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Construction of a Gene Construct for Transfer to Yeast to Degrade Cellulose

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ABSTRACT

Cellulose is the major cell wall polysaccharide of plants, consisting of -D-β glucopyranose units with 1-β and -4 glycoside bonds. Cellulose degradation requires the co-operation of three types of enzymes, including cellulasesendogluconases, exoglucanases, and -β glucosidases. Thermobifidiafusca is a cellulase-producing enzyme and the Cel6B gene is involved in cellulose degradation as an exocellulose. In this study, in order to analyze cellulose and ethanol production under anaerobic conditions with the yeast Pichiapastoris, the first cellobiohydrolase gene of the bacterium Thermobifiadfuscawhich was on plasmid PSZ143. Designing the appropriate primers was amplified by PCR and after digestion of the yeast palsy Phil-S1, the 1861 bp fragment of the Cel6B gene was inserted into the palsy. The confirmation of the recombinant construct was confirmed by cloning PCR with the gene primers and the Phil-S1 plasmid primers. Following gene transfer to yeast, gene expression will be examined.

KEYWORDS: *Cellulose, Gene Structure, Ethanol Production*

I. INTRODUCTION

Cellulose is the major cell wall polysaccharide in plants that is present in the cell wall structure of bacteria, fungi and algae (Valerie et al., 2111; Werich& Jane, 2114; Olicosander et al., 2118). In the cell wall of plants, cellulose is combined with lignin and hemicellulose polymer carbohydrates (Werisch& Jane, 2114). Cellulose is a water-insoluble polymer compound in which -D-β-glucopyranose units are linked by 1-β and -4 glycoside bonds. Plant biomass contains a significant amount of cellulose that can be used as a carbon source for the production of valuable chemicals. To this end, depolymerization of cellulose with glucose is a prerequisite for microbial fermentation. There are two ways to convert cellulose to glucose: chemical and enzymatic hydrolysis. The use of cellulases for hydrolysis of cellulose under mild conditions is more attractive without bio-products because enzyme depolymerization is more environmentally friendly (Werisch& Jane, 2114). In principle, three cellulose enzymes with synergistic effect are used to hydrolyze cellulose. These are endogluconases (E.C 4.2.1.4), exoglucanases and -β glucosidases. Cellulose genes cloned and sequenced from Thermobifidiafusca The Cel6B gene from T. fusca is a 6.8 kDaexocellulose that belongs to the B cell family and contains conserved amino acids. These amino acids are essentialfor catalytic activity in cellobiohydrolases II from Trichodermareesei and

Cel6B from T. fusca. Therefore, the Cel6B exocellular activity is very similar to the fungal exocellulos of this class of amino acids, but the enzyme is highly heat-resistant and has an optimal pH range of 7 and 7, whereas the fungal enzymes have an optimum pH of 4. Cel6B also has a longer encapsulated and longer active site than its fungal type (Wu et al., 2114). Cellulases are widely used in the industry, including applications in the textile, pulp and paper industries, the food industry as well as enhanced detergents and improved digestibility of livestock feed.

The aim of this study was to clone the Cel6B cellobiohydrolase gene from Thermobifidiafusca in the yeast palsy Phil-S1 for its extracellular secretion.

II. Methodology

In this study, we used the PSZ143 construct as a carrier for the Cel6B gene, which is 71.7 bp in size and was donated by Mr Wilson from Cornell University. First, by primer design containing the cleavage site for the EcoRI and BamHI cleavage enzymes (Table 1 shows the sequence of primers (Cel6B gene amplified using PCR with the materials listed in Table 2). Table 4 shows the temperature conditions of the PCR and its cycles. After amplification, the purified PCR product was digested with shear enzymes for 8 h at 47 ° C and then extracted from the gel. Phil-S1 plasmid was first extracted from E. coli TOP10 by alkaline lysis followed by confirmation of extraction by electrophoresis. In the next step, EcoRI and BamHI were digested at 47 ° C for 8 h and then purified by denaturing gel extraction.

Table 1 Sequences of primers used to amplify and cut the Cel6B gene from the vector (PSZ143 primer sequence containing EcoRI truncation site and back sequence containing the BamHI shear site)

Primer length (nucleotide)	Oligonucleotide sequence	Primer
25	6 / -TCCATACGAATTCGCCGGCTGCTCG-4 /	Went
25	6 / -TCACTCCGGGATCCAGAGGCGGGTA-4 /	Return

Table 2. Materials needed for PCR reaction in a volume of 22 µl

Required Materials	Value (µl)
PCR buffer 2x	2
The primer went initiator (11 micro Molar)	1
Return initiator (11 micro Molar)	1
DNA samples (2500 ng/µl)	0.5
MgCl2 (25 mM)	1.2
dNTP (40 mM)	0.5
Pfu enzyme (5 µl)	0.25
Distilled Water	13.55

The fragment binding reaction to yeast plasmid was carried out after recombinant structural binding reaction was transferred to E. coli TOP10. After transfection of the constructs to the bacterium, the next day, the colonies observed via PCR were confirmed for recombination with the gene primer and the vector primer. The colonies were then cultured in liquid medium to extract the plasmid from the bacteria and the extracted plasmid was sent to globe for sequencing.

Table 3 Thermal Cycle for PCR with Specific Primers

Number of cycles	Reaction stage	Time (seconds)	Temperature (C°)
1 cycle	Early Denaturation	300	94
	Denaturation	60	94
34 cycles	Primer binding	60	57
	Expansion	120	72
1 cycle	Final Expansion	300	72

III. RESULTS

After designing the primers to amplify the gene from the PSZ143 plasmid, gene amplification was performed using gradient PCR (Figure 1). According to Figure 1, the gene amplified by PCR using a gradient at 66 ° C produced 1861 bp. The PCR product was then purified, and after 8 h digestion with the enzymes, it was purified using denaturing gel extraction kit (Figure 2). Figure 2 shows that the fragment was successfully cleaved by shear enzymes and gel purification was also successful.

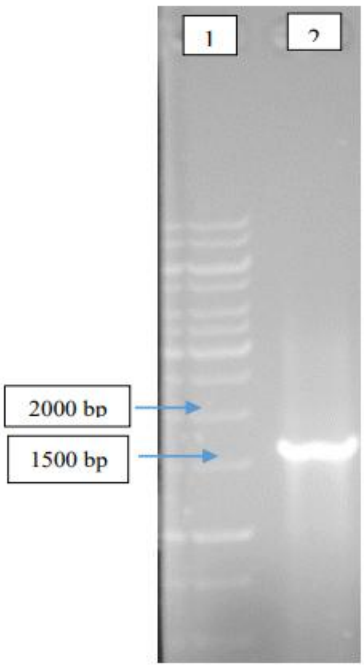


Figure 1
Gene amplification by PCR from palmsid and the amplified gene

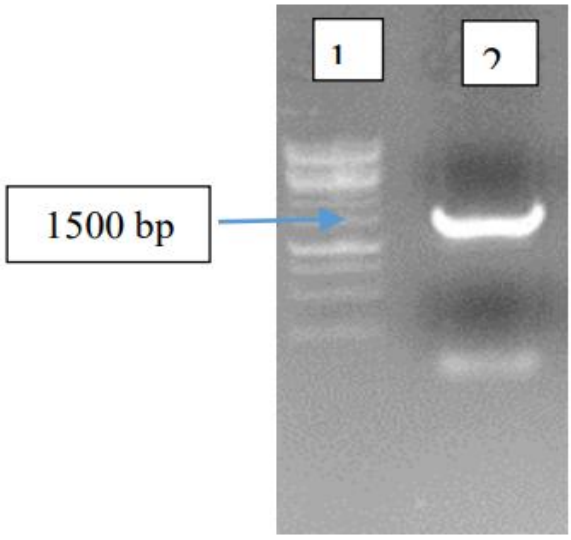


Figure 2
Gene product after purification, digestion and extraction from the gel

Phil_S1 plasmid was also digested after extraction by alkaline lysis and the product digested with gel was purified by denaturing gel extraction kit (Figure 4). Since the cleavage distance between the two cleavage enzymes is 10 bp, therefore, the cleaved fragment on the gel is not observed. after digestion of Phil-S1 plasmid with EcoRI and BamHI shear enzymes, a cleaved fragment was placed opposite the 8000 bp band.

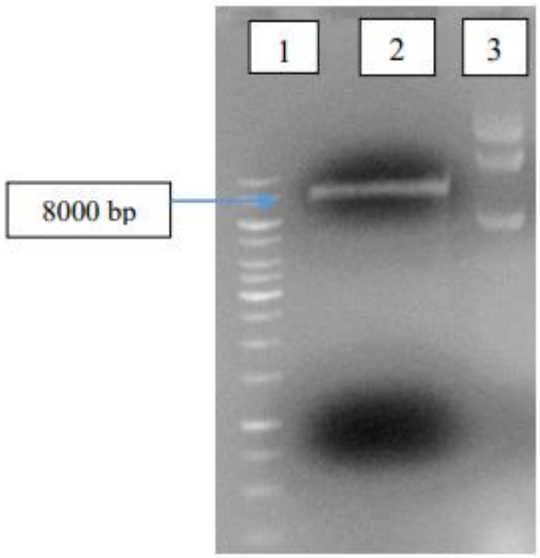
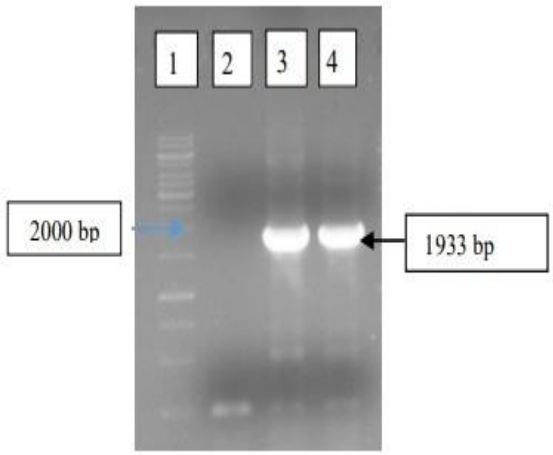


Figure 3
Result of digestion of S1-Phil plasmid with the enzymes

The ligation reaction was then carried out using T4 ligase and transferred to E. coli TOP10, which was observed the next day. Colony PCR was confirmed on two of the colonies grown with vector primers (Figure 4). The result of PCR amplification on gel electrophoresis showed 1.44 bpfragment, which was the same result with respect to gene sequence and cleavage site on plasmid. plasmid extraction was then performed and the product was sent for sequencing in Korea, Figure 6 shows the result of gene sequencing ballast with 100% similarity to the original NCBI gene sequence.

Figure 4 Colony PCR using S1-P primers



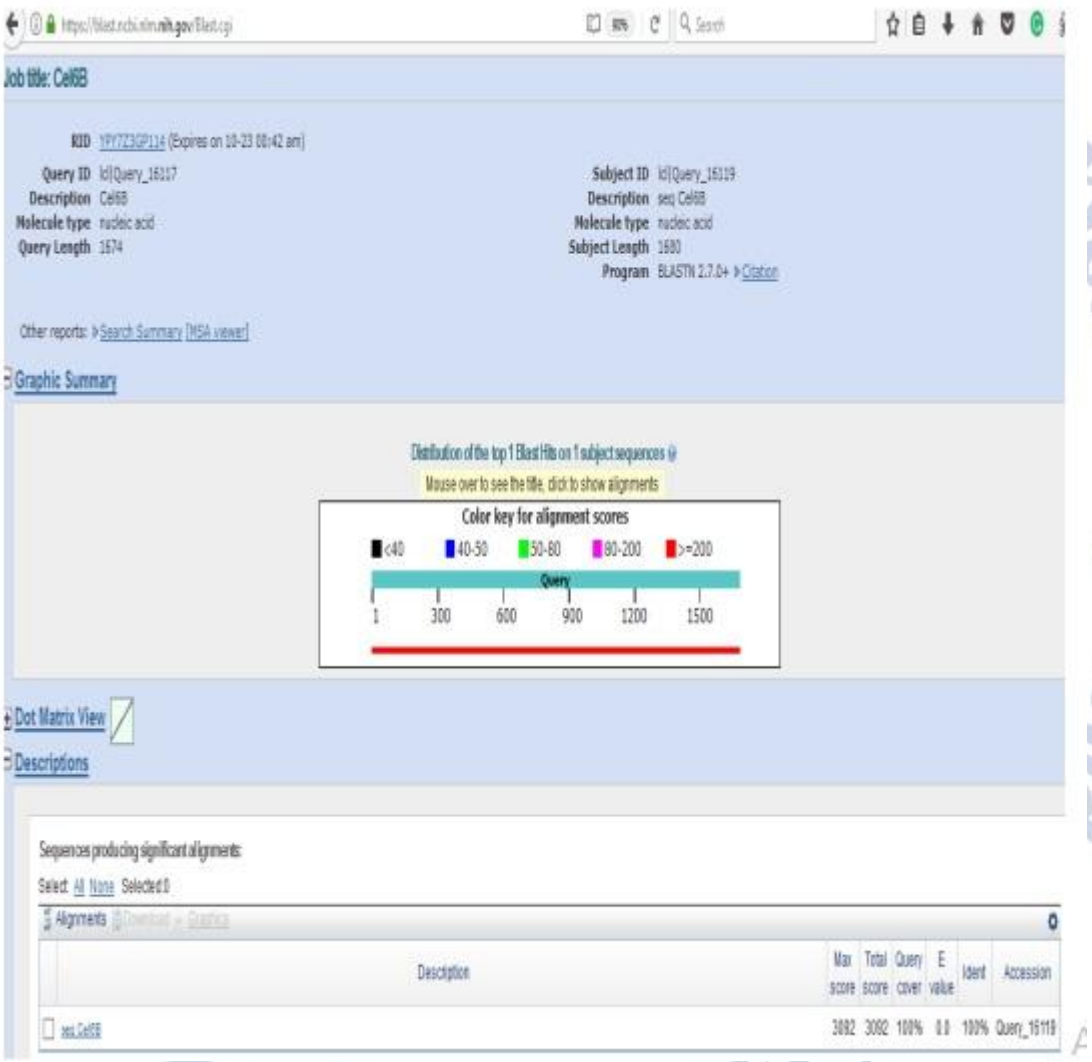


Figure 5
The result is the sequencing of the resulting sequence with the Cel6B genein the NCBI database

III. CONCLUSION

Gene amplification was performed using primers designed by gradient PCR and then the PCR product was digested with EcoRI and BamHI shear enzymes. The yeast plasmid Phil-S1 was then digested using bacterial extraction enzymes and ligation was performed by T4 ligase. Transfected E. coli TOP10 was then transfected the next day. Then, PCR colonies were performed on the colonies and cultured in liquid LB medium to extract plasmid sent to globe for sequencing. The sequencing result showed that the gene sequence was the same as the 111% sequencing result and the cloning was done correctly.

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