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Expression of chitinase (*chi42*) gene from *Trichoderma asperellum* in *Saccharomyces cerevisiae*

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ABSTRACT

We cloned a gene coding chitinase (chi42) from strains Trichoderma asperellum SH 16 isolated from Thua Thien Hue province, Vietnam. Expression of the chitinase in Saccharomyces cerevisiae INVSc1 produced a fusion protein of approximately 50 kDa (42 kDa of mature chitinase, 4 kDa of signal peptide and 3.4 kDa of fusion tag of pYES2/NT(C)/chi42 vector). The effect of induction time on the chitinase production was investigated. The results showed that the highest total activity (0.012 unit/mL) of chitinase occurred after 12 h of induction with 2% galactose.

Key words: *Chi42*, expression, 42 kDa chitinase, *Trichoderma asperellum*

INTRODUCTION

Chitinases (EC 3.2.1.14) are a heterogeneous group of enzymes that catalyze the hydrolytic reaction of chitin, which is the second most abundant polysaccharide (after cellulose) in the world [3] and is long-chain polymer of *N*-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) linked by β-1,4 bonds [4]. Chitin is absent from vertebrates and plants but is commonly found in the exoskeletons of insects and crustaceans as well as in fungi and some algae [15].

Chitin is degraded by chitinases into oligosaccharides, mainly (GlcNAc)2, which is further degraded by N-acetylhexosaminidases (EC 3.2.1.52) to GlcNAc. Chitinases are produced by a variety of organisms and may play important physiological and ecological roles. Numerous genes encoding chitinase have been cloned from a variety of organisms. Chitinases have been classified into two glycoside hydrolase families, 18 and 19, based on amino acid sequence similarity. Family 18 chitinases cleave the β-1,4-glycosidic linkage of not only GlcNAc-GlcNAc but also GlcNAc-GlcN, whereas family 19 chitinases cleave GlcNAc-GlcNAc and GlcNAc-GlcN linkages [11, 12]. Therefore, it is generally accepted that chitinases cannot cleave the β-1,4-glycosidic linkage of GlcN-GlcN [7].

Because of the important applications of chitinases in the fields of pest control, pollution abatement and in basic and commercial biology [5], many have been characterized, mostly from plants and bacteria and in minor proportion from fungi. The production of chitinase has received attention as one step in a bioconversion process to treat shellfish waste and produce single-cell protein for animal and aquaculture feed. The production of chitinase enzyme is thought to be one of the primary economic variables in the bioconversion of chitin, estimated to account for 12% of the total production cost [2] and is presently uneconomic due to the high prices of the commercially available chitinase. A more efficient and economically viable process is essential for chitin utilization and the management of shellfish wastes. DNA recombinant technology may provide a technique to approach this problem.

In this paper, we described the expression of the gene encoding chitinase (*chi42*), this enzyme was isolated from *Trichoderma asperellum* SH16, in *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Subcloning the chitinase gene

The chitinase gene (*chi42*) of *Trichoderma asperellum* SH16 were digested from recombinant pGEM-T Easy vector [9] by *Eco*RI and *Bam*HI and purified by Wizard® SV Gel and PCR Clean-Up System Kit (Promega) after electrophoresis on 0.8% agarose gel. The gene was then ligated into *Eco*RI and *Bam*HI sites downstream of the GAL1 promoter of pYES2/NT(C) vector (Invitrogen) and transformed into *E. coli* DH5α competent cells. The resulting plasmid (pYES2/NT(C)/*chi42*) was characterized by restriction analysis.

Transformation of *Saccharomyces cerevisiae*

The pYES2/NT(C)/*chi42* plasmid was transformed into *S. cerevisiae* INVSc1 (MATa *his3Δ1 leu2 trp1-289 ura3-52*/MATα *his3Δ1 leu2 trp1-289 ura3-52*), which was obtained from Invitrogen (Carlsbad, California), by the lithium acetate method, according to the manufacture's instruction. The pYES2/NT(C) vector is designed for native expression of protein of interest in *S. cerevisiae*. It contains the *URA3* gene for selection in yeast.

Expression of the chitinase gene

S. cerevisiae INVSc1 cells containing the recombinant vector with the coding sequence of chitinase gene, *chi42*, were grown at 30^{o}C, on a shaker with rotation speed of 250 rpm, to an OD_{600} of 0.4, then added galactose to a final concentration of 2% for induction of expression of the *chi42* gene and incubated at 30^{o}C. Culture was then further grown for 4 to 24 h, and the cells were harvested by centrifugation at 15000 ×g for 5 min at 4^{o}C. Sonication was carried out for 10 min to break cells and recombinant chitinase was obtained by extraction buffer (20 mM Tris.HCl pH 7.4, 5 mM imidazole, and 500 mM NaCl). Expression level of chitinase was assayed by electrophoresis on 12% (w/v) polyacrylamide gel. The gel was then stained with Coomassie Blue R-250 and image was analyzed by Quality One software (ver 4.1, BioRad).

Enzyme assay

Chitinase activity was assayed by Tsujibo's method [14] with a slight modification. Reaction was performed by mixing a 70 µL of diluted enzyme with 140 µL of 2.5 mM *p*-nitrophenyl-β-*N*-acetylglucosaminide (*p*-NP-β-GlcNAc) in 50 mM acetate buffer (pH 5). After incubation at 50°C for 10 min, the reaction was terminated by adding 1.4 mL of 0.2 M Na_2CO_3. Chitinase activity was measured at 420 nm with *p*-nitrophenol standard curves. One unit of chitinase activity was defined as the amount of enzyme that liberated 1 µmol of *p*-nitrophenol in 1 min under the conditions described above.

RESULTS AND DISCUSSION

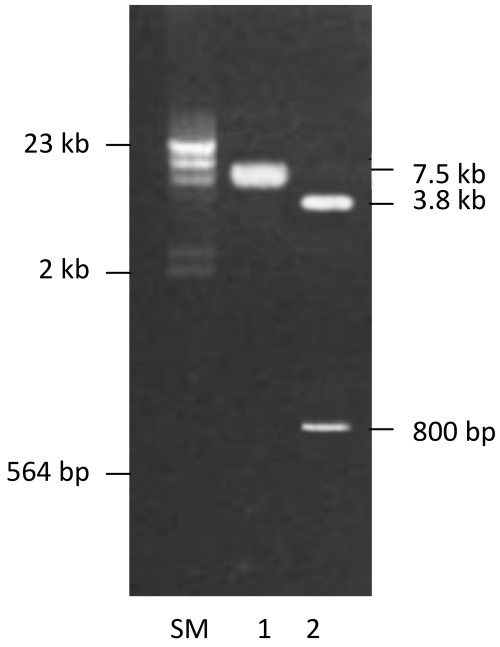

Figure 1. Digestion by *Sal*I for pGEM-T Easy/*chi42* and pYES2/NT(C)/*chi42*. SM: DNA size marker (λ/*Eco*RI), 1: pYES2/*chi42* cut by *Sal*I, 2: pGEM-T Easy/*chi42* cut by *Sal*I.

Subcloning *chi42* gene of *T. asperellum* SH16

The *chi42* gene of *T. asperellum* SH16 was recovered from recombinant pGEM-T Easy vector and ligated into pYES2/NT(C), employing *E. coli* DH5α competent cells. The resulting vector (pYES2/NT(C)/*chi42*) was subjected a digestion by *Sal*I. This digestion produced a linearized DNA fragment of 7.5 kb in length, as expected indicating that the gene were indeed inserted with the vector. While the digestion of pGEM-T Easy/*chi42* vector by *Sal*I produced two DNA bands of approximately 3.8 kb and 800 bp (Fig. 1).

Expression of *chi42* in *S. cerevisiae*

pYES2/NT(C)/*chi42* expression vector, together with empty pYES2 vector as a control, were transformed into *S. cerevisiae* (Fig. 2). The transformed yeast cells were cultured in minimal medium with 2% glucose as carbon source and then used to inoculate rich medium containing 2% galactose, where induction of the gene took place. From these data, it is apparent that a substantial increase of chitinase activity was produced after 4 h of growth using galactose as an inducer.

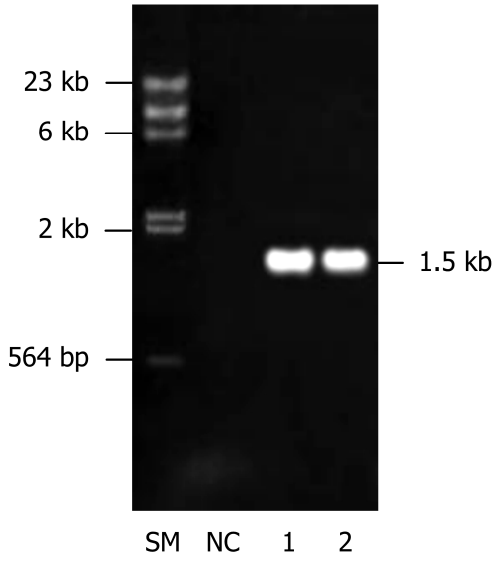

Figure 2. PCR amplification of *chi42* gene. SM: DNA size marker (λ/*Eco*RI), NC: negative control (yeast without pYES2/NT(C)/*chi42*), 1: pYES2/NT(C)/*chi42*, 2: yeast transformed with pYES2/NT(C)/*chi42*.

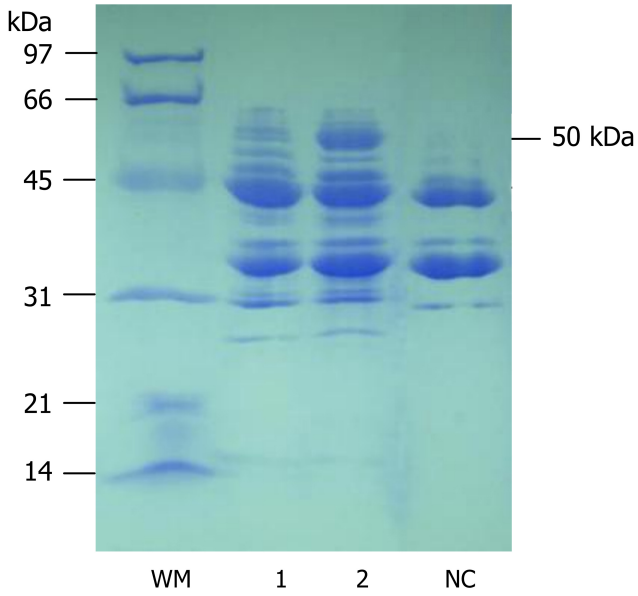

Figure 3. Expression of chitinase in yeast cells were induced by 2% galactose. WM: protein weight marker (97-14 kDa). NC: non-induced yeast cells. 1 and 2: expression of chitinase after 24 and 12 h of induction, respectively.

Expressed recombinant chitinase was analyzed through SDS-PAGE. For the inclusion body fraction, a strong band

of appoximately 50 kDa (42 kDa of mature protein, 4 kDa of signal peptide [1] and 3.4 kDa of fusion tag of pYES2/NT(C)/*chi42* vector) was obtained in culture induced with galactose (Fig. 3). Protein bands of chitinase in unsoluble fractions are very weak (data not shown). For the uninduced cells, no protein band of expected sizes was observed, suggesting that induction with galactose is required for expression of chitinase genes.

There were some reports on expression of chitinase gene from *Trichoderma* such as *ech42* gene from *T. harzianum* [1], *ech42* gene from *T. aureoviride* [8], a novel chitinase from *T. atroviride* strain P1 [6], *chit33* gene from *T. atroviride* [10], *ech42* gene from *Trichoderma* spp. [13]. However, there are no data in the literature concerning expression of the chitinase *chi42* from *T. asperellum* in *S. cerevisiae*.

Profile of chitinase activity in batch culture

A batch culture of recombinant *S. cerevisiae* cells was performed in 250-mL Erlenmeyer flasks for the intracellular chitinase production. Figure 4 displayed a typical curve of enzyme production based on the total activity. Generally, the total activity of the chitinase increased continuously from the 4 h to 12 h of cultivation with a maximum value of 0.012 unit/mL, and it rapidly decreased after 12 h. This enzyme was absent in the extract from the control (non-recombinant *S. cerevisiae*).

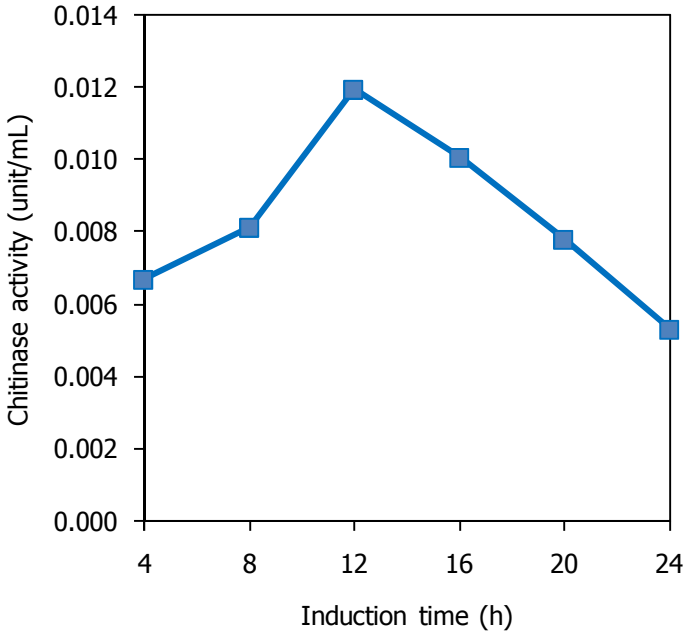

Figure 4. Effect of induction time on total chitinase activity of recombinant *S. cerevisiae* cells.

In conclusion, our experiments show that the chitinase 42 kDa from *T. asperellum* SH16 was expressed and functioned in *S. cerevisiae* cells. This recombinant *S. cerevisiae* cell is relatively convenient, therefore, its application can be further exploited after optimization for culture conditions will be investigated.

Acknowledgments

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REFERENCES

[1] C. Carsolio, A. Gutiérrez, B. Jiménez, M. Van Montagu, A. Herrera-Estrella, *Proc. Natl. Acad. Sci.* **1994**. 91(23): 10903-10907.
[2] I.G. Casio, R.A. Fisher, P.A. Carroad, *J Food Sci*. **1982**. 47: 901-911.
[3]S.P.M. Castro, E.G.L. Paulín (**2012**). Is Chitosan a New Panacea? Areas of Application. Pages 3-46 in Karunaratne DN, ed. The Complex World of Polysaccharides, vol. 9. 51000 Rijeka, Croatia. InTech, Janeza Trdine.
[4] A. Catalli, M. Kulka, *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*. **2010**. 4: 175-189.
[5] P.A. Felse, T. Panda, *Bioprocess Eng*. **2000**. 23: 127-134.
[6] I.A. Hoell, S.S. Klemsdal, G. Vaaje-Kolstad, S.J. Horn, V.G. Eijsink, *Biochim. Biophys. Acta.* **2005**. 1748(2): 180-190.
[7] M. Ike, K. Nagamatsu, A. Shioya, M. Nogawa, W. Ogasawara, H. Okada, Y. Morikawa, *Appl. Microbiol. Biotechnol.* **2006**. 71(3): 294-303.

[8] S. Jinzhu, Y. Qian, L. Beidong, C. Dianfu, *Appl. Microbiol. Biotechnol.* **2006**. 69: 39-43.
[9] N.H. Loc, H.T. Quang, N.B. Hung, N.D. Huy, T.T.B. Phuong, T.T.T. Ha, *Mycobiol*. **2011**. 39: 182-186.
[10] S. Matroudi, M.R. Zamani, M. Motallebi, *Brazilian J. Microbiol.* **2008**. 39: 433-437.
[11] M. Mitsutomi, A. Ohtakara, T. Fukamizo, S. Goto, *Agric. Biol. Chem.* **1990**. 54: 871-877.
[12] A. Ohtakara, H. Matsunaga, M. Mitsutomi, *Agric. Biol. Chem.* **1990**. 54: 3191-3199.
[13] P. Sharma, K. Saravanan, R. Ramesh, P. Vignesh Kumar, D. Singh, M. Sharma, M.S. Henry, S. Deep, *Afr. J. Biotechnol.* **2012**. 11: 12930-12938.
[14] H. Tsujibo, H. Orikoshi, K. Shiotani, M. Hayashi, J. Umeda, K. Miyamoto, C. Imada, Y. Okami, Y. Inamori, *Appl. Environ. Microbiol.* **1998**. 64: 472-478.
[15] W.F. Zhong, J.C. Fang, P.Z. Cai, W.Z. Yan, J. Wu, *Genet. Mol. Biol.* **2005**. 28: 821-826.