AgRePaper and Ecolink Project
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Abstract

Paper is essential for our lives, but the preparation of paper is required of environmental cost such as tree and water. For environmental preservation, making paper without wood is required. Agrobacteria, one of soil bacteria, produces glucans such as cellulose and curdlan that can be used as raw materials for paper. Our team examined various ways to regulate the amount of glucans produced by modification of agrobacteria. For this purpose we have isolated *CelA-C* genes that function in synthesis and degradation of cellulose, and *CrdS* gene encoding synthetase of curdlan. To modify agrobacteria genetically, pBI107 shuttle vector that encodes selection marker and replicates in agrobacteria was also developed.

We had next planned to develop an ink that is biologically recyclable. For preparation of the ink, the myoglobin, a red-colored component of meat which color was changeable by chemical materials, was isolated from sperm whale followed by optimization of codon usage in *E. coli*. The modified myoglobin expressed in *E. coli* showed reddish color. Also, to produce myoglobin massively, T7 cassette was constructed.

▪ AgRePaper Project

A. What's Agrobacterium tumefaciens?

***Agrobacterium tumefaciens* has been used to transform plant cells. The bacteria attached to plant cell with glucans and It made tumor that was named crown gall.**

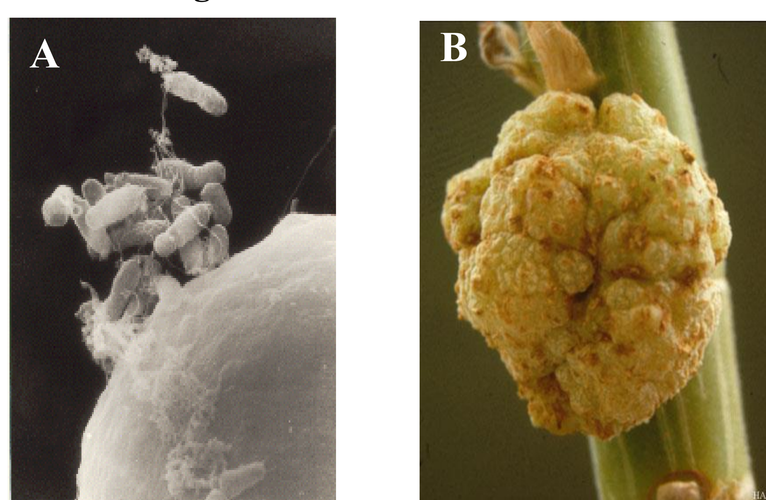


Fig.. 1 A: Scanning electron microscopy copy of several *Agrobacterium tumefaciens* that infect a carrot cell, During the process, the bacterial genetic material will enter in to the plant cell (A. G. Matthysse, K. V. Holmes and R. H. G. Gurlitz)
B: Crown gall caused by *Agrobacterium tumefaciens*.(Halvor Aarnes)

B. Induction of glucans from agrobacteria

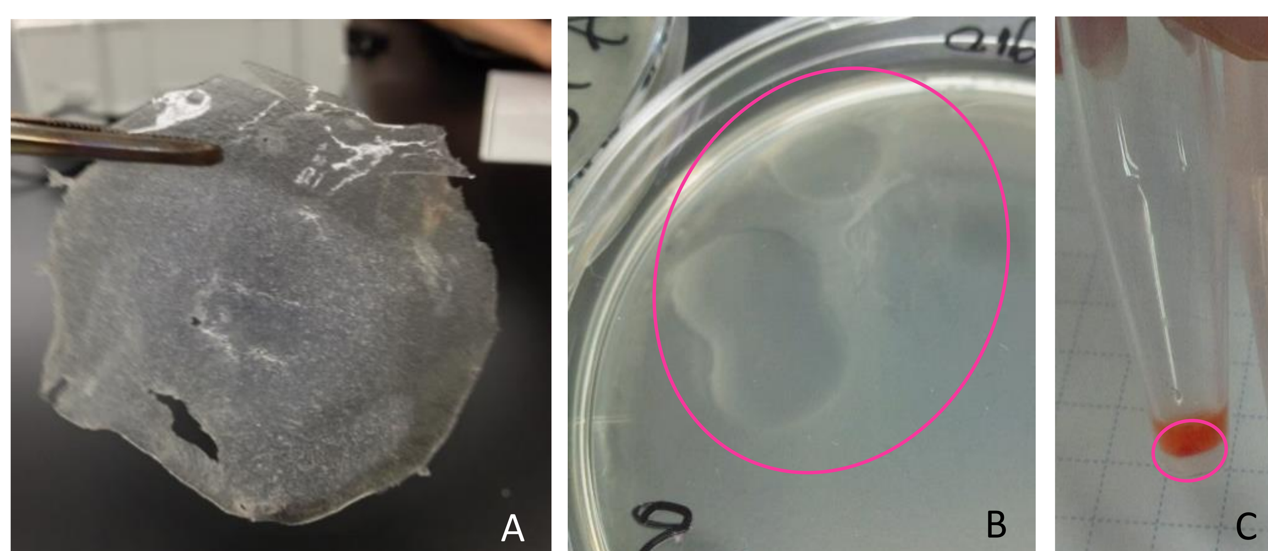


Fig. 2 A: Gel-like glucan sheet prepared with commercially available curdlan
B: White film induced by agrobacteria on LB medium containing soy milk
C: Congo-red-stained film suggest the presence of glucans because the Congo-red binds to glucans

C. Synthesis and degradation system of cellulose and curdlan

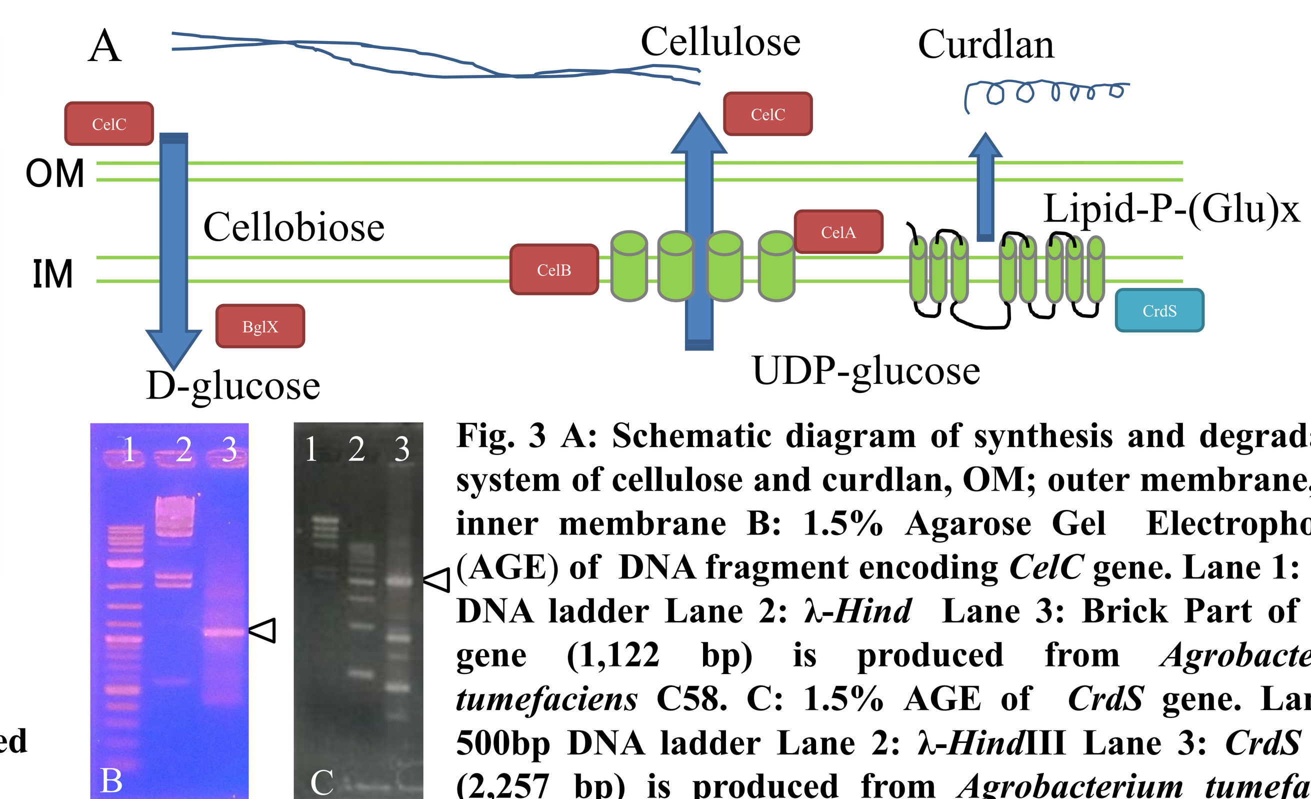
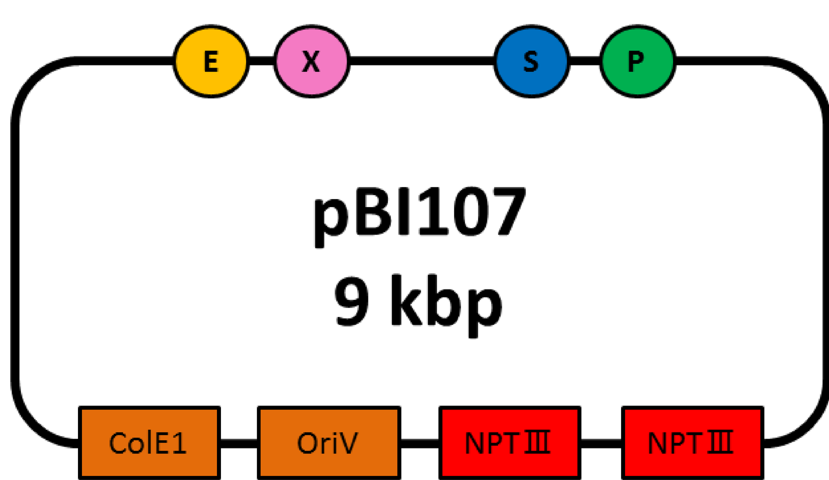


Fig. 3 A: Schematic diagram of synthesis and degradation system of cellulose and curdlan, OM; outer membrane, IM; inner membrane B: 1.5% Agarose Gel Electrophoresis (AGE) of DNA fragment encoding *CelC* gene. Lane 1: 2-log DNA ladder Lane 2: λ-*Hind* Lane 3: Brick Part of *CelC* gene (1,122 bp) is produced from *Agrobacterium tumefaciens* C58. C: 1.5% AGE of *CrdS* gene. Lane 1: 500bp DNA ladder Lane 2: λ-*Hind*III Lane 3: *CrdS* gene (2,257 bp) is produced from *Agrobacterium tumefaciens* C58.

D. What's shuttle vector?



Notation	Function
E X	BioBrick cloning site : prefix
S P	BioBrick cloning site : suffix
CoiE1	replication origin in *E.coli*
OriV	replication origin in agrobacteria
NPTⅢ	kanamycin resistance gene

Fig. 4: pBI107 shuttle vector

Shuttle vector has characteristic to replicate in *E. coli* and agrobacteria. To construct the shuttle vector, replication origin and kanamycin resistance gene of pBI333 plasmid that is used for transformation of plant cell, was isolated and ligated to prefix and suffix sequences, resulting to pBI107 plasmid (fig. 4). To check the replication in *E. coli*, RFP(BBa_J04450) was cloned in pBI107 plasmid following to transformation in *E. coli* JM109. Fig. 5 shows red fluorescence, indicating to replicate in *E. coli*. The same plasmid can replicate in *A. tumefacience* C58 as shown in fig. 6.

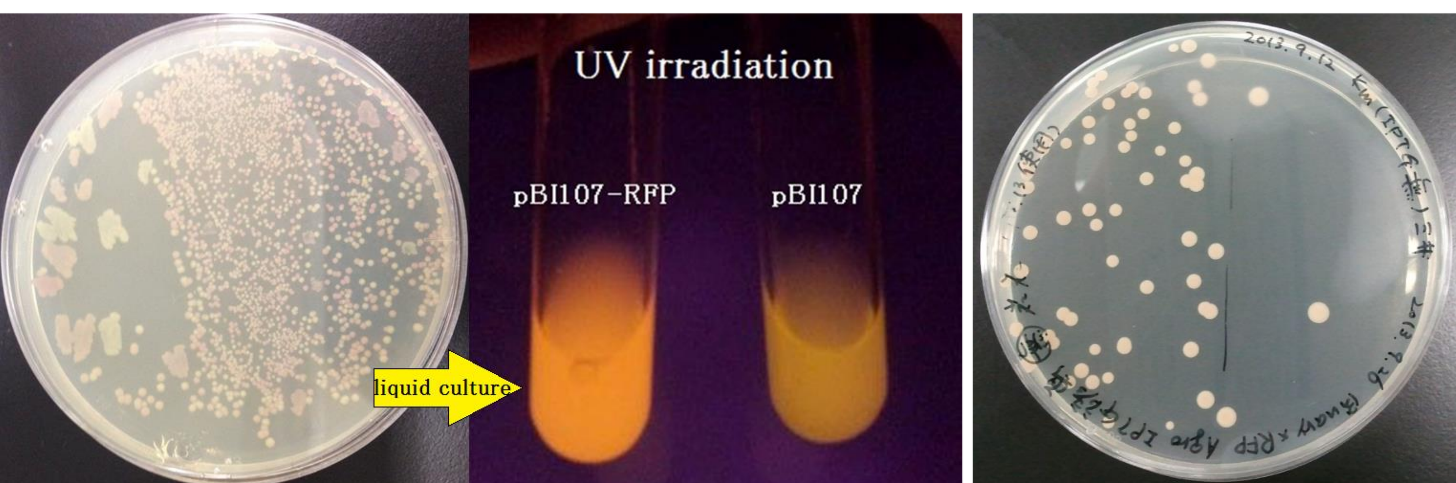


Fig. 5: *E. coli* JM109/pBI107-RFP

Fig. 6: *A. tumefaciens*/pBI107-RFP on kan plate

Synthesis of Glucan
Agrobacteria
Degradation
Synthesis of Myoglobin
E. coli
Ecolink
Paper
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E. What's myoglobin?

Myoglobin (Mb) is one of meat components showing red color. The color can be changed by binding of oxygen and so on. These features make the myoglobin as ink. The Mb gene was isolated from sperm whale with modification of codon usage suitable to *E. coli* (mMb). The mMb gene was submitted as BBa_K1044002.

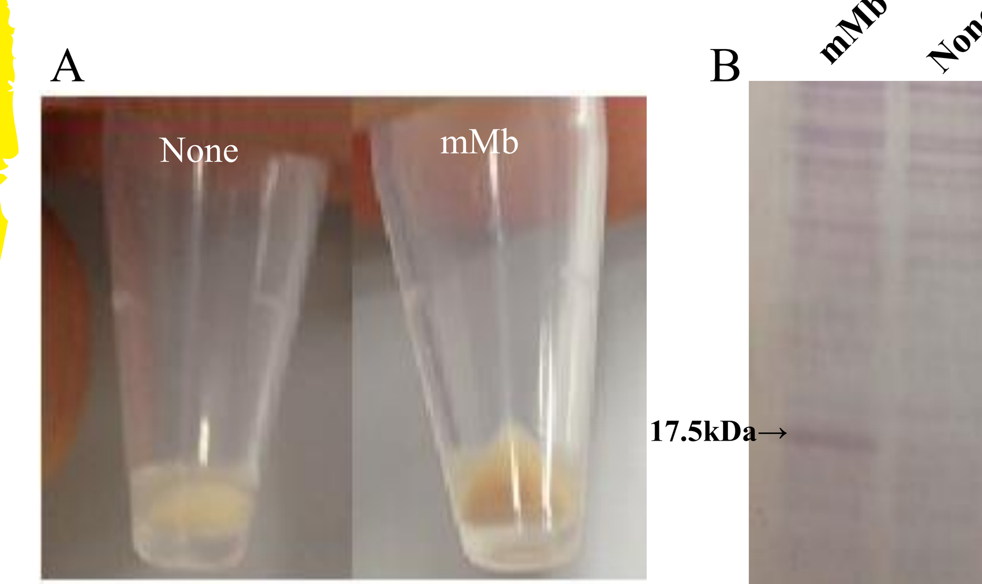

Fig. 7 A:
None; *E. coli* JM109 /pUC18
mMb; *E.coli* JM109/pUC18-mMb
The mMb pellet shows reddish color
B: SDS-PAGE
mMb; *E.coli* JM109/pUC18 -Mb
None; *E. coli* JM109/pUC18

F. T7 cassette

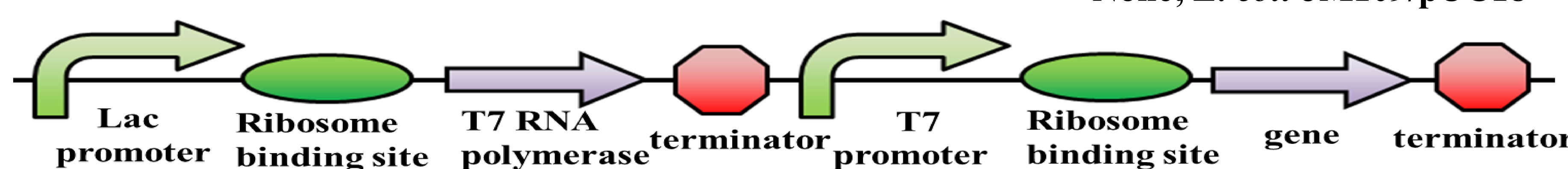

Fig. 8: Construct of T7 cassette

The pET system is the most famous system for massive protein production. But, the pET system relies on special strain of *E. coli* such as BL21 (DE3) encoding T7 RNA polymerase in genomic DNA. Because this cassette contains T7 RNA polymerase, usual strain of *E. coli* such as JM109 can produce massive protein after transformation of this plasmid encoding interested protein.

Future work

We have confirmed the production of glucans by agrobacteria. Because *CrdS* and *CelA-C* genes may be required for production of the glucans, the next planning is to transform agrobacteria with *CrdS* and *CelA-C* genes that were constructed as Biobrick by pBI107 shuttle vector resulting overproduction of cellulose and curdlan in agrobacteria as AgRePaper. After making the paper, activity of *CelC* protein, that may be cellulase, should be measured for recycling the AgRePaper. We are also planning to change myoglobin's color such as pink, green, purple, etc. The procedure for changing color of myoglobin has already been published (Ref: http://meat.tamu.edu/ansc-307-honors/meat-color/). We would like to use T7 cassette to produce myoglobin massively following to create Ecolink.

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