Personal Notebook of Simen Boxuan Zhao

7.4-7.11

1. PCR and Subcloning of Lpp-OmpA-MBP-MerR (LOM-MerR):
Collaboration with Junyi Jiao.

2. Promoter Analysis and Alignment of Other Proteins in Family
2-1. Search NCBI for wild type promoter for PbrR, CueR and CupR respectively.
2-2. Amino acid sequence alignment of proteins for similarity analysis.

7.12-7.18

1. Subcloning of LOM-MerR into commercial plasmid (with His-tag for localization):
Collaboration with Junyi Jiao.

2. Structure Prediction and Design of Lead Bioabsorbent:
2-1. Structure prediction and domain functional analysis of PbrR based on alignment with MerR.
2-2. MBP-PbrR design and primer design for the construction of PbrR.

7.19-7.25

1. LOM-MerR Expression:
1-1. Transform pET21a/pSB1a3-LOM-MerR into BL21(DE3), plating, overnight culture.
2-2. Pick single colony, amplify culture overnight.
2-3. Secondary amplification, grow until OD600=~0.6, add 1mM IPTG, expression 5h at 30℃.
2-4. Run SDS-PAGE, verify protein expression.

7.26-8.1

1. LOM-MerR Expression Optimization:
1-1. Transform pET21a/pSB1a3-LOM-MerR into BL21(DE3), plating, overnight culture.
2-2. Pick single colony, amplify culture overnight.
2-3. Secondary amplification, grow until OD600=~0.6, add 1mM IPTG, expression 24h at 16℃.
2-4. Run SDS-PAGE, verify protein expression.

2. Functional Test of Mercury Bioabsorbent:
Dithizone Assay:
Collaboration with Junyi Jiao.

8.2-8.15

1. Localization of Lpp-OmpA-MBP-MerR:
Western Blotting
Collaboration with Xin Teng.

8.16-9.5

1. Functional Test of Mercury Bioabsorbent:
ICP-AES Sample Preparation:

1-1. Grow 10mL E.coli to OD600=0.6
1-2. +1mM IPTG, transfer to 30°C, 30min.
1-3. +10 uM HgCl2, 30°C overnight expression.
1-4. Centrifuge and collect 10mL bacteria at 12000rpm, discard the medium, wash the pellet with ddH2O for a few times, collect by centrifugation.
1-5. Add 3 mL fuming nitric acid, heat at 65°C for 4h. Wait till NO_2 complete release.
1-6. Freeze-dry the sample, measure the weight of bacteria pellet.
1-7. Resuspend sample with 5mL 2% nitric acid, send for inspection.

9.6-9.12
1. Expression and Localization of Lpp-OmpA-MBP-PbrR (LOM-P):
1-1. Transform pET21a-LOM-P into BL21(DE3), plating, overnight culture.
1-2. Pick single colony, amplify culture overnight.
1-3. Secondary amplification, grow until OD600=0.9, add 1mM IPTG, expression overnight.
1-4. Collect bacteria from 1mL medium. Lyse with Bugbuster (Novagen, USA) according to manufacturer's protocol.
1-5. Perform high speed centrifugation to separate membrane protein with cytosolic protein.
1-6. Add 4×sample buffer, boil at 95°C for 5 min.
1-7. Run SDS-PAGE and western blotting. Verify protein expression and localization.

9.13-9.17
1. Expression of Mercury and Lead Bioabsorbent:
1-1. Transformation, plating, picking and culturing BL21(DE3) expressing LOM-PbrR or LOM-MerR respectively.
1-2. First and secondary amplification, grow to OD600=0.63, add 1mL IPTG, transfer to 30°C, culturing 30min.
1-3. Add different amounts of mercury/lead: 0, 0.1 uM, 1uM, 10uM, respectively. Overnight expression at 30°C.

9.18-9.25
1. Functional Test of Mercury Bioabsorbent LOM-MerR:
1-1. Protein expression according to previous protocol. Amplify bacteria to OD600=0.75, add 1mM IPTG, transfer to 30°C, culturing 30min.
1-2. Divide bacteria into 100mL aliquots, add different amount of mercury. (0, 0.01uM, 0.1uM, 1uM) Overnight expression at 25 °C.

2. ICP-AES Measurement:
2-1. Sample preparation according to previous protocol. Collect bacteria, wash for 4 times, freeze dry overnight.
2-2. Precise weighing bacteria pellet in digestion tube. Resuspend with 8mL fuming nitric acid. Microwave digestion.
2-3. Resuspend sample to 25mL with water. ICP-AES measurement for three parallel times.

9.26-10.2

1. Functional Test of Mercury Bioabsorbent MBP+DsbA-MBP+Lpp-OmpA-MBP-MerR (PML-MerR):
1-1. Protein expression according to previous protocol. Amplify bacteria to OD600=0.5, add 1mM IPTG, transfer to 30°C, culturing 30min.
1-2. Divide bacteria into 100mL aliquots, add different amount of mercury. (0, 0.01uM, 1uM) 24 h expression at 37 °C.
1-3. ICP-AES sample preparation according to previous protocol. Collect bacteria, wash for 4 times, freeze dry overnight. Digest with fuming nitric acid.

10.3-10.12
Full Range Functional Test of Mercury Bioabsorbent (MBP, DsbA-MBP, LOM, PML-MerR):
1. Protein expression according to previous protocol:
1-1. PML-MerR: Amplify bacteria to OD600=0.6, add 1mM IPTG, transfer to 30°C, culturing 30min. Then add different amount of mercury. (0, 0.1uM, 1uM, 10uM) ~40h expression at 30°C.
1-2. MBP, DsbA-MBP, LOM-MerR: Amplify bacteria to OD600=~1, add 1mM IPTG, transfer to 30°C, culturing 30min. Then add 10uM mercury. ~40h expression at 30°C.
1-3. Blank-1: Add 1mM IPTG only. Blank-2: Add 1mM IPTG and 10uM mercury.

2. ICP-AES Measurement:
2-1. Sample preparation according to previous protocol. Collect bacteria, wash for several times, freeze dry overnight.
2-2. Precise weighing bacteria pellet in digestion tube. Resuspend with 8mL fuming nitric acid. Microwave digestion.
2-3. Resuspend sample to 10.00mL with water. ICP-AES measurement for three parallel times.

10.13-10.19
1. Synthesis of Organic Heavy Metal Indicator TritonX-100-PAN-S (TPS):
1-1. Mix 0.2g PAN with 5mL sulfuric acid in a 50mL beaker. Stirring reaction overnight at room temperature.
1-2. Add excessive ethyl ether into reaction mixture, perform suction filtration, wash with acetone and water for several times.
1-3. Collect crude product on the filter paper. Parching overnight on watch glass at 100°C. Collect final product PAN-S.
1-4. Mix 1mg PAN-S with 20mg TritonX-100 and 1mL ddH_2O, dissolve thoroughly to get final working solution with orange color. Store final working solution at room temperature.

2. Characterization of Organic Heavy Metal Indicator TritonX-100-PAN-S:
2-1. pH and concentration titration: Add TPS into different pH solution at different mercury concentration to decide proper color transition point. Result shows at pH=7-8, the lower limit of color transition metal concentration is 0.8×10^{-5}M. Color changes from rosy color to bright yellow.

3. Direct Visualization of Mercury Absorbent Function:
3-1. Culturing bacteria with PML-MerR and constitutive promoter overnight.

3-2. Prepare three groups of solution: A: 500uL PBS buffer +10uL TPS +10uM mercury + bacteria pellet (collect from 500uL medium); B: 500uL PBS buffer +10uL TPS +10uM mercury; C: 500uL PBS buffer +10uL TPS.
3-3. 37°C culturing for 1h. After centrifugation, collect upper solution to compare the color change.
3-4. Similar results repeated in HEPES buffer at pH=~8. Pictures taken for view.

10.20-10.26
Full Range Functional Test of Lead Bioabsorbent (MBP, DsbA-MBP, LOM, PML-PbrR):
1. Protein expression according to previous protocol:
1-1. PML-MerR: Amplify bacteria to OD600=0.6, add 1mM IPTG, transfer to 30°C, culturing 30min. Then add different amount of lead. (0, 0.1uM, 1uM, 10uM) ~40h expression at 30°C.
1-2. MBP, DsbA-MBP, LOM-MerR: Amplify bacteria to OD600=~1, add 1mM IPTG, transfer to 30°C, culturing 30min. Then add 10uM lead. ~40h expression at 30°C.
1-3. Blank-1: Add 1mM IPTG only. Blank-2: Add 1mM IPTG and 10uM lead.