CLONING OF ENDO- β-1,4 GLUCANASE FROM *ASPERGILLUS NIGER* AND ITS EXPRESSION IN *SACCHAROMYCES CEREVISIAE*

Pham Viet Cuong[1, *], Quyen Dinh Thi[2], Vu Thi Quyen[1], Nguyen Thi Kim Cuc[1]

[1]*Institute of Marine Biochemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[2]*Institute of Biotechnology, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

[*]Email: *cuongwg@yahoo.com*

Received: 2 July 2011; Accepted for publication: 17 November 2012

ABSTRACT

Endoglucanase is one of the three forms of cellulase enzymes, widely used in different industries, and has been synthesized by animals, plants and microorganisms. β-glucanase is one of the feed enhancing enzymes, so adding of β-glucanase to the feed originated from plants, it can not only enhance their nutritional quality greatly, but also resolve the digestion problems of chickens/pigs due to high amount of β-glucan in these kinds of feed.

In this work, β-glucanase gene from *A. niger* was ligated in expression vector pESC-His and expressed in *S. cerevisiae* KY117.

*Egl*B gene was cloned in pCR2.1 vector, then ligated in expression vector pESC-His using T4 ligase enzyme after cleaving both recombinant pCR2.1/ *EglB* and pESC-His by *Bam*HI and *Xho*I. Using protoplast fusion method, recombinant pESC-His/*EglB* was transformed into competent *S. cerevisiae* KY117 strains. Transformants were verifyed by PCR using specific primers GAL10 for pESC-His plasmid.

Recombinant *S. cerevisiae* KY117 was cultured in SC-His and induced by galactose. Total protein was subjected to SDS-PAGE and the obtained result showed that after 60 hours incubation, the protein with molecular weight about 17kDa was induced, corresponding with theoretical EglB.

Biological activity of the expressed protein was tested based on CMC-ase activity of recombinant KY117. The strains without recombinant plasmid did not digest CMC in the medium, meanwhile recombinant *S. cerevisiae* KY117 made clearly unstained circular zone around the wells after staining CMC plates with Congo red.

Keywords: cloning, expression, EglB, glucan, *S. cerevisiae*

1. INTRODUCTION

Endoglucanase is one of the three forms of cellulase enzymes, which randomly hydrolyzes the β-1,4-glucosidic bonds in cellulose, and other similar β-glucan subtances. As a result, the polymer rapidly decreases in length, and the concentration of the reducing sugar increases slowly. Endo-glucanase can strongly hydrolyze amorphous cellulose [1].

Endo-β-1,4-glucanase has been widely used in the pulp and paper industry, waste treatment industry, and microbial fertilizer production. The endo-β-1,4-glucanase is synthesized from different sources including animals (mollusk, pig, chicken); plants (sprouts of cereals like barley, oats, wheat, rye); and microorganisms (*Aspergillus niger, A. oryzae, A. aculeatus, Trichoderma viride* and *Bacillus* strains) [2, 3, 4].

Cereals and soybean meal are mainly traditional animal feed diets, i.e. pigs and chickens, and other alternative products such as peas, beans, cereal by-products have been used widely in recent years. But the β-glucan in these types of feed can give problems for the digestion of the chicken and pigs.

β-glucanase is one of the feed enhancing enzymes that improve feed digestibility, resulting in increasing the efficiency of the feed utilization. By adding of β-glucanase to the feed, the nutritional quality of soybean and other cereal grains can be greatly enhanced and the digestion problems of chickens/pigs can be resolved.

Most enzyme products for feed purposes are produced by fungi rather than bacteria. Fungi prefer a low pH for their growth and produce an enzyme spectrum with the desired lower pH preference. Recently, the use of genetically mofified organisms (GMOs) for production of industrial enzymes have received increasing interest. The advantage of GMO's as applied to feed enzymes has been the capacity to achieve higher production levels of specific enzymes.

In this paper, the β-glucanase gene from *A.niger* was cloned in expression vector pESC-His and expressed in the yeast *Saccharomyces cerevisiae*.

2. MATERIALS AND METHODS

All the instruments used were sterilized with DEPC.

The common chemicals used in molecular biology were purchased from Sigma, Bio-Rad, Pharmacia-Biotech. The restriction enzymes *Bam*HI, *Xho*I, the ligation enzyme T4 DNA ligase, Taq DNA polymerase were from Fermentas. The expression vector pESC-His (Stratagen); cloning vector pCR2.1 and *E.coli* DH5α (Invitrogen). *Saccharomyces cerevisiae* KY117 yeast strain was a gift from Genetic engineering Lab., Institute of Biotechnology, VAST.

Culture media: LB; YPD; SD (Bacto-yeast extract 1%; Bacto-peptone 2%; D-glucose 2%); SC-his(Yeast Nitrogen base without amino acids, Difco, 0.67%; adenine, uracil, arginine, cysteine, lysine, threonine 0.01% each; aspartic acid, isoleucine, leucine, methionine, phenylalanine, proline, serine, tryptophan, tyrosine, valine 0.005% each).

Other chemicals: Sorbitol, EDTA, DTT, Sorbitol, Sodium citrat buffer, Tris buffer pH 7.5; Zymolyase, Lyticase, $CaCl_2$, PEG/Tris pH 7.5.

Primers for partial sequence of *Elg*B gene:

Forward primer: 5' – GC **GGATCC** TGT GCC CAC CAG CCA TTG (*Bam*HI)

Reverse primer: 5' - GC **CTCGAG** CTC GAG TGT TCC ATC TG (*Xho*I)

Extraction of total RNA

Total ARN was extracted from *A.niger* biomass by using Trizol reagent. After 24 hours culture, the mycelium was collected and washed carefully by sterilized water, then the biomass was ground in liquid nitrogen. Transfering ground biomass to a tube and adding 1ml trizol, pipetting 2-3 times till the mixture becomes transparent. Added 0.3 ml chloroform, inverting the tube gently 2-3 times and keeping about 10 minutes at room temperature. Centrifuged 5 minutes

at 10000 rpm; the supernatant was collected and added isopropanol, keept at room temperature 10 minutes and centrifuged at 10000 rpm, collected dregs, washed by ethanol 70 % twice and then stored in sterilized water.

Cloning of ElgB gene

PCR product was cloned into the pCR®2.1 vector. Recombinant plasmid was transformed into competent *E.coli* Top10, isolated and verified on agarose 1 % [5].

Construction of pESCHis/EglB

Cloning vector pCR®2.1 and pESCHis both were digested with *Xho*I and *Bam*HI. The PCR product and the opened pESCHis were purified from 1 % agarose gel using QiaQuick kit. The ligation was carried out using T4 ligase with following components: 0.75 µl T4-ligase buffer, 3 µl pESCHis (about 250 ng/ µl), 3.5 µl PCR product (about 60ng/ µl), 0.25 µl T4 ligase. The ligation mixture was desalted and then transformed into *E.coli* DH5α. After recovery in SOC medium, the transformation mixture was plated on LB plates containing 40 µg/ml kanamycine. Plasmids were isolated from liquid culture supplemented with kanamycin and were screened by restriction digestion. The sequence was verified by dideoxynucleotide chain-termination DNA sequencing, using ABI 310-PRISM Genetic Analyzer (Perkin Elmer).

Transformation pESCHis/EglB into S. cerevisiae by protoplast fusion method (Zymolyase™ protocols; www.amsbio.com)

Expression EglB in S.cerevisiae

S.cerevisiae strain carrying pESCHis/*EglB* plasmid was grown in SC-His medium supplemented with leucine for 12 hours with shaking, then galactose was added at final concentration 2 % as gene inductor. The incubation was continued and the same amount of inductor was added into induction medium after each 24 hours. At 60 hours, the culture was centrifuged at 6000 rpm and the pellet and supernatant fractions were separately collected. The pellet was subjected to ultrasonication and cell extract was collected by centrifugation. Fermantation broth and cell extract were incubated at 100 ^{o}C in 10 minutes for protein degeneration, then the samples were centrifuged at 4000 rpm for 5 minutes and the supernatants were subjected to SDS-PAGE electrophoresis for protein expression assessment.

3. RESULTS

3.1. Cloning EglB gene

cDNA was synthesized by reverse transcriptase reation with following components: 10 µl 5X RT buffer, 2 µl random primer – hexamer (60 ng/ µl), 5 µl DTT – dithio threitol (0.1 M), 8 µl dNTPs (2.5 mM), 0.5 µl Rnasin (40 U/ µl), 2 µl enzyme reverse trancriptase – MMLV reverse transcriptase (200 U/ µl), 18.5 µl sterilized distilled water and 4 µl total RNA (250 ng/ µl). The reaction cycle was: 25 ^{o}C in 5 minutes, 42 ^{o}C in 60 minutes, 70 ^{o}C in 5 minutes. Then the product (cDNA synthesized) was diluted 10 times by distilled water and stored at -20^{o}C.

The PCR reaction (10.2 µl sterilized water; 2.5 µl 10 x Pfu buffer; 2 µl dNTPs-2.5 mM; 8 µl RT product deluted 10 x; 1 µl each primer-10pmol and 0.3µl polymerase-0.5 U/µl) was carried using synthesized cDNA as the template to amplify the *ElgB* gene. The PCR cycle was optimized as denaturate at 94 ^{o}C for 2 minutes, 94 ^{o}C - 45 seconds, 54 ^{o}C - 45 seconds and 72 ^{o}C - 2 minutes with 35 cycles; ending at 72 ^{o}C for 10 minutes.

The PCR product was verified on 1 % agarose gel (Fig. 1). The result showed that the PCR product was specific with about 500 bp in size.

Figure 1. PCR product of *EglB* gene
Lane 1: DNA marker 1 kb plus
Lane 2: PCR product

The PCR product was ligated into pCR 2.1 vector (Invitrogen) by standard method of Sambrook et al. (2001). The recombinant plasmids were transformed into competent *E. coli* Top10 and transformed clones were screened based on colony colour. Plasmids were isolated from liquid culture grown overnight at 37 ^{o}C of blue and white colonies picked from the LB supplemented with kanamycin plates and verified on agarose 1 % (Fig 2). For ascertaining of PCR product insertion into pCR2.1, the choosing plasmids were used as template for PCR reaction with designed primers (Fig. 3).

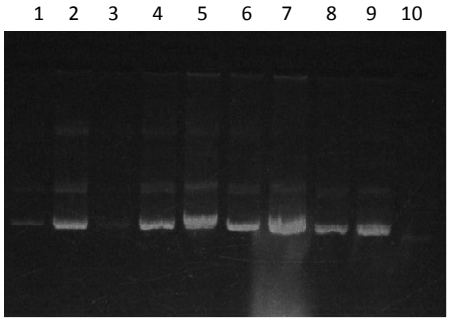

Figure 2. Agarose gel analysis of plasmids isolated from the white colonies (1-9) and blue colony (10)

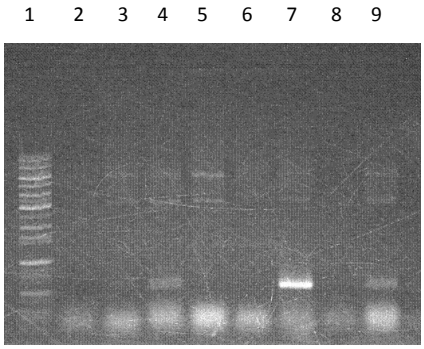

Figure 3. PCR products from white colonies (2-9); 1- DNA marker

It was obviously from Fig. 3 that, the clones 4, 7, 9 harboring exotic gene about 500 bp in size. The clone 7 was picked out and grown overnight in liquid LB medium containing kanamycin. Plasmid DNA was isolated using MiniPrep kit and sequencing. The deduced protein from the sequence was compared with other glucanases in GenBank by BLASTx and it was 98 % identified with *A.niger* Egl*B* (Assession N^{o} XP001391969.1) .

3.2. Construction pESCHis/EglB

After the *ElgB* gene was ligated into pESCHis vector, and recombinant plasmids were transformed into *E. coli*, the efficiency of the reactions was checked by cutting of isolated plasmids with *Bam*HI and *Xho*I (Fig. 4).

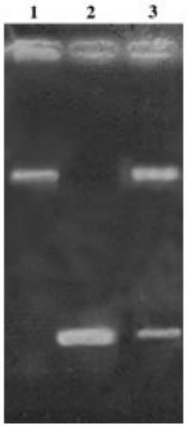

Figure 4. Agarose gel analysis of pESCHis/*EglB* cutting with *Bam*HI and *Xho*I; Lane 1: pESCHis
Lane 2: PCR product of *EglB* gene
Lane 3: pESCHis/*EglB* after cutting

3.3. Expression of EglB in *S. cerevisiae*

By protoplast fusion method, the pESCHis/*EglB* plasmid was transformed into *S. cerevisiae* K117 (10 ng pESCHis/*EglB* plasmid was incubated with 100 µl *S. cerevisiae* K117 protoplasts for 10 minutes at RT). After washing with 1 ml Sorbitol 1 M , the transformants were cultured on SC-His medium and incubated at 30 ^{o}C for one week.

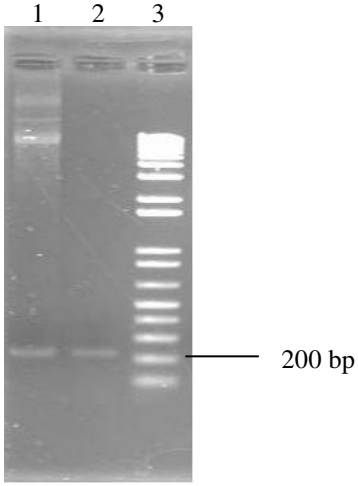

Figure 5. Picture of PCR products with GAL primers and template were plasmids isolated from transformed *S. cerevisiae* in 1.5 % agarose gel;
Lanes 1, 2: PCR products
Lane 3: Marker 1kb plus (Invitrogen)

The plasmids were isolated from liquid culture of *S. cerevisiae* K117 colonies picked from SC-His plates and used as template for PCR reaction with the GAL10 primers specific for pESCHis plasmid. This step was done for verifying the present of pESCHis/*EglB* plasmid in *S. cerevisiae* K117. The expected band is about 220 bp (Fig. 5).

The recombinant yeast strain was grown in SC-His medium with 2 % inoculum and the culture was induced as described in method. After degeneration, the presence of induced protein was tested by SDS-PAGE (Fig. 6).

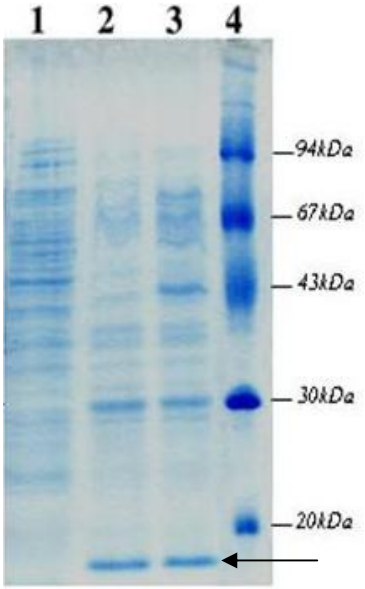

Figure 6. Analysis of protein expression on SDS-PAGE gel

Lane 1: Non-induced strain *S. cerevisiae* K117

Lane 2: Fermentation broth of induced *S. cerevisiae* K117 at 60 hours

Lane 3: Cell lysate of induced *S. cerevisiae* K117 at 60 hours

Lane 4: Protein marker

The expressed protein with expected molecular mass about 17 kDa (arrow) and it was obviously on SDS-PAGE gel (Fig 6) that the *EglB* from *A.niger* was expressed in *S. cerevisiae* K117. The protein was secreted into culture medium (lane 2) as well as in cells (lane 3).

3.4. Protein activity assay

Expressed protein activity was assayed based on CMCase activity of recombinant *S. cerevisiae*. The transformant after 12 hours culture in SD-his medium was induced with galactose and continuing it's growth for following 36 hours. The 100 µl culture aliquotes were put into holes of 1.5 % CMC plates and incubating at 30 ^{o}C for 3 days. Staining the plates with Congo red for identifying the enzymes activity by means of unstained circular zone around the wells in which the enzyme has degraded CMC to destroy its dye affinity. This contrasts strongly with the purple background of undigested substrate (Fig. 7).

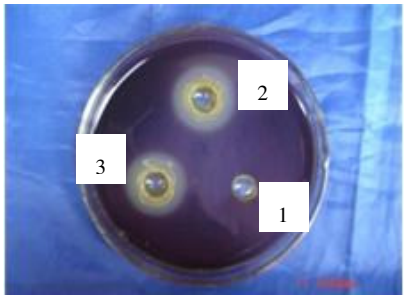

Figure 7. CMCase activity of *rSaccharomyces cerevisiae*

1. *S. cerevisiae* without pESCHis/*EglB*

2 and 3. *S. cerevisiae* with pESCHis/*EglB*

4. DISCUSSION

Cellulase and hemicellulase have different applications in animal feed, food, textile, laundry, pulp and paper industries. These enzymes are mostly from filamentous fungi such as *Trichoderma* and *Aspergillus* and account for approximately 20 % of the world enzyme market [6]. There are 3 classes of cellulase: (i) endoglucanase or β-1,4-endoglucanase EC 3.2.1.4, randomly cleaving β-1,4-glucosidic bonds in cellulose molecules; (ii) Cellobiohydrolases EC 3.2.1.91, degrading celluloses from non-reduction ends, releasing cellobiose; and (iii) β-glucosidases EC 3.1.2.21, hydrolysing cellobioses and cellodextrins with low molecular weight, releasing glucose. Carboxymethyl cellulose (CMC) are hydrolysed by endoglucanase, so this property can be used for determination of enzyme activity *in vitro* [1].

The endoglucanase gene from different microorganims has been cloned and expressed in *E.coli* [7, 8, 9, 10, 11, 12], in yeast [13, 14, 15, 16, 17] and in other systems [2, 18].

Some scientists suggested that in *A. niger* there are 3 endoglucanase genes, two of them, *eglA* and *eglB* lack a CBD and the associated linker region. *EglC* is unique among the *A. niger* endoglucanases because it exhibits its greatest activity towards xyloglucan, meanwhile *EglA* and *EglB* exhibit the greatest activity towards β-glucan [3, 6]. The yeast *S.cerevisiae* is a simple unicellular eukaryote and has been utilized for hundreds of years in making food and beverages. Metabolomics and molecular methods have been applied for yeast, thus it become the model eukaryote in cell and molecular biology and is a useful tool in studying other eukaryote genes. Several fungal endoglucanases have been cloned and expressed in *S.cerevisiae*. Salohaimor *et al*., (1997) expressed *egl4* gene with endoglucanase activity in *S.cerevisiae* Sf750-14Da [14]. Hong *et al*., (2001) had cloned a highly stable endo-β-1,4-glucanase (*eglI*) gene from *A.niger* [5]. The deduced amino acid sequence encoded by *eglI* showed high homology with that encoded by *eglB*. By Congo red staining method on agar plates, the authors noticed that the recombinant enzyme was secreted by the yeast *S.cerevisiae*. Qin *et al*., (2008) expessed wild-type Cel5A cDNA carrying the native Cel5A signal sequence of *T.reesei* in *S.cerevisiae* H158 [19]. Although our eglB protein expressed in *S.cerevisiae* was only 158 amino acid, but it possessed CMCase acivity. Result of comparing it's nucleotide sequense and deduced amino acid sequence lets us to conclusion that the cloned gene was endoglucanase.

5. CONCLUSION

The *EglB* gene from *A. niger* was cloned into pCR2.1 and the expression vector pESC-His/*EglB* was successfully created. By protoplast fusion method, the recombinant plasmids were transformed into *S. cerevisiae* KY117 and PCR was confirmed the presence of pESC-His/*EglB* in the yeast. Recombinant KY117 was induced by galactose and the expressed protein with MW of 17 kDa was corresponded with theoretical EglB protein. Based on CMC-ase activity of recombinant KY117 and results of BLASTn and BLASTp, it was obviously that the cloned gene was endoglucanase.

Acknowledgements. This work was done with the finance support from Ministry of Agriculture and Rural Development for research project:" Production and application of multienzyme preparation from recombinant microorganisms in improving animal feed utilizing effect".

REFERENCES

1. Hurst P. L., Jan Nielsen, Patrick A. Sullivan and Maxwell G. Shepherd - Purification and Properties of a Cellulase from *Aspergillus niger,* Biochem. J. **165** (1977) 33-41.
2. Baird Stephen D., Douglas A. Johnson, and Verner L. Seligy - Molecular Cloning, Expression, and Characterization of Endo-3-1,4-Glucanase Genes from *Bacillus polymyxa* and *Bacillus circulanst,* J. Bacteriol **172** (3) (1990) 1576-1586.
3. Hasper Alinda A., Ester Dekkers, Marc van Mil, Peter J. I. van de Vondervoort, and Leo H. de Graaff. EglC - A new endoglucanase from *Aspergillus niger* with Major activity towards xyloglucan, Appl. Environ. Microbiol. **68** (4) (2002) 1556-1560.
4. Beshay U., G. Miksch, K. Friehs, E. Flaschel - Improved β-Glucanase Production by a Recombinant *Escherichia coli* Strain Using a Zinc-Ion Supplemented Medium, Eng. Life Sci. **7** (3) (2007) 253-258.
5. Sambrook J., Russell D. W. - Molecular Cloning: A Laboratory Manual, Third edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York, 2001.
6. Coral G., Burhan Arikan, M. Nisa Unaldi, Hatice Guvenmez. Some Properties of Crude Carboxymethyl Cellulase of *Aspergillus niger* Z10 Wild-Type Strain Turk J Biol. **26** (2002) 209-213.
7. Cantwell BA and DL McConnell. Molecular cloning and expression of a *Bacillus subtilis* β-glucanase gene in *Escherichia coli,* Gene **23** (1983) 211-219.
8. Robson Lori M and Glenn H Chambliss. Cloning of the *Bacillus subtilis* DLG 1-1,4-Glucanase Gene and Its Expression in *Escherichia coli* and *B. subtilis* J Bacteriol **165** (2) (1986) 612-619.
9. Lee D. S. and Hun-Gil Chang - Cloning and expression of a β-1,3-glucanase gene from *Bacillus circulans* KCTC3004 in *Escherichia coli,* Biotech Lett. **17** (4) (1995) 355-360.
10. Miksch G., Neitzel R., Fiedler E., Friehs K., Flaschel E. - Extracellular production of a hybrid *β*-glucanase from *Bacillus* by *Escherichia coli* under different cultivation conditions in shaking cultures and bioreactors, Appl Microbiol Biotechnol **47** (1997) 120-126.
11. Kim Ji-Yeon, Han-Bok Kim & Dong-Seok Lee - Cloning and expression of the *Bacillus circulans* endo-*β*-1,3-1,4-glucanase gene (*bglBC1*) in *Escherichia coli,* Biotechnol Lett **24** (2002) 53-57.
12. Teng Da, Jian-hua Wang, Ying Fan, Ya-lin Yang, Zi-gang Tian, Jin Luo, Guan-pin Yang, Fan Zhang - Cloning of *β*-1,3-1,4-glucanase gene from *Bacillus licheniformis* EGW039 (CGMCC 0635) and its expression in *Escherichia coli* BL21 (DE3), Appl. Microbiol Biotechnol **72** (2006) 705-712
13. Perez-Gonzalez JA, Ramon Gonzalez, Amparo Querol, Jose Sendra, and Daniel Ramon - Construction of a Recombinant Wine Yeast Strain Expressing β-(1,4)-Endoglucanase and Its Use in Microvinification Processes, Appl. Environ. Microbiol **59** (9) (1993) 2801-2806.
14. Saloheimo M. Tuna Nakari-Setala, Maija Tenkanen and Merja Penttila - cDNA cloning of a *Trichoderma reesei* cellulase and demonstration of endoglucanase activity by expression in yeast Eur, J. Biochem. **249** (1997) 584-591.

15. Hong J., Hisanori Tamaki, Shunichi Akiba, Kenji Yamamoto and Hidehiko Kumaga - Cloning of a Gene Encoding a Highly Stable Endo-β-1,4-Glucanase from *Aspergillus niger* and Its Expression in Yeast, J. Biosci Bioengineering 92 (5) (2001) 434-441.
16. Beshay U., Hesham El-Enshasy, Ismail I. M. K., Hassan Moawad, Ewa Wojciechowska, Sawsan Abd-El-Ghany - β-Glucanase production from genetically modified recombinant *Escherichia coli*: Effect of growth substrates and development of a culture medium in shake flasks and stirred tank bioreactor, Process Biochemistry **39** (2003) 307-313.
17. Bai Y., Jianshe Wang, Zhifang Zhang, Pengjun Shi, Huiying Luo, Huoqing Huang, Chunliang Luo and Bin Yao - Expression of an extremely acidic β-1,4-glucanase from thermoacidophilic *Alicyclobacillus* sp. A4 in *Pichia pastoris* is improved by truncating the gene Sequence. Microbial Cell Factories **33** (9) (2010).

    http://www.microbialcellfactories.com/content/9/1/33.
18. Seok L. D. and Pack M. Y. - Use of bacilli for overproduction of exocellular endo-β-1,4-glucanase encoded by cloned gene Enzyme Microb Technol **9** (1987) 594-597.
19. Qin Y., Xiaomin Wei, Xiangmei Liu, Tianhong Wang, Yinbo Qu. - Purification and characterization of recombinant endoglucanase of *Trichoderma reesei* expressed in *Saccharomyces cerevisiae* with higher glycosylation and stability, Protein Expression and Purification **58** (2008) 162-167.

APPENDIX

Alignment of deduced amino acid sequence of cloned *EglB* gene in *S.cerevisiae* (Query 1) with that in database (98% identity)

ref|XP_001391969.1| endoglucanase B *eglB-Aspergillus niger*

```
Query  1    YYDMDQDLVLNLNQAAINGIRAAGASQYIFVEGNSWTGAWTWVDVNDNMKNLTDPEDKIV  180
            YYDMDQDLVLNLNQAAINGIRAAGASQYIFVEGNSWTGAWTWVDVNDNMKNLTDPEDKIV
Sbjct  161  YYDMDQDLVLNLNQAAINGIRAAGASQYIFVEGNSWTGAWTWVDVNDNMKNLTDPEDKIV  220
Query  181  YEMYQYLDSDGSGTSETCVSGTIGKERITDATQWLKDNKKVGFIGEYAGGSNDVCRSAVS  360
            YEM+QYLDSDGSGTSETCVSGTIGKERITDATQWLKDNKKVGFIGEYAGGSNDVCRSAVS
Sbjct  221  YEMHQYLDSDGSGTSETCVSGTIGKERITDATQWLKDNKKVGFIGEYAGGSNDVCRSAVS  280
Query  361  GMLENMANNTDVwkgaswwaagpwwgDYIFSLEPPDGT  474
            GMLE MANNTDVWKGASWWAAGPWWGDYIFSLEPPDGT
Sbjct  281  GMLEYMANNTDVWKGASWWAAGPWWGDYIFSLEPPDGT  318
```

TÓM TẮT

TÁCH DÒNG GEN ENDO- β -1,4 GLUCANASE TỪ *ASPERGILLUS NIGER* VÀ BIỂU HIỆN GEN TRONG *SACCHAROMYCES CEREVISIAE*

Phạm Việt Cường[1, *], Quyền Đình Thi[2], Vũ Thị Quyên[1], Nguyễn Thị Kim Cúc[1]

[1]*Viện Hóa sinh biển, Viện KHCNVN, 18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội*

[2]*Viện Công nghệ sinh học, Viện KHCNVN, 18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội*

[*]Email: *cuongwg@yahoo.com*

Endoglucanase là một trong ba dạng của enzyme cellulase và được sử dụng rộng rãi trong nhiều ngành công nghiệp khác nhau, được tổng hợp bởi động vật, thực vật và vi sinh vật.

B-glucanase là một trong những enzyme làm tăng khả năng tiêu hóa thức ăn của động vật và vì vậy, khi bổ sung enzyme này vào thức ăn nguồn gốc thực vật, ngoài việc làm tăng giá trị dinh dưỡng của chúng mà còn giải quyết được vấn đề tiêu hóa của gà hoặc lợn do hàm lượng β-glucan cao trong các loại thức ăn này.

Trong bài báo này, gen β-glucanase từ chủng nấm mốc *A.niger* đã được nhân dòng trong vector biểu hiện pESC-His và biểu hiện trong chủng *S.cerevisiae* KY117.

Gen *Egl*B trước tiên được tách dòng bằng vector pCR2.1, sau đó sử dụng enzymes giới hạn *Bam*HI và *Xho*I để gắn gen vào vector biểu hiện pESC-His. Bằng phương pháp dung hợp tế bào trần, plasmid tái tổ hợp pESC-His/*Egl*B đã được biến nạp vào chủng *S. cerevisiae* KY117, chủng biến nạp được kiểm tra bằng phương pháp PCR với cặp mồi đặc hiệu của plasmid.

Chủng nấm men tái tổ hợp được nuôi trong môi trường SC-His và được cảm ứng bằng galactose. Protein biểu hiện được kiểm tra trên gel SDS-PAGE và kết quả nhận được cho thấy, sau 60 giờ nuôi cấy đã nhận được protein với khối lượng phân tử khoảng 17 kDa, tương ứng với protein của gen *Egl*B theo lí thuyết.

Hoạt tính của protein biểu hiện cũng đã được kiểm tra dựa trên hoạt tính CMC-ase của chủng *S. cerevisiae* tái tổ hợp. Chủng nấm men không mang plasmid tái tổ hợp không tạo vòng phân giải của CMC trên môi trường thử nghiệm, trong khi đó chủng *S. cerevisiae* mang plasmid pESC/*Egl*B tạo vòng phân giải rất rõ.

Từ khóa: biểu hiện, EglB, glucan, *S. cerevisiae*, tách dòng gen.