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Construction of the Cellulosome for Targeting the Cell Wall Degradation in Red Algae and Its Application to Marine Biomass

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Research aims

Marine biomass is comprised of a variety of polysaccharides as structural or storage compounds. The biological degradation to marine biomass occurs through polysaccharolytic enzymes that are produced by organisms ranging from eukaryotes to microorganisms. Thus, biodegradation is important for natural and industrial cycling of environmental compounds. Furthermore, its biological degradation plays a major role in the turnover of CO_2. The components of cell walls in marine seaweed, especially for red algae include quite unique sugar polymers such as β-1,4-mannan, β-1,3-xylan, and agar, while those in terrestrial plants consist of cellulose, hemicellulose, and lignin. In particular, *Porphyra* is an important red seaweed which has familiarly been edible in Japan.

Glycosidases, which generally play a major role in the turnover of polysaccharides in plant biomass, have been found either as free enzymes that work synergistically or as an enzyme complex called cellulosomes. In this project, we tried to construct a cellulosome for targeting the cell walls of marine seaweed, especially for red algae, *Porphyra*.

Methods

To construct the cellulosome for degrading the cell walls of red algae, we used mini-*cbpA* and *engE* genes from *Clostridium cellulovorans*[1] and AgaB from *Vibrio* sp. strain PO-303, ManA from *Vibrio* sp. strain MA-138, and TxyA from *Alcaligenes* sp. strain XY-234. Instead of an original cellulose-binding domain (CBD) in mini-CbpA, we changed a carbohydrate-binding domain (CBM) of TxyA from *Alcaligenes* sp. strain XY-234. Dokerin domain of EngE from *C. cellulovorans* was amplified by PCR to create expression plasmids pET-AgaB, pET-ManA, and pET-TxyA, respectively (Fig. 1). The amplified fragments containing each gene and dokerin domain were inserted between *Nco*I and *Xho*I sites of pET22b. Four recombinant proteins were purified from each of the *E coli* BL21 strains harboring expression plasmids. To determine the protoplast formation ability of enzymes from three kinds of marine bacteria, we used a red algae, *Porphyra yezoensis*.

Results

E. coli BL21 harboring each pET expression plasmid was grown in 300 ml of LB medium at 37°C to optical density at 600 nm of 0.9. Isopropyl-β-D-thiogalactoside (IPTG) was then added to a final concentration of 1 mM, and the culture was grown at 30°C for 3 h. After collection of *E. coli* cells by centrifugation, the periplasmic proteins were extracted into 50 ml of extraction buffer (20 mM Tris-HCl [pH 8.0], 10 mM EDTA) by the osmotic method. A 50-ml aliquot of the supernatant was applied to an Ni_2^+-nirilotriacetic acid agarose column. Each protein was eluted with 200 mM imidazole in 50 mM sodium phosphate buffer

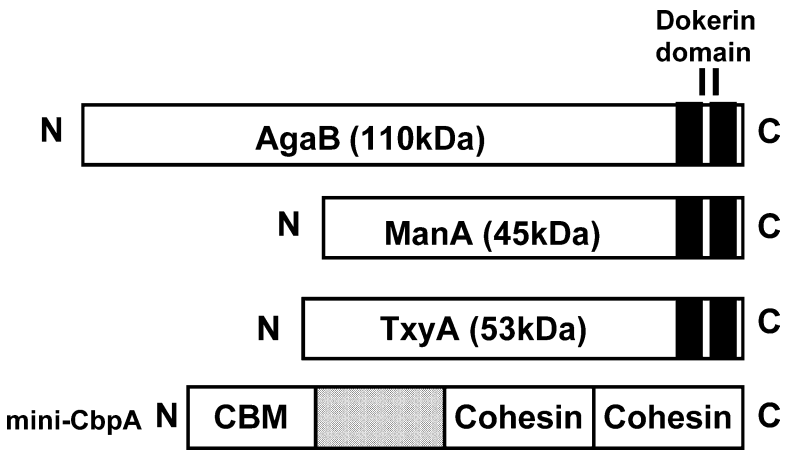

Fig. 1. Construction of expression plasmids for cellulosome.

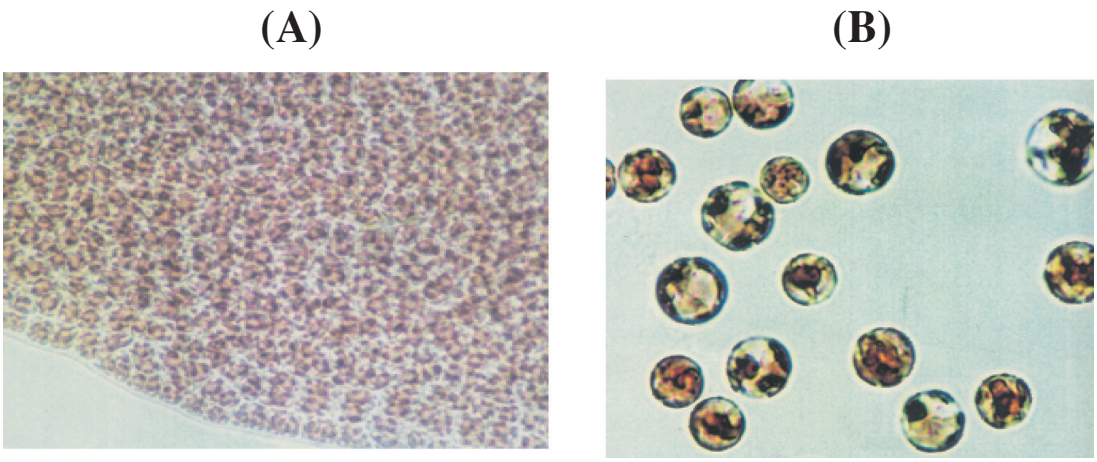

Fig. 2. Protoplast formation from *Porphyra yezoensis* using the recombinant enzymes from marine bacteria.

(pH7.0), and fractions containing each protein were collected. After purification and SDS-PAGE, the final preparation yielded a single band. By using these purified enzymes, we examined the interaction between mini-CbpA and three kinds of enzymatic subunits. As the result, no interaction bands were detected by native PAGE, indicating that dokerin domain of each subunits might have been lacked by proteolysis in *E. coli* cells. Next, in order to confirm that three kinds of recombinant enzymes are able to release the protoplasts from *Porphyra yezoensis*, the three enzymes were tested for protoplast formation. As the result, approximately 4.0×10^4 protoplasts were released in 20 mM MES buffer (pH 7.5) containing 0.5 M mannitol and one unit each of three kinds of recombinant enzymes (AgaB, ManA, and TxyA) and under incubation at 22°C for 60 min with gentle agitation.

Conclusion

We have succeeded in protoplast formation from *Porphyra yezoensis* by using three kinds of recombinant enzymes from marine bacteria. However, the cellulosome complex between mini-CbpA and these enzymes was not found. Since this problem is probably caused by proteolysis in *E. coli*, it therefore seems that it is necessary to choose the host strain as protease-deficient one.

Reference

1) Kosugi, A., Murashima, K., Tamaru, Y., and Doi, R. H. (2002) Cell-surface-anchoring role of N-terminal surface layer homology domains of *Clostridium cellulovorans* EngE. J. Bacteriol., **184**: 884–888.