NOTEBOOK

BC Group（Bacteria cellulose）

Silks Reinforcement Experiment

4/23/2019

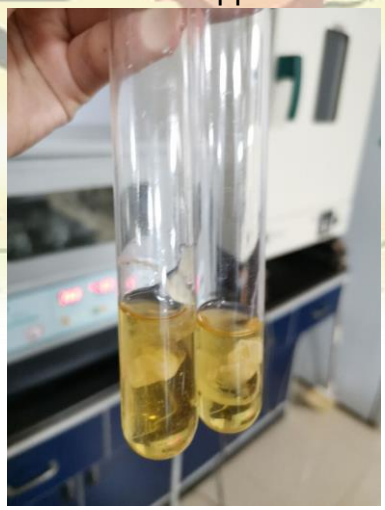
1. Explore the aging methods of silk (2x2cm small pieces):
   (1) Silk was irradiated by ultraviolet in the culture dish.
   (2) UV control group: the untreated silk was placed in a culture dish and kept away from light.
   (3) The silk was treated with NaOH in the culture dish.
   (4) The silk was treated with acetic acid in the culture dish.
   (5) Control group: the silk was treated with H2O in the culture dish.
2. Sterilized Gluconacetobacter. xylinus resuscitation medium and prepared for experiments.

4/24/2019

1. Silk (2x2cm small piece) aging: observed the results of the previous day and find that the effect is not good. Continue to explore best method of aging: follow the method used yesterday but increase the processing time.
2. Recovery of *G. xylinus.*

4/26/2019

1. Activated *G. xylinus.* White floccules appeared in *G. xylinus* test tube.



2. Continued to explore best method of aging silk. Tried to dye silk with Coomassie brilliant blue for better observation but failed.

4/27/2019

1. Activated *G. xylinus.*
2. Explored the methods of aging silk.

4/28/2019

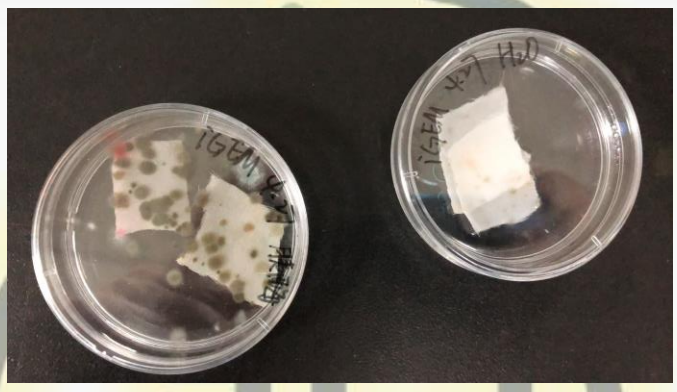
1. Activated *G. xylinus.* And white gelatinous substance increased.
2. Preliminary results have been obtained in exploring aging silk.

5/2/2019

1. *G. xylinus* had been activated for about one week. The culture had nearly turned to solid. Kept activating *G. xylinus* and subcultured.
2. Slope culture of *G. xylinus*.

5/3/2019

1. Aged silks became mildewed.



2. Thin biofilm formed on the slope culture of *G. xylinus.*

5/6/2019

1. Identified the Congo Red stained cellulose pellets produced by *G. xylinus.*

5/8/2019

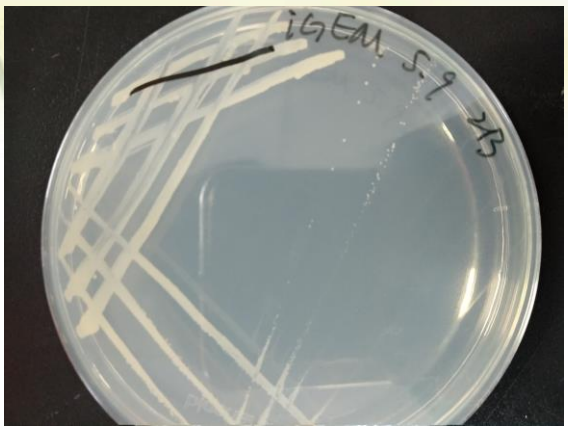
1. Streaked a plate to separate single colonies of *G. xylinus.*
2. Reinoculated *G. xylinus* into seed media and cultured for 3h. Diluted the media and streaked plates.

5/11/2019

1. The result of streaking plates were failed.

5/13/2019

1. Small colonies appeared on plates.



2. A little floc formed in the seed media.

5/14/2019

Keep researching the growth cycle of *G. xyllinus.* Inoculated a single colony into seed medium. Diluted the previous media and streaked plates.

5/15/2019

1. Small colonies appeared on plates.
2. Inoculated a single colony into liquid medium.

5/17/2019

Small colonies appeared on the plates of 5.14.

5/18/2019

1. Tried to Identify cellulose by Congo Red Staining. From left to right is the plate of *G. xylinus,* blank plate and Congo Red dye liquor.

We could use *Escherichia coli* as a control in later experiments.

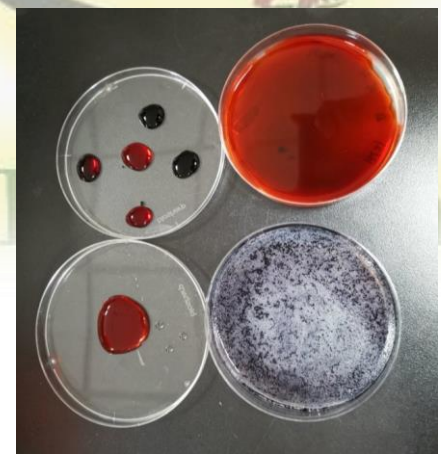
2. Small pellets appeared in the liquid media inoculated on May 16th.

5/20/2019

1. Inoculated *G. xylinus* on a slope.
2. We found the black Congo Red stained plate of *G. xylinus* could turn back to red by washing.

5/22/2019

1. We found the Congo Red stained plate showed black because the pH was lower than 7. The plate of *E. coli* showed red after being stained by Congo Red. This showed the plate of *G. xylinus* was acidic.



5/24/2019

The result of slope culture was successful.

6/17/2019

We tried to dry Bacterial Cellulose and found the dried cellulose will attached

to the wall of beaker tightly.

6/23/2019

Inoculated *G. xylinus* into seed media.

7/16/2019

Aged Silks for 4h, drying at 60℃, 1h and then sterilized silks.

7/17/2019

Inoculate *G. xylinus* to 50ml seed medium and cultured at 28℃, 150rpm, 72h.

7/18/2019

Ex-situ reparation:

(1) Negative control: dry and aged silk, sprayed solvent to soak.

(2) Positive control: none-aged silk, sprayed solvent to soak.

(3) Experimental group: three parallel of dry and aged silks, sprayed BC homogenate.

The immersed samples were cultured at 30℃ and 90% relative humidity for about 15 hours.

7/19/2019

1. Observed silks by microscope and test tensile strength.
2. Inoculate the colony of *G. xylinus* to seed medium.

7/20/2019

Transfer the *G. xylinus* suspension from seed medium to fermentation medium at a ratio of 1:10. Then cultured at 150rpm, 30℃ for 5h

In-situ reparation:

(1) Negative control: dry and aged silk, sprayed solvent to soak.

(2) Positive control: none-aged silk, sprayed solvent to soak.

(3) Experimental group: three parallel of dry and aged silks, sprayed *G. xylinus* suspension.

The immersed samples were cultured at 30℃ and 90% relative humidity for about 15 hours.

7/21/2019

Observed silks by microscope and test tensile strength.

7/22/2019

1. Observed repaired silks (totally dried) and test tensile strength again.
2. Inoculated *G. xylinus* to seed medium and cultured for 3 days. Then conserved.

9/6/2019

1. Silk Aging:

(1) Cut the plain colored silk into pieces of 2cm*5cm. And cut patterned silk into pieces of 4cm*4cm. Soak the pieces with 10% NaOH solution for 3 hours to age the silk.

(2) Discard NaOH solution and wash the silk with distilled water once. Add hydrochloric acid to adjust pH to 6.5. Wash twice with distilled water.

(3) Divide the aged silk pieces into three groups. Sterilize in an autoclave.

(4)Dry the pieces in 60℃ for 1 hour. Then dry them in the air.

2. Phenomena:

Aged silk shows faint yellow on the surface, and more roughness. In general, the silk gets more transmittance and stiffness. The fiber bundles have gotten loosen under an inverted microscope.

9/7/2019

1. Preparation of BC

(1) Shatter the commercial BC into homogenate in a blender.

(2) Heat to boil the homogenate and add 10% NaOH in the meantime. Boil the mix for 1 hour. Supply water to maintain the liquid level.

(3) Cool the mix and adjust the pH to 6.5.

2. Phenomena:

The suspension of BC is homogenous and shows transmittable white.

9/10/2019

1. Preparation of *G. xylinus* suspension:

(1) Inoculate *G. xylinus* into 5mL seed medium and culture for 3-4 days in 30℃, 150rpm, until cellulose pellets of a diameter of 1cm appear.

(2) Dilute the seed medium with the ratio of 1:10 and inoculate into fermentation medium. Culture at 30℃ for 4 hours.

2. Phenomena:

G. xylinus growed slowly. At the first day in seed medium, there was no obvious phenomena. Slight turbidity could be seen, but generally the medium was clear. On the second day, more flocculent precipitant appeared in the medium. White transparent pellets started to form. The pellets got bigger in size but less in quantity on the fourth day. 4 hours after inoculating into fermentation medium, a little floc formed.

9/12/2019-9/18/2019

1. Reinforcement:

(1) prepare sterilized plain colored and patterned silks separately, BC homogenate, suspension and distilled water.

In-situ reinforcement group: spray *G. xylinus* suspension to wet silks.
Ex-situ reinforcement group: spray BC homogenate to wet silks.
Aged group (negative control): spray distilled water
Unaged group (positive control): spray distilled water

(2) Culture for 6 days in 30℃. Supply corresponding liquid every two days to make sure the silks are wet.

(3) After reinforcement, wash silks with distilled water for 3-4 times.

(4) Dry the silks in 60℃ oven for 1 hour. Then dry totally in the air. Take out some samples and dry in oven for 3 hours for electron microscopic observation.

2. Phenomena:

(1) In in-situ reinforcement group, many membranous substances adhered to the surface. Washing could shed some of the substances.

(2) In ex-situ reinforcement group, less membranous substances were observed. There was a sour smell on the sample.

(3) All the reinforced samples showed a better water-holding ability and were more difficult to dry.

9/18/2019

1. Mechanical Strength Test (Universal Testing Machine)

(1) Prepare the silk and set up parameters: choose rubber clamp to fix the ends of silk and 2kN detector. Set the stretching speed at 10mm/min.

(2) Determine tensile strength and percentage of breaking elongation. Determine 6 sets of data in each group.

2. Phenomena:

Unaged silk was extremely hard to break and had a high percentage of breaking elongation.

Aged silk had a 1/10 tensile strength of unaged silk. The percentage of breaking elongation was also lower. But the breaking point percentage declined slower than tensile strength.

Reinforced silks showed better tensile strength. In-situ reinforcement had a better effect on tensile strength than ex-situ reinforcement. Both in-situ and ex-situ reinforcement silks showed higher percentage of breaking point than aged silk. The difference in breaking point percentage between in-situ and ex-situ reinforcement was not significant.

9/19/2019

1. Chromatic Aberration Analysis

Take photos on patterned silk. Observe with naked eyes and analyze with software.

2. Phenomena

Unaged silks showed more gloss. Aged, in-situ reinforced and ex-situ reinforced silks were fainter in color. In general, the difference was not significant.

9/20/2019

1. Electron microscopic observation

(1) Sample preparation: put the dry plain colored silk sample onto loading stage with conducting resin. Spray gold dust.

(2) Observation

2. Phenomena

(1) Unaged silk showed smoothness and tightness.

(2) On aged silk, damages could be seen. The connections between fibers were loose.

(3) On ex-situ reinforced silk, there were membranous or agglomerate substances wrapping protein fibers.

(4) On in-situ reinforced silk, less membranous and agglomerate substances could be seen. More substances showed fibrous, adhering to single cellulose fiber or between cellulose fibers.

BC Expression in *E.coli*

9/15/2019

Transfer EV#51 plasmids (pQE-80L plasmids containing cesA, cesB) and EV#84 plasmids (pBAD33 plasmids containing DGC) into DH5α competent cells separately.

9/16/2019

Preserve transformant of EV#51 and EV#84.

9/17/2019

Extract EV#51 plasmids.

9/18/2019

Transfer EV# 51 plasmids into DH5α strain containing EV#84 plasmids, yielding DH5α strain containing both EV#51 and EV#84 plasmids.

9/21/2019

Induce expression by adding IPTG to 0.4mM and L-arabinose to 0.2% (v/v).

9/22/2019

There was no obvious result after inducing DH5α.

Prolong induction time.

9/23/2019

There was still no obvious result in DH5α medium.

Transfer EV#51 plasmids into BL21(DE3) strain.

9/24/2019

There was still no obvious result in DH5α medium.

9/25/2019

Transfer the second plasmids (EV#84) into BL21(DE3) strain, yielding BL21(DE3) cells containing both EV#51 and EV#84.

9/27/2019

Induce the expression of bacterial cellulose in BL21(DE3) by adding 0.4mM IPTG and 0.2% L-arabinose.

9/28/2019

There was no obvious result after overnight induction.

Repeat the induction of BL21(DE3)

9/29/2019

No obvious result after induction.

10/3/2019

White solid appeared on the walls of conical flasks. We assumed that a sign of success induction.

10/4/2019

Prepare a flask of induced BL21(DE3) and a flask of uninduced BL21(DE3) separately.

10/5/2019

Prepare 3 flasks of induced BL21(DE3) and a flask of uninduced BL21(DE3) separately.

10/6/2019

The previous 4 flasks of induced BL21(DE3) medium appeared to produce white solid.

No solid observed in 2 flasks on negative control medium.

10/12/2019

Prepare anthrone reagent for the quantification of synthesized cellulose.

The resulting of absorbance in 620nm were extremely low. We assumed the dilution rate was too high.

10/14/2019

The first step of synthesized cellulose quantification was successful. The absorbance of an induced group was 0.749, with a negative control group 0.288.

10/15/2019

Inoculate 3 flasks of engineered E. coli BL21(DE3) strain and 3 flasks of G. xylinus. When the OD600 of E. coli reaches about 0.7, add IPTG and L-arabinose to induce the synthesis of cellulose. Culture both BL21(DE3) and G. xylinus in 28℃, 220rpm for 48 hours.

10/17/2019

Measure the bacteria cellulose concentration of induced BL21(DE3) and *G. xylinus* with anthrone method. We didn't know the ability of cellulose synthesis of *G. xylinus* and the measure result was out of the range of the spectrophotometer.

10/19/2019

Remeasure the cellulose concentration with the anthrone method. Change the dilution multiple of *G. xylinus*.

We got reasonable concentration of induced BL21(DE3), *G. xylinus* and negative control. According to the measurement, though the synthesis ability of *G. xylinus* was much higher than engineered BL21(DE3), the *E. coli* strain still had significant cellulose secreted.

Preserve 40mL of induced BL21(DE3) and 40mL of *G. xylinus* medium for the electron microscopy.

10/20/2019

Compare the ex-situ reinforcement ability of engineered *E. coli* and *G. xylinus*. Spray the medium of induced BL21(DE3) and *G. xylinus* onto silk pieces. Let stand for about 1 hour. Dry the pieces in the oven for 2 hours.

10/21/2019

Observe the surface of reinforced silk pieces with electron microscopy. The surface of silk pieces reinforced by *G. xylinus* had a much larger quantity of bacteria cellulose attached. However, the silk reinforced by engineered *E. coli* still had cellulose fibers attaching on the silk fibroins and connecting between silk fibroins.