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Expression and Application of Heterologous Genes in *Saccharomyces cerevisiae*

Soo-Wan Nam
Dept. Biotechnology & Bioengineering, Dong-Eui University, Busan 614-714, Korea

Abstract

Cyclodextrin glucanotransferase (CGTase) and endoxylanase genes of *Bacillus* sp. were subcloned down-stream of yeast *ADH1* promoter and expressed in *S. cerevisiae*. Most of the CGTase and endoxylanase expressed were detected in the extracellular medium with activity of 0.6 and 7-8 unit/ml, respectively. The recombinant enzymes were secreted as *N*-linked-glycosylated forms, resulting an enhanced thermal stability. CGTase predominantly produced α-cyclodextrin from starch and endoxylanase produced xylobiose and xylotriose from xylan.

Introduction

Cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) converts starch and related α-1→4-glucans into cyclodextrins (CDs) through an intramolecular transglycosylation reaction. CDs are now used in food, pharmaceutical, chemical, cosmetic, and agricultural industries because of their ability to form inclusion complexes with a wide variety of hydrophobic compounds, resulting in a prolonged stability and increased water solubility of these guest molecules. Xylan is a major component of the cell walls of monocots and hardwoods, representing up to 35% of the dry cell weight of these plants. Hydrolysis of xylan is of considerable interest for various biotechnological applications (biobleaching, food, and animal feed). Endoxylanase (EC 3.2.1.8) acts on xylans and produces xylo-oligosaccharides.

The yeast *Saccharomyces cerevisiae* can neither utilize nor degrade xylan or starch, but it possesses a number of advantages that render it an attractive host for the expression and production of CGTase and endoxylanases. The expression of heterologous genes in yeast has often resulted in an increase of thermal stability due to the glycosylation and/or a change of enzymatic characteristics such as optimal pH or temperature, etc.

In this paper, we describe the expression of *Bacillus* CGTase and endoxylanase genes in *S. cerevisiae*, and the production of CDs or xylo-oligosaccharide from starch or xylan by the recombinant enzymes.

Materials and methods

Yeast strains and plasmids : Yeast host strain used was *S. cerevisiae* SEY2102 or 2805. The

plasmid pKBR1 and pJHKJ4 were used for the source of CGTase and endoxylanase genes, respectively. For the construction of yeast expression plasmid, *E. coli*-yeast shuttle vector pVT103-U was used, in which the yeast *ADH1* promoter (*ADH1*p) and the *ADH3* terminator were connected with MCS region. The two genes, including its signal sequences, were subcloned downstream of *ADH1*p. The resulting plasmid, pVT-CGTS (9.0 kb) and pAEDX-1 (7.63 kb), were individually transformed into *S. cerevisiae*.

Media and culture conditions : For the selection of yeast transformant, YNBCAD medium (6.7 g/l yeast nitrogen base without amino acids, 5 g/l Casamino acid, 20 g/l glucose) was used. The seed culture was grown on YNBCAD 6 unit/ml and inoculated at 5% (v/v) into a fermentor containing 2 l YPD medium.

Analyses : The yeast culture supernatant was used for the source of enzymes. The periplasmic and intracellular fractions were obtained by treatment of Zymolyase and glass beads. One unit of CGTase activity was defined as the amount of enzyme liberating 1 μmol α-CD from starch per min at 50℃. One unit of endoxylanase activity was defined as the amount of enzyme liberating 1 μmol reducing sugar from oat spelt xylan per min. The denatured enzymes by boiling for 10 min were treated with endoglycosidase H for 2 h at 37℃ and then electrophorezed. The reaction samples were withdrawn at appropriate time intervals, and then immediately boiled. After filtration (0.45 μm) of the reaction mixture, the filtrate was analyzed by HPLC equipped with RI detector and TSKgel Amide-80 column.

Results and discussion

Expression and localization of enzymes in yeast : The yeast transformants were further selected on YPD plate containing starch azure (0.25%) or xylan (1%). To investigate the expression level and localization of the enzymes, the transformants, *S. cerevisiae* SEY2102 or 2805 harboring pVT-CGTS or pAEDX-1, were cultured in fermentor. As shown in Table 1, the CGTase activity in the extracellular medium reached to a maximum level of 0.6 unit/ml at 48 h. Also, the total expression level of endoxylanase was about 7-8 unit/ml, and most (>65%) of enzyme was successfully secreted into the culture medium. This high secretion efficiency indicates that the signal peptides of CGTase and endoxylanase functioned well in the yeast secretory pathway.

SDS-PAGE analysis and thermal stability of recombinant enzymes : By SDS-PAGE analysis, the MW of purified CGTase was estimated to be 120 kDa, whereas that of wild-type enzyme was 74 kDa. After endo H treatment, the MW of recombinant CGTase decreased to 81 kDa. This result indicates that the recombinant CGTase was produced as an *N*-linked glycosylated. This hyperglycosylation of CGTase significantly increased the thermal stability as that the half life of recombinant CGTase at 55℃ was about 60 h, whereas that of unglycosylated enzyme expressed in *E. coli* was about 24 h.

Through the active staining of recombinant endoxylanase, three active bands with 42, 49, and 111 kDa were detected. Taking into account the MW of 20.4 kDa of *Bacillus* endoxylanase, the recombinant enzyme seems to be glycosylated: 111 kDa band is a heavily and 42 and 49 kDa bands

are lightly glycosylated forms. When the recombinant enzyme was treated with endo H, three active bands migrated as three sharp bands with 25, 29, and 49 kDa, indicating that the glycosylation occurred via *N*-linked type. The endoxylanase activity was remained over 80% at 80℃, resulting in 4-fold increase of thermostability than that of the *Bacillus* enzyme. This result indicates that the enhancement of thermostability seems to the *N*-linked glycosylation.

Formation of CDs and xylo-oligosaccharide : Through the CGTase reaction with 5% (w/v) soluble starch at 55℃, total CD was produced at 16.7 g/l after 48 h, of which the fractions of α-, β-, and γ-CD were 69% (11.5 g/l), 23% (3.8 g/l), and 8% (1.3 g/l), respectively. From this result, the conversion yield of starch to CD was about 33% and the production ratio of α-, β-, and γ-CD was 9:2:1. The predominant formation of α-CD might be caused by a conformational change of glycosylated CGTase in the active site cavity or flexibility of the enzyme. With the recombinant endoxylanase, the xylotriose was the main product at all reaction temperatures (40℃ ~ 70℃). This result indicates that the recombinant endoxylanase produced in yeast can be used for the production of xylo-oligosaccharide with high-content xylotriose.

Table 1. Comparison of cell growth, plasmid stability, and CGTase or endoxylanase expression in yeast transformants. Yeast cells were grown on YPD medium at 30℃ for 48 h.

Strain	Cell Conc. (OD_{600})	Plasmid Stability (%)	Enzyme Activity (unit/ml)			Secretion Efficiency (%)
			Medium	Periplasm	Cytoplasm	
SEY2102/pVT-CGTS	32.4	98	0.63	0.04	0.01	93
2805/PVT-CGTS	31.4	90	0.59	0.08	0.01	87
SEY2102/pAEDX-1	36.7	99	6.46	2.00	1.30	66
2805/pAEDX-1	37.2	98	5.98	1.84	1.11	67

References

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