NOTEBOOK

Functional Peptides Group

AMP Experiments (Antimicrobial Peptide)

6/24/2019

Got the strains of Aspergillus japonicus and Penicillium funiculosum, they were inoculated to PDA slant medium and recover under 30 degree after dissolved with 500ul ddH2O.

6/26/2019

The recovered fungi were transferred to new culture mediums as preserved strains.

6/28/2019

1.Pre-experiments to test the antimicrobial peptides. Tried the three methods in antifungal test:

(1) Dropwise: after the colonies' radius grew to 3cm, added 10ul tested solution (dissolved antimicrobial peptides) 0.3cm from the edge of the colony.

(2) Diffusing: dig a hole with a 0.3cm radius after the molds were inoculated and added 10ul tested solution into the hole.

(3) Diffusing after cultured: dig a hole with a 0.3cm radius 0,.5cm far from the edge of the colony after the colonies had grown to 3cm in radius.

2.Found a way to test antimicrobial ability on E.coli:

Filter paper test: plated the solution of *E.coli* (OD=0.6) on the LB plate and put a piece of filter paper soaked with tested solution.

PS: the tested solution used in pre-experiments contain 20ug/mL antimicrobial peptides metchnikowin2.

6/29/2019

1.Observed the result of the pre-experiments: no inhibition zone was spotted on the *E.coli* while the molds needed more time to grow.

2.Dissolved antimicrobial peptide magainin with different concentration: 160. 80. 40. 20. 10. 5ug/mL (dissolvent: LB liquid)

6/30/2019

1.Recovery of the glycerol strains (TOP10) containing ULP1 gene: plated them on LB plates containing kanamycin and culture at 37 degree.

2. Tested magainin's antimicrobial ability on *E.coli*: used the filter paper test in pre-experiments and change the concentration of the tested solution into 5\20\40\80\160ug/mL .

3. Tested metchnikowin2's antifungal ability on molds: used two methods in pre-experiments (dropwise and diffusing) and repeated the operation.

7/1/2019

1. Amplification of the ULP1 gene (in TOP10): chose three single colonies on the plate cultured for 1 day and transferred into 5mL LB liquid (contains kanamycin), cultured under 37 degrees and 220rpm.
2. Tested magainin's antimicrobial ability on *E.coli* strain BL21: used the same method as 6.30 to test the affection magainin has on *E.coli*, using ddH_2O as a contrast.
3. Tested magainin's antifungal ability on *Aspergillus japonicus*: used the diffusing method in pre-experiment and added filter paper test (put the filter paper soaked with tested solution 0.3cm far away from the edge of the colony, the colonies should have a radius at least 3cm when tested) to test the affection magainin has on the mold. ddH_2O is a contrast.

7/2/2019

1. ULP1(TOP10) preservation and extracted plasmids.
2. The result of the *E.coli* test (magainin): the groups that added magainin solution of different concentration have no difference with the contrast.
3. The result of the mold test (magainin): no obvious inhibition was spotted.
4. Tested magainin's antifungal ability on *Penicillium funiculosum*: used a new method—plate test (add the tested solution directly into the melted PDA culture medium and then make the plate and inoculate the molds) to test the affection magainin has towards *Penicillium funiculosum.*

7/3/2019

1. The result of the mold test made with *Penicillium funiculosumII*: an unsure inhibition was found on the plate.
2. Dissolved antimicrobial peptide magainin with different concentration: 160. 80. 40. 20. 10. 5ug/mL (dissolvent: ddH_2O)
3. Tested magainin's antimicrobial ability on *E.coli* strain BL21: with the same method we had used, the purpose was the find the solution's effect when solvent is water.
4. Tested magainin's antifungal ability on *Penicillium funiculosumII*: positive contrast: 10%NaOH solution, negative contrast: ddH_2O. experiment group: 1.6mg/mL magainin solution. Plated the tested solution on the PDA plate, divided the plate into 4 parts evenly and inoculated spores on each part.
5. Tested magainin's antifungal ability on *Aspergillus japonicus*: used the same method above and increased the concentration to 1.6mg/mL

7/4/2019

Double digested the ULP1 plasmid with BamHI and EcoRI.

7/5/2019

1.The result of the *E.coli* test (magainin) on 7.3: the situation around the filter paper is shown in the chart:

	ddH_2O	5ug/mL	20ug/mL	40ug/mL	80ug/mL	160ug/mL
1	Water	Water	Water	Water	Water ring and	Inhibition

	ring	ring	ring and a thin inhibition zone	ring	inhibition zone	zone
2	Water ring	Water ring	Water ring and a thin inhibition zone	Water ring and a thin inhibition zone	Inhibition zone (comparatively big)	Inhibition zone

PS: the water rings were caused by the diffusing of the water which took away some bacteria when the paper was added to the plate, so the bacteria growing there seems to be fewer.

2. Electrophoresis of the double digest sample: no target stripe was found on the gel.

7/7/2019

1. Tested magainin's antifungal ability on *Aspergillus japonicus*: new method: liquid culture test (make a mixture of 4.5mL culture medium, 0.5 tested solution and 50uL suspension of spores).

Used 1.6mg/mL magainin solution as experiment group, 10% NaOH solution as positive contrast and ddH_2O as negative contrast.

2. Preparation to make BL21 competent cell: inoculated BL21 into 5mL liquid culture medium and cultured under 37 degree overnight.

3. Double digested ULP1 plasmid with BamHI and EcoRI.

4. Extracted ULP1 plasmids.

7/8/2019

1. Electrophoresis of the double digest sample: got the target band.

2. Made competent BL21 cells.

3. Transferred the ULP1 plasmids into BL21 competent cells.

7/9/2019

1. Dissolved antimicrobial peptide metchnikowin2 with different concentration: 160. 80. 40. 20. 10. 5ug/mL (dissolvent: ddH_2O).

2. Tested metchnikowin2's antimicrobial ability on *E.coli*: use the same method above, adding a group with 10% NaOH solution as a positive contrast.

3. Tested metchnikowin2's antifungal ability on *Aspergillus japonicus*: use the same method above, adding a group with 10% NaOH solution as a positive contrast.

7/10/2019

1. Chose three single colonies on the transforming plate and transferred them into 5mL LB liquid (containing kanamycin 50μg/ml) to amplify the plasmids, then cultured under 37 degrees and 220rpm.

2. Used the amplified BL21 to extract plasmids and did electrophoresis: the plasmid was found in the bacteria, which meant success in transformation.

3. The result of the *E.coli* test (metchnikowin2) on 7.9: the positive group showed an obvious inhibition while the situation of the experiment group was

hard to judge because of uneven plating.
4. Preparation for the induced expression of ULP1: inoculated BL21(containing Ulp1 plasmid) into 5mL liquid culture medium and cultured under 37 degree overnight.

7/11/2019

1. The result of the mold test (metchnikowin2) on 7.9: the positive group showed an obvious inhibition while the experiment group showed no evident difference comparing with negative contrast.
2. Induced expression of ULP1: added the bacteria fluid prepared the last day into 150mL LB liquid (contain kanamycin) and continued culturing till the OD (at 600nm) reached 0.6. Preserved some fluid as the 'uninduced sample', and added IPTG to the rest to start inducing. After 2.5 hours, got another sample as 'induced for 2.5h', the induction ends 4.5 hours after its start. Centrifuged to collect the bacteria in the samples and purify the protein with Ni column later.
3. Used the three samples to do SDS-PAGE.
4. Recovery of the MJ-2(BL21): plate on the LB medium with chloromycetin and cultured under 37°C.

7/12/2019

ULP1 protein purifying, SDS-PAGE and Coomassie Brilliant Blue dyeing.

7/13/2019

1. Got the result of SDS-PAGE.
2. Amplification of the MJ-2(BL21): chose three single colonies on the plate cultured for 1 day and transfer into 5mL LB liquid (contains chloromycetin), cultured under 37 degrees and 220rpm.
3. Extracted MJ-2 plasmids and do electrophoresis to examine.

7/14/2019

Preparation for the induced expression of ULP1.

7/15/2019

1. Induced expression of ULP1.
2. Recovery of the Magainin (TOP10), metchnikowin2 (TOP10): plated on the LB plate with chloromycetin and cultured under 37°C.
3. Double digested the MJ-2 plasmids and do electrophoresis.

7/16/2019

1. Purification of the ULP1 protein: used Ni column to purify the sample and did SDS-PAGE.
2. Preparation for making competent DH5α cell: inoculated DH5α into 5mL liquid culture medium and cultured under 37 degree overnight.

7/17/2019

1. Result of the ULP1 purification: failed. No target band was found in the eluent.
2. Amplification of the metchnikowin2 (TOP10): chose three single colonies on the plate cultured for 1 day and transferred into 5mL LB liquid (contains chloromycetin), then cultured under 37 degrees and 220rpm.
3. Made DH5α competent cells.
4. Transformed the pET-28a vector into DH5α to amplify the vector.

5. Extracted metchnikowin2(TOP10) plasmids and did electrophoresis to examine.

7/18/2019

1. Recovery of the Magainin (TOP10)
2. Amplification of the pET-28a (DH5α)
3. PCR: used PCR to get the sequence containing MJ-2, metchnikowin2, magainin genes from the vectors.
4. Tried purifying the ULP1 protein again with the rest sample from 7.17

7/19/2019

Did SDS-PAGE with the new samples made the last day.

7/22/2019

Linearized the vector pET-28a with double digestion using BamHI and EcoRI.

7/24/2019

Preparation for the induced expression of ULP1

7/25/2019

1. Induced expression of ULP1: set three concentration of IPTG: 0.1mM, 0.5mM, 1mM. Got 4 samples in each group at time 1h, 2h, 3h and 4h, each have 20mL bacteria fluid.
2. Did SDS-PAGE with the samples.

7/26/2019

1. Got the result of the SDS-PAGE.
2. Purified the samples with Ni column.

7/27/2019

1. Did SDS-PAGE with the purified samples.
2. PCR: used PCR to get the sequence containing MJ-2, metchnikowin2, magainin genes from the vector SB1C3.
3. Linearized the vector pET-28a with double digestion using BamHI and EcoRI.

9/4/2019

Transformed plasmids containing PgD5 and NaD2 genes into BL21 and cultured overnight.

9/5/2019

Amplified the colonies of PgD5 and NaD2 by culturing in LB. Then used IPTG (0.4mM) to induce the production of the two peptides (16°C).

9/6/2019

Collected the bacteria into 50mL EP tubes by centrifuge after 21 hours' induction. Put into -20°C overnight.

9/7/2019

Dissolved the bacteria with 15mL lysis buffer and break their cell wall with ultrasonic breaking machine. Used Ni column to purify the supernatant of the fluid to get samples and then examine by SDS-PAGE. Added glycerol to the elution sample at a 10% ratio. Stored the samples at -80°C after frozen with liquid Nitrogen.

9/8/2019

Recovered the molds to prepare for the plate culturing experiment of the AMPs

Tris-tricine SDS-PAGE

9/9/2019

Transformed the vector pGEX6P-1 into JM109 and culture overnight.
Transformed NaD2 and PgD5 plasmids into BL21 and culture overnight.

9/10/2019

Induced expression of NaD2 and PgD5 (0.4mM IPTG, 16°C).
Amplified pGEX6P-1 in LB.

9/11/2019

Extracted plasmid pGEX6P-1 and digest with BamHI and EcoRI.
Digested NaD2 and PgD5 gene with BamHI and EcoRI.
Collected the bacteria induced for 22h by centrifuge and store overnight at -20°C.
Observed the antifungal experiment.
Inoculated Aspergillus japonicus.

9/12/2019-9/15/2019

Extracted plasmids containing PgD5 and NaD2 genes and used the restriction enzymes and T4 DNA ligase to insert the genes into the vector pGEX6P-1.

9/12/2019

Examined the digested product and the induced product with electrophoresis
Digested again.

9/13/2019

Extracted, digested and examined the genes and vector again.
Regained the product from the gel.
Western blot the purified product from 9.12.
Linked the PgD5 gene and vector pGEX6P-1 and transformed it into JM109.

9/14/2019

Linked the NaD2 gene and vector pGEX6P-1 and transformed it into JM109
Amplified GST-PgD5 (JM109) and extracted plasmids.

9/15/2019

Amplified GST-NaD2 (JM109) and extract plasmids.
Double digested the GST-PgD5 and examine the product.
Transformed GST-PgD5 and GST-NaD2 into BL21.

9/16/2019-9/23/2019

Linked the PgD5-avitag gene with the vector pGEX6P-1 (and sent to sequencing).

9/16/2019

Induced the expression of GST-PgD5 and GST-NaD2

9/17/2019

Collected the induced bacteria and stored overnight.

9/18/2019

Used GST column to purify the proteins in collected bacteria and do SDS-PAGE.
Transformed pGEX-PPase into JM109 to amplify.

9/19/2019

Extracted pGEX-PPase plasmid.

9/19/2019-9/26/2019
Linked the NaD2-avitag gene with the vector pGEX6P-1 (and sent to sequencing).
9/21/2019
Transformed pGEX-PPase into BL21.
9/22/2019
Induced the expression of PPase.
9/23/2019
Collected the induced bacteria and store overnight.
Transformed GST-PgD5 and GST-NaD2 into BL21.
9/24/2019
Induced the expression of GST-PgD5 and GST-NaD2.
Purified the proteins in the collected bacteria and examined by SDS-PAGE.
9/25/2019
Collected the induced bacteria and stored overnight.
9/26/2019
Purified the proteins in the collected bacteria and digested the product with the purified PPase.
9/27/2019
Chose some more colonies and send to sequence.
Collected the digested product and did SDS-PAGE.
9/28/2019
Transformed GST-PgD5, GST-NaD2 and GST-PgD5-avitag into BL21.
9/29/2019
Induced the expression of GST-PgD5, GST-NaD2 and GST-PgD5-avitag.
9/30/2019
Collected the induced bacteria and stored overnight.
10/1/2019
Used GST column to purify the proteins in collected bacteria and did SDS-PAGE.
Reloaded the purified fused protein GST-PgD5 and GST-PgD5-avitag to the column and digested for 16h.
10/2/2019
Collected the digested product and did SDS-PAGE.
Reloaded the passing fluid fused protein GST-PgD5 and GST-PgD5-avitag to the column and digested for 16h.
10/3/2019
Collected the digested product and did SDS-PAGE.
Reloaded the passing fluid fused protein GST-PgD5 and GST-PgD5-avitag to the column and digested for 16h.
10/4/2019
Collected the digested product and did SDS-PAGE.
Reloaded the passing fluid fused protein GST-PgD5 and GST-PgD5-avitag to the column and digested for 16h.

10/5/2019
Collected the digested product and did SDS-PAGE.
Reloaded the passing fluid fused protein GST-PgD5 and GST-PgD5-avitag to the column and digested for 16, 20, 24 and 28h.
10/6/2019
Collected the digested product and did SDS-PAGE.
Transformed GST-PgD5 and GST-PgD5-avitag into BL21.
10/7/2019
Induced the expression of GST-PgD5 and GST-PgD5-avitag.
10/8/2019
Collected the induced bacteria and stored overnight.
Used GST column to purify the proteins in collected bacteria and digested.
10/9/2019
Collected the digested product and did SDS-PAGE.
10/10/2019
Transformed GST-PgD5, PgD5, PgD5-avitag and GST-PgD5-avitag into BL21.
10/11/2019
Induced the expression of GST-PgD5-avitag and PgD5.
Transformed GST-PgD5 and PgD5-avitag into BL21.
10/12/2019
Collected the induced bacteria and stored overnight.
Examined the product from 10.8 by silver staining.
Induced the expression of GST-PgD5 and PgD5-avitag.
10/13/2019
Collected the induced bacteria and stored overnight.
Purified the proteins in induced bacteria with according column and did SDS-PAGE.
10/14/2019
Collected the induced bacteria and stored overnight.
Concentrated the purified proteins from 10.8 and did SDS-PAGE.
Inoculated Aspergillus japonicus.
10/15/2019
Observed the result of the SDS-PAGE.
Tested the inhibition effect of the PgD5 on the molds.

Verification of Light System

10/2/2019

Inoculated the colony containing blue-light system's genes to a new plate to amplify and recover the bacteria (including 4 strains: prototype, YF2, tac2, T7c) .

10/3/2019

1. Inoculated the 4 strains of the blue-light system into 10ml LB (with kanamycin), set the positive and negative contrast, culture at 37°C to prepare for light induced expression.
2. Extracted the plasmid of red-light system to prepare for link

10/4/2019

1. Light induction experiment: Divided the 10ml bacteria fluid into two equal parts, one was put under the blue light while the other one was kept in dark (by wrap it up with silver paper), both of them were put into incubator to culture overnight.
2. Plate culturing experiment: Plated 150ul bacteria fluid from two kinds of the experiment groups (light and dark), used silver paper to wrap up the culture plate and cut some special shapes to let the light goes through, expecting the bacteria will turn green at the place exposed to light.

10/5/2019

1. Result of the plate culture: no difference was found between the two groups.
2. Inoculated the prototype and YF2 strains and did the light and dark experiment again
3. Repeated the plate culturing experiment, changing silver paper into newspaper (to reduce the reflection of the light).

10/6/2019

1. Result of the light induction experiment (10.5): difference cannot be found with naked eyes. Collected 1ml sample from each test tube with samples. Got the lysis of the samples with the ultrasonic lysate machine.
2. Did the plate culturing experiment again with the prototype and YF2 strains, wrapped with newspaper.

10/7/2019

1. The result of the plate culturing was not ideal; no special shape can be recognized on the plate. Considering the newspaper was too thin to keep the light out, so a silver paper layer was added in the repeated experiment
2. Measured the florescence intensity of the samples from the light induction experiment, the paralleled groups were not in good condition, so the experiment was repeated (this time added tac2 and T7c strains).

10/8/2019

Checked the result of the light induction experiment (10.7) and found T7c didn't

become green.

10/9/2017

Inoculated the prototype into culture medium.

10/10/2017

Did the light induction experiment with the prototype

10/11/2017

1. Measured the florescence intensity of the bacteria lysis and found that there was little difference between the data. The leakage of the prototype blue-light system was severe.
2. Began culturing YF2 strain.

10/12/2019

1. Light induction for YF2.
2. Inoculated tac.

10/13/2019

1. Measured florescence intensity, and found that YF2 strain did well in controlling the expression of GFP.
2. Light induction of the tac2 strain.
3. Inoculated the strain containing red-light system

10/14/2019

1. According to the florescence intensity, tac2 can also control the expression well, but not as good as YF2.
2. Transformed the red-light system plasmid (pCCK-1) to BL21.

10/15/2019

Began the light induction of the newly transformed BL21 strain.

10/16/2019

1. Collected sample for the first time, no difference in florescence intensity between the two groups.
2. Collected sample for the second time, no difference in florescence intensity between the two groups.
3. Made a plate culturing experiment adding a column made with card to limit the light's path in order to reduce the diffraction of the light.

10/17/2019

1. Collected sample for the third time, no difference in florescence intensity between the two groups
2. Began light induction of the red-light system on another vector: pBSc
3. Did SDS-PAGE to see if GFP had been produced by the bacteria induced by the red light.

10/18/2019

1. Result of the light induction: no obvious difference had been found.
2. Plate culturing experiment observation: couldn't see obvious difference in color under microscope.

Verification of pR+dlacO Experiment

8/15/2019
Recovery of TOP10 with synthesized pR or pR+dlacO plasmids.
8/16/2019
Inoculated a single colony of TOP10 strain containing pR or pR+dlacO into 5mL liquid medium.
8/17/2019
Preserved the strain in 4 degree.
8/29/2019
Built linearized plasmid pet-28a without T7 promoter by PCR.
8/30/2019
Added homology arms to target genes (pR+sfGFP, pR+dlacO+sfGFP) by PCR.
9/2/2019
Recombined the target genes with linearized vectors by Gibson assembly.
9/4/2019
Transformed the recombined plasmids into DH5α.
9/6/2019
Extracted plasmids from transformed colonies and examined by PCR and sequencing.
9/7/ 2019
Electrophoresed the PCR products but the results showed no plasmid correct.
9/9/2019
Extracted plasmids from other plates of transferred colonies.
9/11/2019
Did same things as 9/5 and 9/6.
9/16/2019 to 9/20/2019
Did same things as 9/2 to 9/6, but target bands appeared.
9/23/2019 to 9/25/2019
Sequencing but the results showed empty vectors.
9/25/2019
Modified primers with new homology arms.
9/27/2019 to 10/1/2019
Did same things as 8/30 to 9/4.
10/8/2019
Sequencing analysis.
10/12/2019
Measured fluorescence intensity.