRKFKHTH) which are a sulfate and heparin-binding domain and conducts the sulfate and heparin binding to alginate hydrogel thereby, it explains the therapeutic usage in healing. Compounds are prescribed in the form of a gel which contains PDGF-BB. Formulations are proper to accelerate the wound healing in some conditions such as ulcers, superficial wounds, surgical wounds, abrasion and bone defects. Nowadays, a gel formulation from rPDGF has been approved to heal full-thickness ulcers in diabetic patients

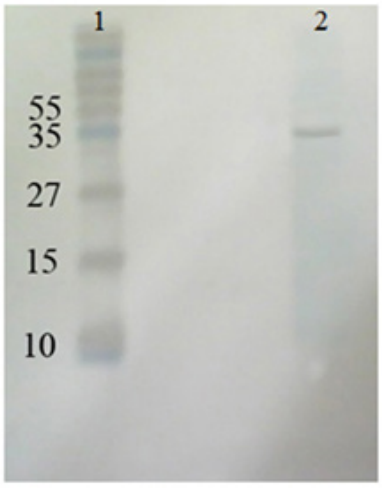

Figure 6. Western blotting analysis of expressed PDGF-Bb using histidine antibody, Lane 1: Protein size marker (sm0671), Lane 2: Purified recombinant protein.

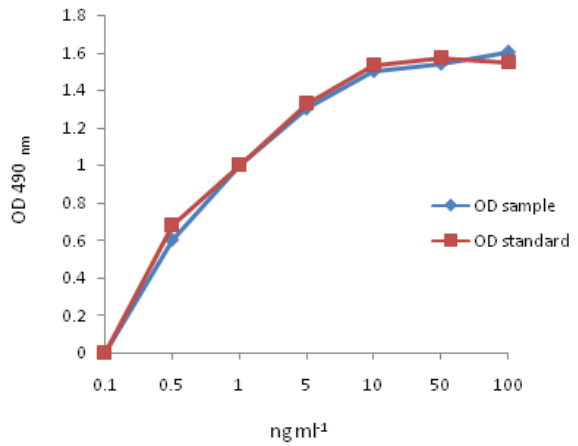

Figure 7. Biological assessment of purified and standard rhPDGF-BB. 3T3 fibroblast cells were treated with different concentration of rh PDGF-BB. The biological activity of the purified recombinant protein was assessed by measuring the proliferation of 3T3 cells using a rapid colorimetric technique. The data are expressed as means ± SD of three independent measurements.

such as the Regranex brand (Smith & Nephew Plc, London, UK). Yeasts are strong industrial fermentative organisms which it is able to express therapeutic proteins more efficiently and compared to other eukaryotic expression systems such as mammalian cells and the baculovirus system. The purpose of this study is that the gene encoding of PDGF protein was extracted from Gene Bank which was followed by gene optimization to express the protein in yeast host cell. Gene was synthesized in the final size of 714 bp and delivered into pGEM vector. Gene was subsequently subcloned in pPinkα-HC vector and its expressed recombinant protein production was purified.

The *Escherichia coli* expression system has many advantages, although it is faced with several challenging issues such as the lack of desired post-translational and post-transcriptional modifications of eukaryotic proteins, the formation of inclusion bodies and also inappropriate folding. It seems that this occurs due to the low amount of intracellular chaperones or reduced environment of cytoplasm. Moreover, proteins expressed in *E. coli* preserve their N-terminal extension which may affect the stability or immunogenicity in hosts (10, 16). Therefore, *E. coli* is not proper for the cloning and expression of the purpose protein. On the other hand, the cloning and expression of recombinant proteins in eukaryotic cells require expensive and complex mammalian cell culture laboratory equipment. On the other hand, the *P. pink* expression system is a *P. pastoris* mutant cell which it has both advantages of *E. coli* expression system, such as high levels of expression, inexpensive medium, expression of proteins with many disulfide bonds and controlled induction, and also, the advantages of eukaryotic systems are included: post-transcriptional and post-translational processes and glycosylation process. These advantages make the system more appropriate for production of eukaryotic proteins (10, 17). Because several common codons are rare or lacking aminoacyl-tRNA in the yeast *Pichia*. The translation process could be incomplete (16, 18). In addition, if the target gene contains AT-rich regions, the transcription may be incompletely terminated (19). Therefore, in order to improve the expression of the desired glycoproteins, several codons with these characteristics were replaced and optimized by similar codons.

Since pPinkα-HC is a large size of plasmid (8 kb) and also for maximizing the plasmid elution yield, the TE buffer needs to be pre-warmed for about 2 min, at 60°C and it is able to be followed by incubation of the column at 37°C for 1 h. Otherwise, the desired concentration would not be obtained. It is recommended to incubate the extracted plasmid at 60°C for 1 h in order to prevent DNA degradation and remove DNAse or use a DNAse free micro tube.

Our observation suggested that transformed cells were incubated in an immovable incubator with sucrose at 37°C which this performance was done after electroporation which it caused to make a higher transformation rate and it seems to occur due to the cell wall reformation.

Multiple growth factors have been produced in the yeast *P. pastoris* host including epidermal growth factor (20), insulin-like growth factor-1 (19), hepatocyte growth factor (21), keratinocyte growth factor 2 (22) and bone morphogenetic protein 6 (23). Our study is the first time to report the hPDGF-BB expression in *P. pink*. The expression of hPDGF-BB was previously studied by other researchers in heterogenous expression systems including bacteria *E. coli* (24, 25), yeast *S. cerevisia* (11), mushroom *Pleurotus eryngii* (12), insect and mammalian cells. The expression levels represent an essential issue in this system. For instance, Wang et al. reported the efficiency of 32 mg/L in *Saccharomyces* with optimization while Karumuri et al. reported the yields of 10-12 mg/L in *E. coli*. In the present study, a high efficiency without optimization was obtained for the rhPDGF-BB protein with using *P. pink* about 30 mg/L. The obtained protein is considerably more purified when it was compared to the previously unpurified proteins.

The rhPDGF-BB protein was produced in this study and it showed that the same pattern of proliferation in fibroblasts as standard PDGF-BB (Sigma-Aldrich, St. Louis, MO, USA). Protein cannot bind to its receptor on fibroblasts and cannot induce the proliferation because of incorrect conformation. Therefore, it seems that the rhPDGF-BB produced in *P. pink* host. In the present stu-

dy, it has conformational and biological similarity with the standard type which was produced by Sigma-Aldrich (St. Louis, MO, USA). The high purity of the rhPDGF-BB was produced in our experiments and was confirmed by SDS-PAGE and Western blotting. The rhPDGF-BB has been recently used as a medicine for various diseases. In particular, rhPDGF-BB was produced from *P. pastoris* which is a homodimer in native form and is biologically active. The SDS-PAGE, Western blotting and cell culturing had confirmed the active structure of the purified protein. These data confirm that the *P. pastoris* is capable of producing a bioactive protein and that protein can be easily purified from yeast extract. We believe that rhPDGF-BB production in the yeast *P. pink* is economically cost-effective. In this study, pPinkα-HC vector was used for the subcloning and expression of target gene. The vector was designed for laboratory and industrial expression and has the following advantages: it has the AOX promoter (which is considered a strong promoter), it is an ampicillin selective marker in *E. coli* and an adenine selective marker in yeast *P. pink.* It is also a highly replicable plasmid. Methanol is used to induce the promoter in BMMY medium. In this medium, methanol is the only carbon source for the recombinant glycoprotein production with a high level of availability.

In this study, the recombinant protein was observed on polyacrylamide gel electrophoresis as a single band with a molecular weight of approximately 35 kDa, based on used protein markers while the molecular weight of PDGF-BB is about 25 kDa. There are a lot of differences due to the lack of glycoprotein production in control samples with using Nickel affinity chromatography and Western blotting experiments with antibodies against His-Taq, which gene was confirmed by sequencing process after it was transformed into yeast. Band formation was expected during the PCR yeast genome with vector and gene primers, Repeat expression steps were induced the correct expression of protein for a colony Starin1 and colony Starin4 and production of a recombinant protein of 35 kD. The only difference can be related to reducing of speed recombinant protein electrophoresis and can not be related to separate secretory signal from recombinant protein into the cytoplasm (signal S, 9 kD) during the passage.

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References

1. Wang Y, Xue L, Li Y, Zhu Y, Yang B, and Wang X. High-level secretory production of recombinant human platelet-derived growth factor-BB by Saccharomyces cerevisiae under the non-selective conditions. Applied biochemistry and microbiology 2009; 45: 156-161.

2. Heldin CH, Eriksson U, and Ostman A. New members of the platelet-derived growth factor family of mitogens. Arch Biochem Biophys 2002; 398: 284-90.

3. Heldin CH and Westermark B. Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 1999; 79: 1283-316.

4. Lin H, Chen B, Sun W, Zhao W, Zhao Y, and Dai J. The effect of collagen-targeting platelet-derived growth factor on cellularization and vascularization of collagen scaffolds. Biomaterials 2006; 27: 5708-14.

5. Li H, Fu X, Zhang L, Huang Q, Wu Z, and Sun T. Research of PDGF-BB gel on the wound healing of diabetic rats and its pharmacodynamics. J Surg Res 2008; 145: 41-8.

6. Embil JM, Papp K, Sibbald G, Tousignant J, Smiell JM, Wong B, and Lau CY. Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. Wound Repair Regen 2000; 8: 162-8.

7. Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. Plast Reconstr Surg 2006; 117: 143S-149S; discussion 150S-151S.

8. Stringa E, Knauper V, Murphy G, and Gavrilovic J. Collagen degradation and platelet-derived growth factor stimulate the migration of vascular smooth muscle cells. J Cell Sci 2000; 113 (Pt 11): 2055-64.

9. Greenhalgh DG, Sprugel KH, Murray MJ, and Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am J Pathol 1990; 136: 1235-46.

10. Andrae J, Gallini R, and Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev 2008; 22: 1276-312.

11. Wang Y, Xue L, Li Y, Zhu Y, Yang B, and Wang X. High-level secretory production of recombinant human platelet-derived growth factor--BB by Saccharomyces cerevisiae under the non-selective conditions. Prikl Biokhim Mikrobiol 2009; 45: 176-80.

12. Choi JH, Kim S, Sapkota K, Park SE, and Kim SJ. Expression and production of therapeutic recombinant human platelet-derived growth factor-BB in Pleurotus eryngii. Appl Biochem Biotechnol 2011; 165: 611-23.

13. Bahrami A, Shojaosadati SA, Khalilzadeh R, Saeedinia AR, Farahani EV, and Mohammadian-Mosaabadi J. Production of recombinant human granulocyte-colony stimulating factor by Pichia pastoris. Iranian Journal of Biotechnology (IJB) 2007; 5.

14. Ningrum RA, Santoso A, and Herawati N. Secretory expression of recombinant human interferon-alpha2b in methilotropic yeast Pichia pastoris. Int. J. Res. Pharm. Sci 2013; 4: 207-210.

15. Amani J, Salmanian AH, Rafati S, and Mousavi SL. Immunogenic properties of chimeric protein from espA, eae and tir genes of Escherichia coli O157:H7. Vaccine 2010; 28: 6923-9.

16. Lueking A, Holz C, Gotthold C, Lehrach H, and Cahill D. A system for dual protein expression in Pichia pastoris and Escherichia coli. Protein Expr Purif 2000; 20: 372-8.

17. Daly R and Hearn MT. Expression of heterologous proteins in Pichia pastoris: a useful experimental tool in protein engineering and production. J Mol Recognit 2005; 18: 119-38.

18. Romanos MA, Scorer CA, and Clare JJ. Foreign gene expression in yeast: a review. Yeast 1992; 8: 423-88.

19. Sreekrishna K, Brankamp RG, Kropp KE, Blankenship DT, Tsay JT, Smith PL, Wierschke JD, Subramaniam A, and Birkenberger LA. Strategies for optimal synthesis and secretion of heterologous proteins in the methylotrophic yeast Pichia pastoris. Gene 1997; 190: 55-62.

20. Clare JJ, Romanes MA, Rayment FB, Rowedder JE, Smith MA, Payne MM, Sreekrishna K, and Henwood CA. Production of mouse epidermal growth factor in yeast: high-level secretion using< i> Pichia pastoris</i> strains containing multiple gene copies. Gene 1991; 105: 205-212.

21. Liu ZM, Zhao HL, Xue C, Deng BB, Zhang W, Xiong XH, Yang BF, and Yao XQ. Secretory expression and characterization of a

recombinant-deleted variant of human hepatocyte growth factor in Pichia pastoris. World J Gastroenterol 2005; 11: 7097-103.
22. Wang Y, Yuan S, Wang P, Liu X, Zhan D, and Zhang Z. Expression, purification, and characterization of recombinant human keratinocyte growth factor-2 in Pichia pastoris. J Biotechnol 2007; 132: 44-8.
23. Dong M, Shuang Y, Fen H, Jie Z, Yuhuan Q, Jun D, and Tianhui Z. Expression and Secretion of Human Bone Morphogetic Protein-6 in Pichia pastoris [J]. Acta Scientiarum Naturalium Universitatis Nankaiensis 2010; 1: 008.
24. Karumuri NN, Gangireddy SR, Narala VR, Majee SS, Gunwar S, and Reddy RC. Simple, rapid, high-purity preparation of recombinant human platelet-derived growth factor-BB. Biotechnol Lett 2007; 29: 1333-9.
25. Ghoshoon M, Ghasemi Y, and Nouri F. Cloning and expression of pdgf-bb gene in eschrishia coli. Research in Pharmaceutical Sciences 2012; 7: S963.