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Biochar: Production, Characterization and Applications

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Developing biosensors as monitoring tools to assess the performance of biochar amended contaminated soil

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Developing Biosensors as Monitoring Tools to Assess the Performance of Biochar-Amended Contaminated Soil

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- **Introduction (Background and Reviews)**
- **Objectives**
- **Research Methods**
- **Results and Discussion**
- **Conclusion**

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BACKGROUND

Inspiration

- **Biochar-Amended contaminated soil for soil restoration purpose**

Biochar

Contaminated Soils → **Biochar Amended Contaminated Soil Complex** → **Treated Soil** → **Reuse**

- **Bioavailability monitoring is required to assess the risk (toxicity) of soil contaminant to living organisms**
- It is a complementary tool to chemical or physical analysis

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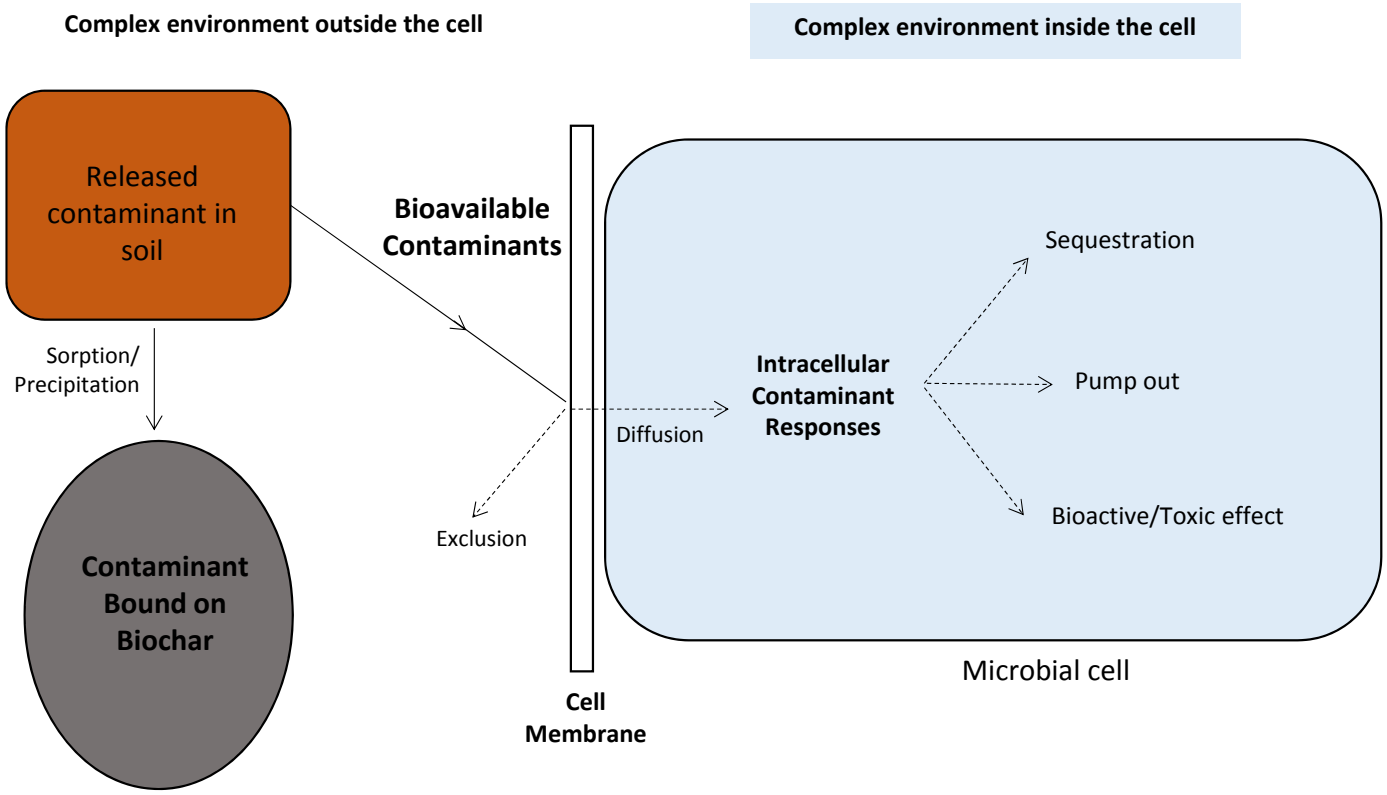
Interaction of Soil Contaminants, Biochar and Soil Microorganisms

- **Interaction of Contaminant, Soil, Biochar and Microbial cell is in a complex manner**

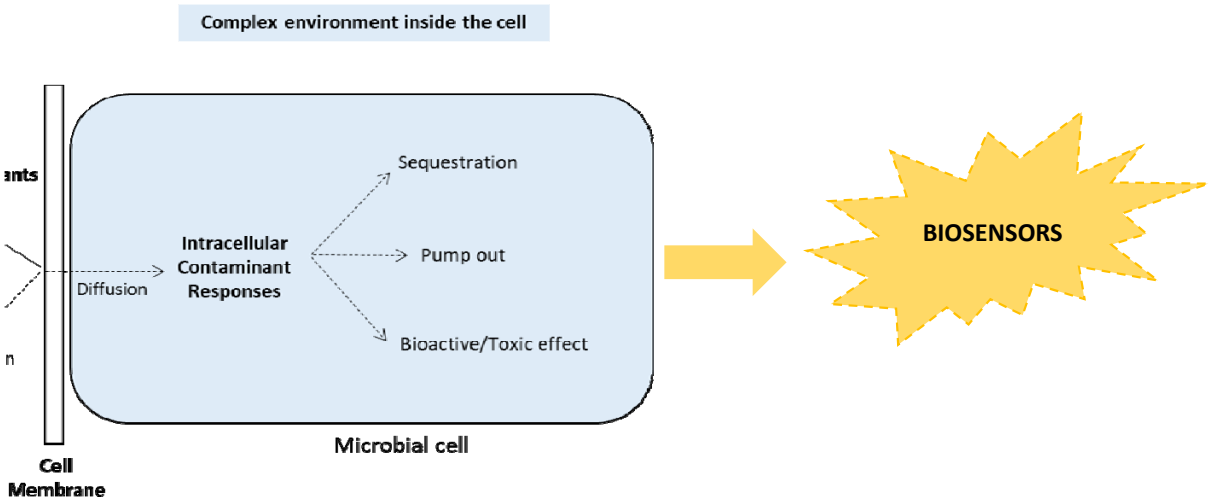
- **Bioavailable contaminants : fraction of contaminant which is available for living cell**

The increase of contaminant adsorption by biochar will reduce the bioavailable contaminant to living cell

How to measure?



Attractiveness of Biosensors



- **Measure the complex interaction of bioavailable contaminant inside the cell directly**

How ?

- ✓ **Exploit the intracellular response towards contaminants**
  e.g Metal binding protein for sequestration : **Metallothioneins**
- ✓ **Utilisation of reporter proteins : Fluorescent proteins**

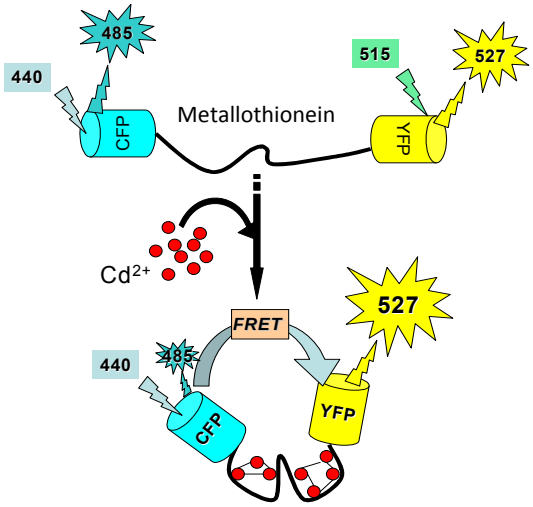
- Biosensors allow us to see the <u>natural process of biological response</u> and <u>report what is happening inside the cell</u>

Biosensors integrate: **Metal binding protein (Metallothioneins)** + **Reporter proteins** → **Signals**

❖ Foster Resonance Energy Transfer (FRET) Biosensor

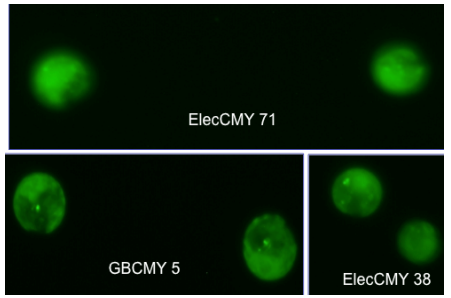
- ✓ Fusion of Metallothionein (MT) with two pairs of donor-acceptor Fluorescent proteins (FPs).
- ✓ The binding of HMs with MT will change the molecular structure of donor-acceptor FPs.
- ✓ This change alters the distance between FPs leading to the increase of light emission intensity (Carter, et.al, 2014).

- **Main advantage : fast response (read out in seconds)**
- **FRET is attractive but has not been widely used for environmental purpose**



❖ Rajamani, et.al, 2014 has developed FRET biosensor to measure the intracellular Cd^{2+}, Pb^{2+}, and Zn^{2+} inside the *Chlamydomonas reinhardtii* cells.

Fig.4 and 5. Model of FRET Biosensor as a fusion of **chicken metallothionein** with **CFP** (donor FP) and **YFP** (acceptor FP) for monitoring of intracellular ion metals (Rajamani, et.al, 2014).



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OBJECTIVES

Inspiration

1. To develop biosensors that exploit the capability of cellular responsive mechanism for HMs (e.g Metallothionein) to measure the bioavailable HMs

2. To apply the biosensors as monitoring tools to determine the effect of biochar amendment on microbial function in HMs contaminated soils

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RESEARCH METHODS

Inspiration

Determine the type of contaminants in soil (HMs)

e.g: Cd, Pb, Zn

Determine the cellular responsive mechanism

e.g: Metallothionein

Lab Test
Construction of gene encoding biosensor inside the soil bacteria (host cells)

Cloning of gene encoding Metallothionein and FRET

Field Test
Biosensor application in biochar-amended contaminated soil

Test of Robustness and Sensitivity

Plots of soil with various conditions :
- **Different types of soil**
- **Various concentration of HMs and PAHs**
- **Various biochar addition**

Assessment of Biochar Performance
Integrate the biosensor analysis with plant-bioavailable contaminants

Monitoring for decision making:
- Bioavailable contaminant
- Soil microbial function
- Plant growth

RESEARCH METHODS

Lab Test
Construction of gene encoding biosensor

❖ FRET Sensor construction

Elements:
1. Metallothioneins gene
2. Donor-Acceptor FPs gene
3. Plasmid (vector) with constitutive promoter
4. Host cells

Cloning approach:
Gateway Recombination Method (Hartley, et.al, 2000)

Biosensor Protein synthesis in the host cells

Biochar amended contaminated soil samples

Mix of soil sample and biosensor cells

Fluoresence Reading

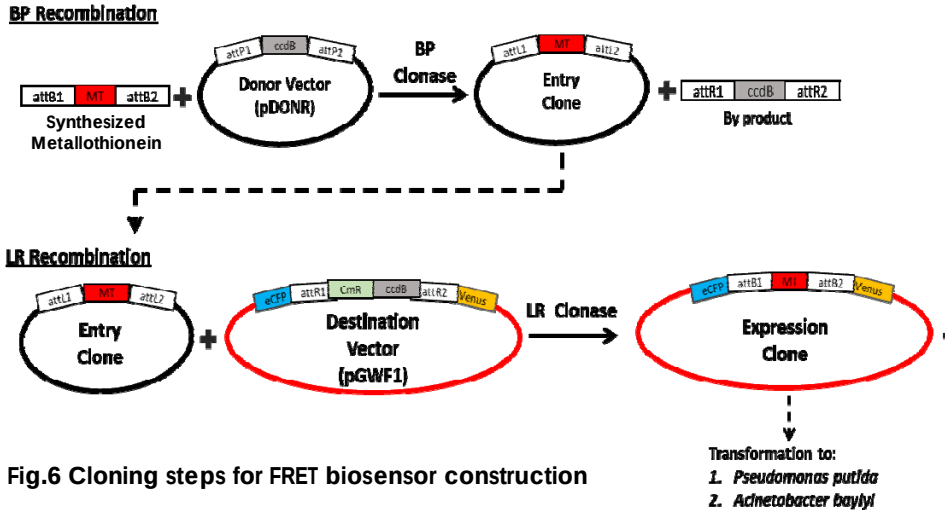
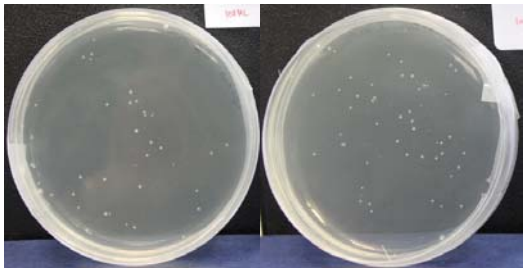


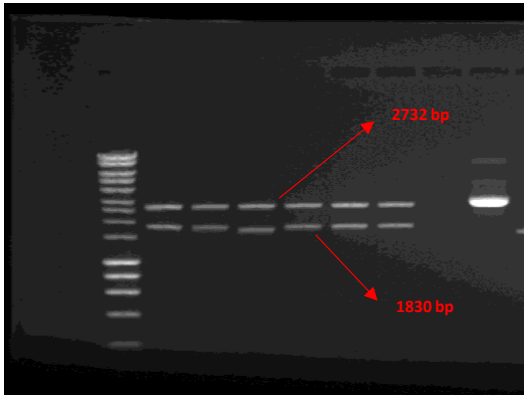
Fig.6 Cloning steps for FRET biosensor construction

RESULTS

- **Constructed gene encoding biosensor will be synthesized inside the host cell**
- **Signals produced when the intracellular heavy metals are present**

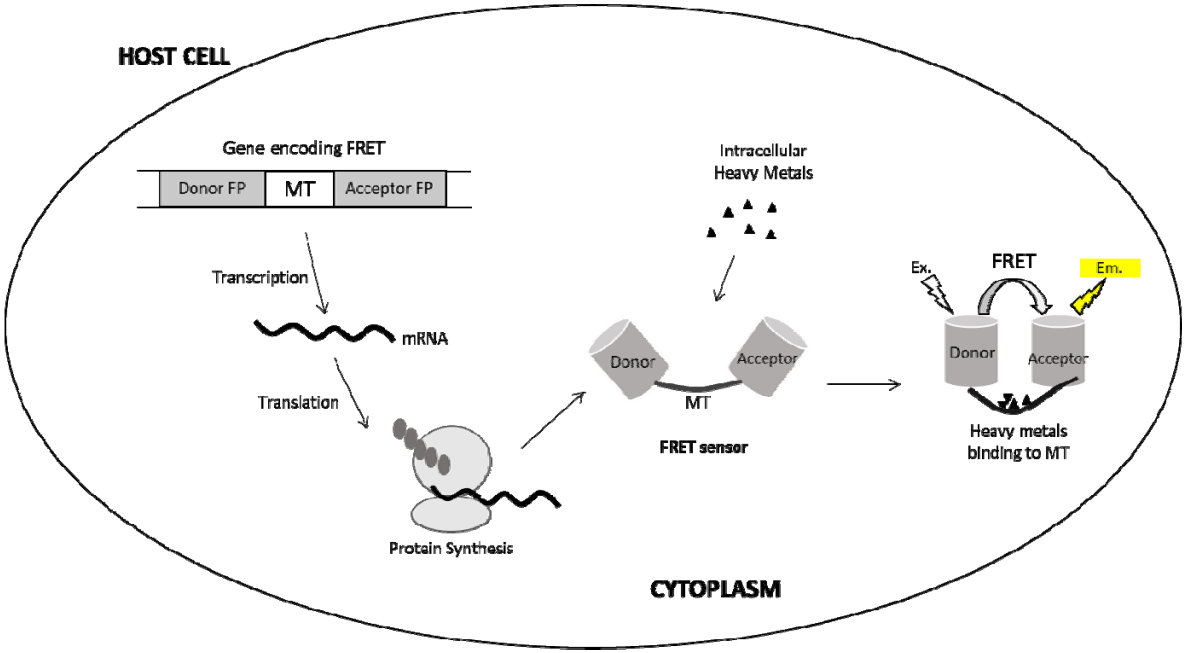
Transformant cell containing Entry Clone (LB+Kan)





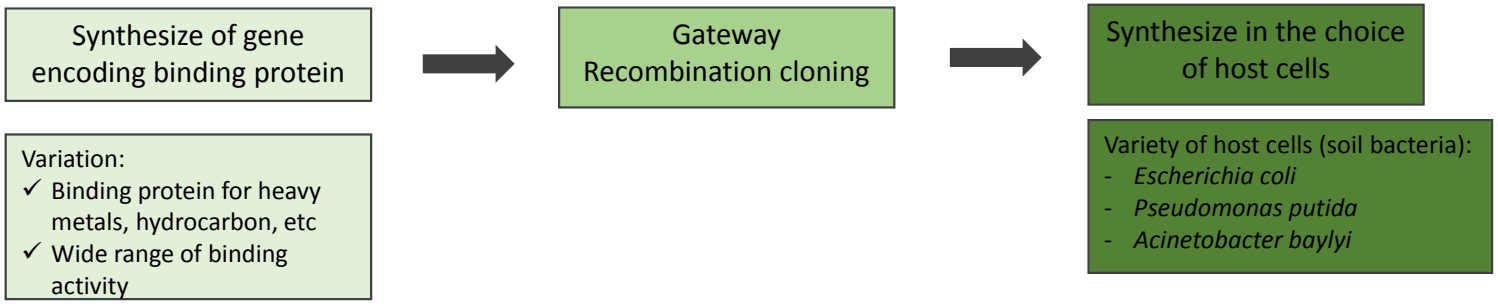
Gel electrophoresis results of Expression clone

Fig.7 Model of FRET sensor synthesis inside the host cell



DISCUSSION

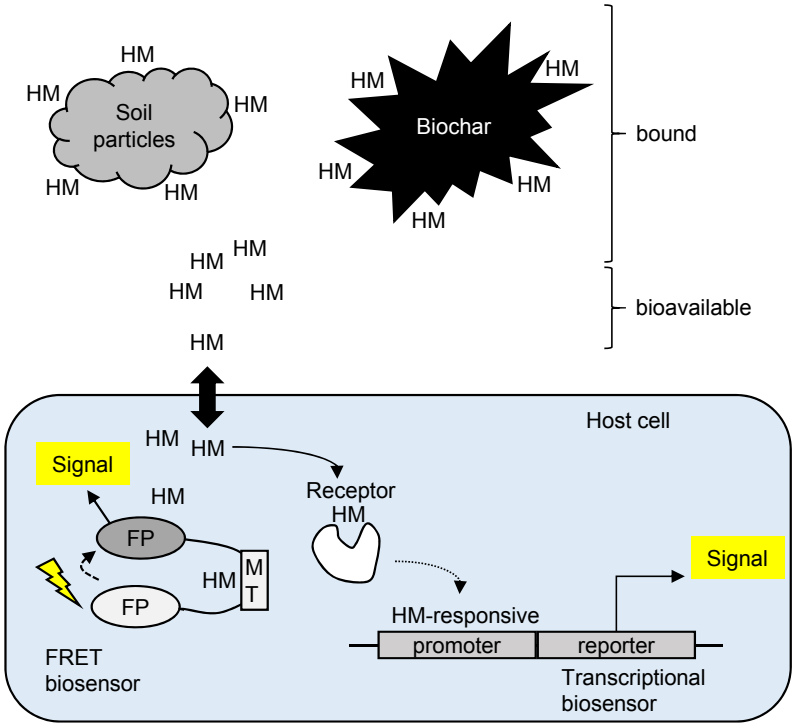
- **Efficient cloning method**
  - ✓ The properties of biosensors can be changed easily for specific contaminants by changing the properties of binding protein



- **Produce biosensors for different applications**
  - ✓ The production of biosensors tailored to specific applications
    e.g utilisation of wide range of binding activity of MT → application for heavily contaminated soil

DISCUSSION

- **Biosensors Application**
  - ✓ **Monitoring Tools to determine the bioavailable contaminants level due to the effect of biochar amendments**



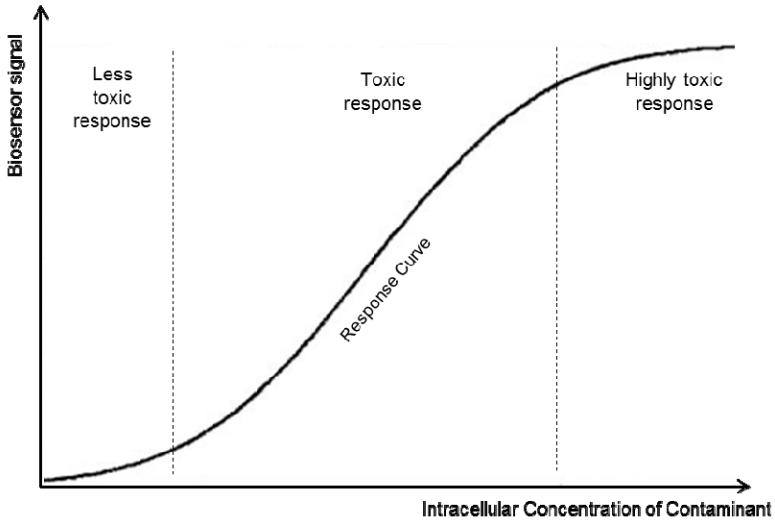
Signals

Hypothetical Response Curve of soil contaminant toxicity

Signal intensity

VS

Intracellular Concentration of contaminant



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DISCUSSION

Inspiration

- **Biochar-Amended contaminated soil for soil restoration purpose**

Biochar

Contaminated Soils

Biochar Amended Contaminated Soil Complex

Treated Soil

Reuse

High bioavailable contaminant (Toxic)

Monitoring by biosensors

Integrated monitoring with chemical, physical analysis and plant bioavailable contaminants

Low bioavailable contaminant (Less toxic)

Integration of biosensor during monitoring will help to assess the soil remediation performance more precisely

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CONCLUSION

Inspiration

1. **FRET biosensors are expected to give rapid measurement of the change in bioavailable contaminants and soil toxicity**

2. **Efficient cloning methods will allow the development of biosensors for different applications**

3. **Biosensors are expected to help identify conditions that enhance soil microbial function and plant growth after biochar amendments.**

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THANK YOU