5th International Conference on Sustainable Solid Waste Management

Assessment of Pb accumulation in roots of fast-growing trees inoculated with endophytic bacteria: Hydroponic culture

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Presentation outline

Introduction: Research background & Objectives

Methodology: Inoculation & Hydroponic test

Results & Discussion: Colonization

Pb accumulation

Conclusion: Fast-growing trees assisted endophytic bacteria

Introduction

- Research background
- Objective

Why Pb must be removed ?

- Soil contamination by Pb is one of the major environmental problems in the global scale.

Pb data

- ✓ It is the **most widespread** contaminant in soils.
- ✓ It is **extremely toxic** to all organisms even at **low concentrations**.
- ✓ It is **non-biodegradation** & can **persist** in the environment for **150-5,000 years.**

http://thaipublica.org

http://kanchanapisek.or.th

Fahr et al., (2013). Frontiers in Plant Science, doi: 10.3389/fpls.2013.00175.

What is the suitable method ?

Conventional method

- ❑ Very expensive
- ❑ Not eco-friendly
- ❑ Sludge Production

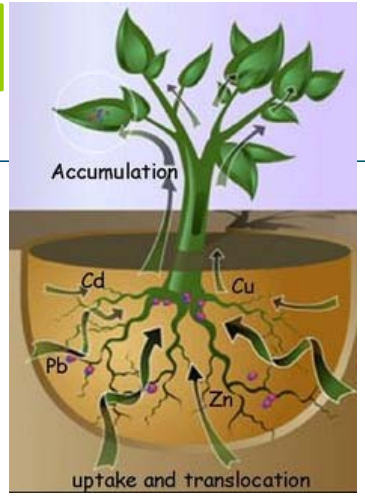
Phytoremediation

- ❑ Cheap
- ❑ Eco-friendly
- ❑ Bioenergy

Using PLANTS to remediate Pb from the environment.

Plants are a KEY FACTOR to succeed.

PLANT SELECTION is very important work.



Ali et al. 2013. *Chemosphere*, 91, 869–881.

http://www.pflanzenphysiologie.uni-bayreuth.de

Why fast-growing trees are interesting ?

- Tools for phytostabilization
- High biomass with deeper & more root systems
- Leaf fall, dead roots, & root exudates improve soil physical characteristics
- Large amount of water removed from soil by transpiration decreases the downward flow through the soil, reducing leaching losses
- Economic value as firewood with high heating value

Environmental Sustainability & Economic Gain

Pulford & Watson. Environ Int 29:529-540 (2003)
Wang et al. PLoS ONE 9(9): e108568. doi: 10.1371/journal.pone.0108568 (2014)
Liu et al. J Zhejiang Univ-Sci B (Biomed & Biotechnol) 16:123-130 (2015)

A. mangium & *E. camaldulensis*

- They are plant candidates
  - High heating value with planting for 2 years
    - *A. mangium* => 4,900 cal/g
    - *E. camaldulensis* =>4,800 cal/g

Department of Alternative Energy Development and Efficiency, Thailand.

  - High Pb accumulation in roots
    - *A. mangium* => 49004.36±6148.98 mg/kg
    - *E. camaldulensis* => 40597.72±2694.13 mg/kg

Constraints of Fast-growing trees for phytoremediation

- Tree accumulate low amounts of heavy metals
- Pb has the least bioavailable form
- Low tolerance

Ali et al. Chemosphere 91, 869-881 (2013)
Yanqun et al. Environ. Int. 30, 567-576 (2004)

They need to be developed

By

ENDOPHYTIC BACTERIA

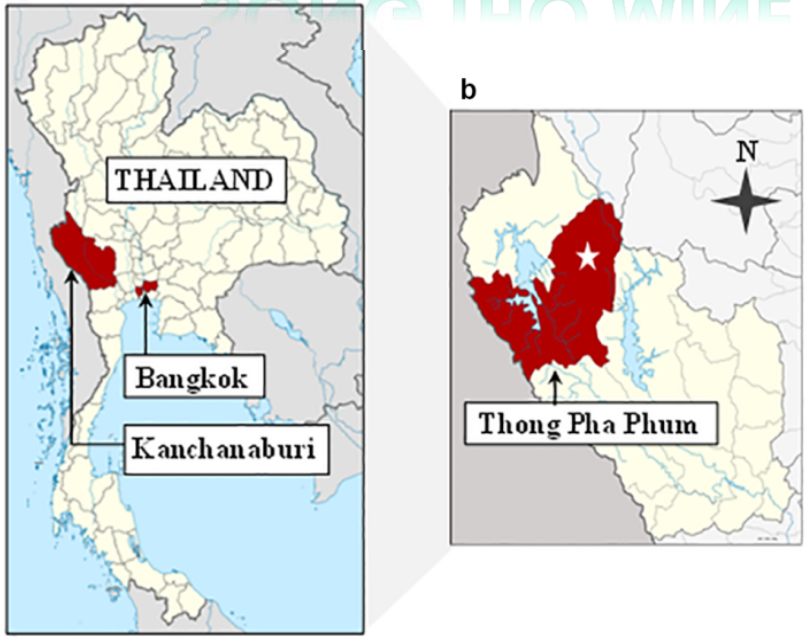
Endophytic bacteria's role in phytoremediation

- Improves growth
  - Atmospheric nitrogen fixation
  - Production of phytohormones & siderophore
  - Solubilisation of phosphorus
  - Synthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase
- Enhance heavy metal solubilisation & bioavailability
- Reduce toxicity

Navarro-Torre et al. Marine. Pollut. Bulletin. 110, 133-142 (2016)
Santoyo et al. Microbiol. Res. 183, 92-99 (2016)

How to get the promising endophytic bacteria

SONG THO MINE



Metallophyte

Pityrogramma calomelanos

Extraction, Isolation, Characterization & Identification

Pseudomonas psychrophila

Pseudomonas psychrophila

- Properties
  - Plant growth promoting traits
    - Produce siderophore
    - Solubilize inorganic phosphate
  - Pb mobilization in the soil (2.23 mg/kg)
  - Pb solubilization in solution (18.23 mg/L)
  - High Pb tolerance (1,875 mg/L of Minimum Inhibitory Concentration).

Objective

To test the effect of *P. psychrophila* inoculation on Pb accumulation of *A. mangium* & *E. camaldulensis* by hydroponic test

Methodology

- Hydroponic test
- Pb analysis

Method: Hydroponic test

- Plants & bacteria preparation
- Inoculation by pruned-root dip
- Pb removal test
- Pb analysis
- Recovery extraction

Plants & Bacteria preparation

1. Fast-growing trees

- ❑ *A. mangium*
- ❑ *E. camaldulensis*

↓

Acclimatization 7 days

↓

Clean

↓

30-50% of roots were cut

2. Endophytic bacteria

P. psychrophila

↓

Inoculum preparation

↓

Centrifugation

↓

Resuspend

↓

10^8 CFU/ml cell suspension

Bressan & Borges BioControl. 49, 315-322 (2004)

Inoculation: Pruned root dip

1. Add 10% of cell suspension (10^8 CFU/ml) in 300 ml of 25% modified Hoagland's solution

2. Pruned-root plants was submerged in solution for 48 h

Trees associated bacteria

Pb removal test: Batch experiment

1. All treatments were cleaned & added in 0 & 30 mg Pb/L in Hoagland's solution for 15 days,
2. [Pb] in roots were analyzed by FASS

Pb Analysis

1. Plant preparation

- Roots were cleaned
- Dried @ 60°C for 3 days
- Ground & Sieved

2. Digestion

- 0.5 g sample + 10 mL HNO_3 in digestion block
- Clear solution

3. Pb analysis

- Solutions were filtrated
- [Pb] was analyzed by FAAS: 217λ, 10 mA

Recovery extraction

- 1. Roots surface disinfection
  - Clean with tap water → 70% ethanol: 40 s
  - 2.5% NaOCl, + 1 drop of Tween 80 : 15 min
  - Rinsed 3 times: sterile dH_2O
  - No colony = success → *P. psychrophila* become the true endophytic bacteria
- 2. Extraction
  - Ground roots (0.5 g) with a sterile mortar & pestle
  - Mix with (5 ml of 0.85% NaCl) to make plant slurry
- 3. Isolation
  - Spread plates on LB+20 mg Pb/L agar & Incubate (30°C, 2 d)

Luo et al. Chemosphere, 85, 1130-1138 (2011)
Shin et al. J. Hazard. Mater. 199-200, 314-20 (2012)

Results & Discussion

- Colonization
- Species identification
- Pb accumulation

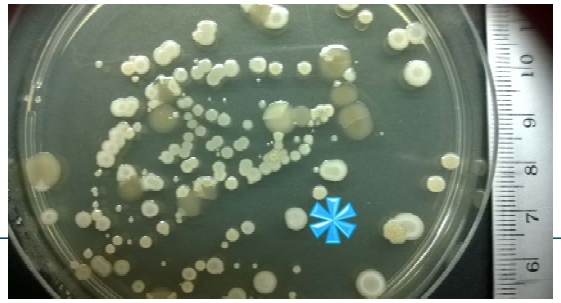
Successful Colonization

Pure PC

E. calmadulensis (cont)

E. calmadulensis + PC

A. mangium (control)



A. mangium + PC

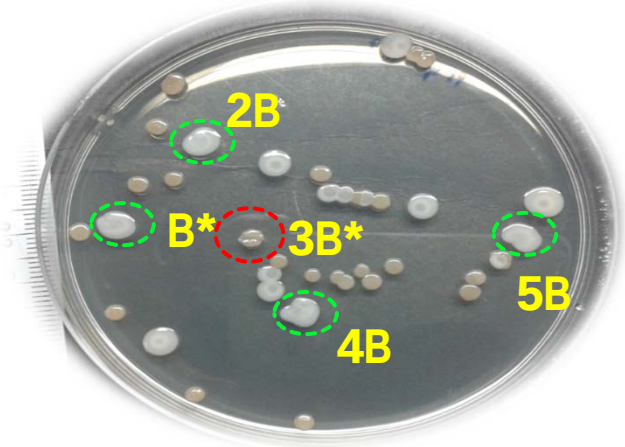
Survival under metal stress is very important factor to produce the beneficial substances for the activity of endophytic bacteria

Ma et al. J. Hazard. Mater. 196, 230-237 (2011)

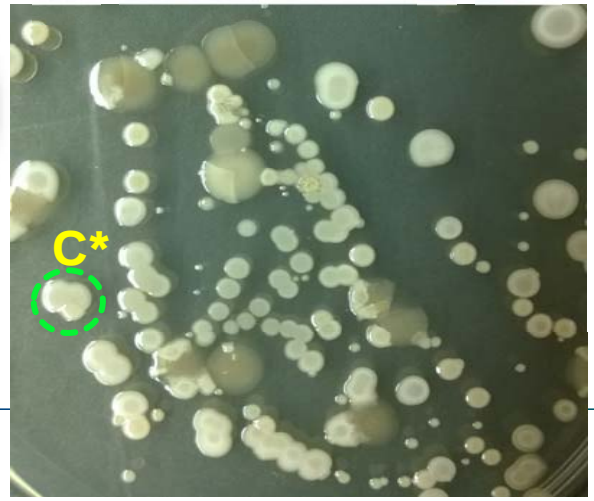
Species identification 16S rRNA gene

Pure PC

***E. camaldulensis* + PC**



***A. mangium*+ PC**



Partial of 16S rRNA gene analysis

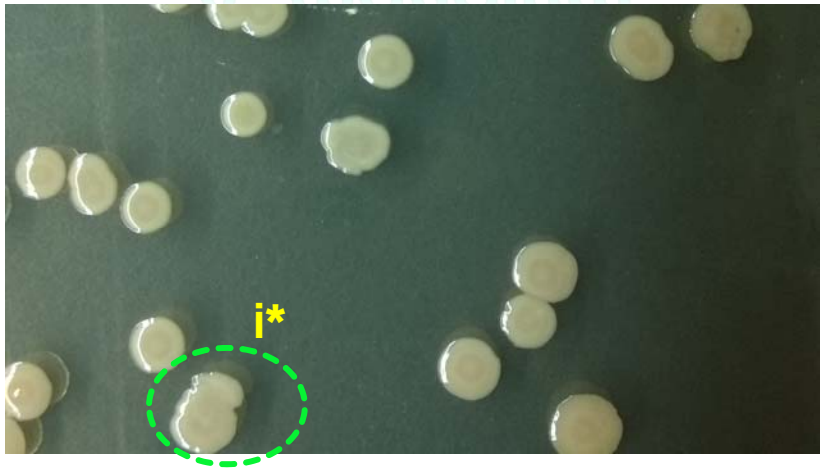
A = P. fragi
B = P. fragi
C = P. fragi

3B = *P. extremaustralis*

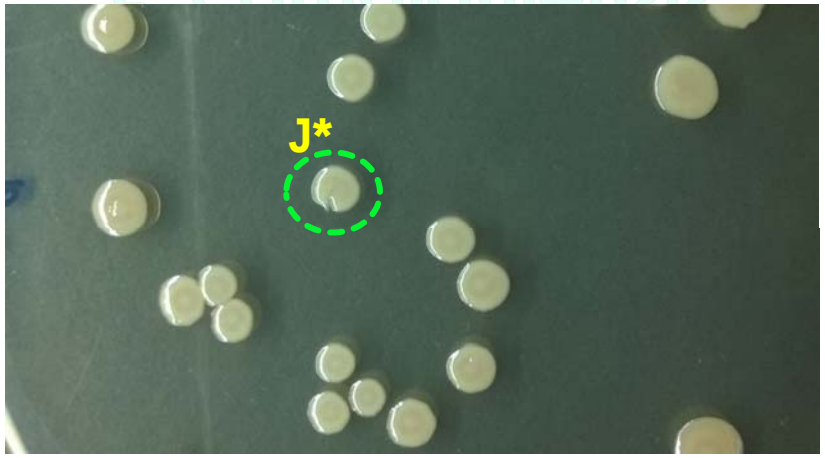
Full sequencing gene analysis =
Pseudomonas psychrophila

Species identification: cont.

A. mangium



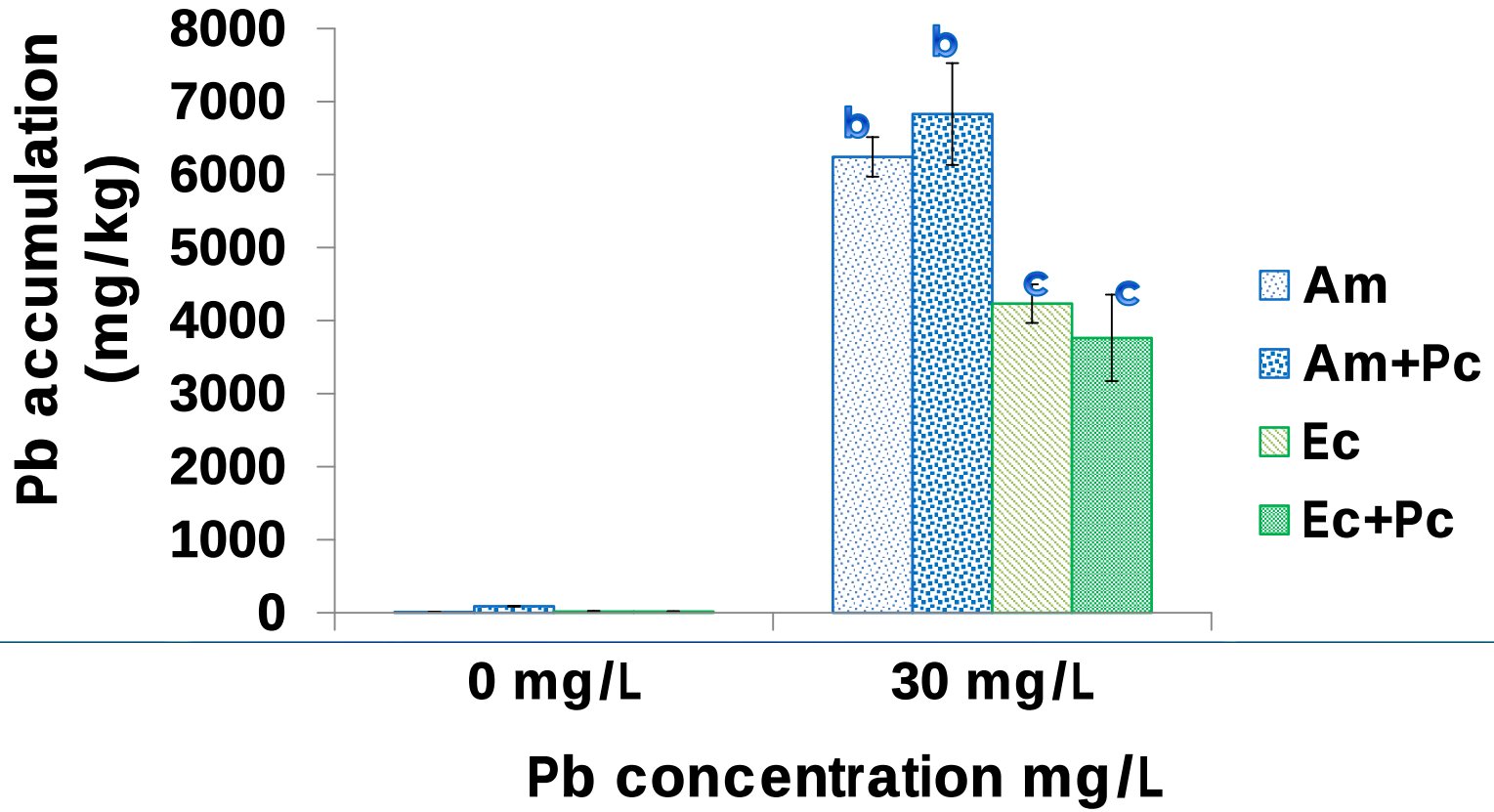
E. camaldulensis



Pure PC

Result from 16S rRNA => *I & J = P. extremaustralis*

Pb accumulation



Inoculated plants show no significant difference from non-inoculated plants

Other results

- The results of Pb accumulation are similar with other works
  - Increase in *A. mangium*
  - Reduce in *E. camaldulensis*

Due to: Bacterial mechanisms

Siderophore production & P solubilizaiton

: Effect of inoculation on metal extraction depends on plant species

Ma et al. J. Hazard. Mater. 196, 230-237 (2011) | Ma et al. J. Environ. Manage. 174, 14-25 (2016)
Li et al. Fungal Divers. 54, 11-18 (2012) | Xu et al. Environ. Sci. Pollut. Res. 22, 5070-5081 (2015)
Madhaiyan et al. Chemosphere. 69, 220-228 (2007)
Sessitsch et al. Soil Biol. Biochem. 60, 182-194 (2013)

Conclusion

- Key knowledge
- Suggestion

The key knowledge gained

- ***P. psychrophila*** **seemed to be effective in promoting the phytoremediation potential of** ***A. mangium***

!!! BUT !!!

It was not useful for Pb phytoremediation of *E. camaldulensis* based on reduction of Pb content in roots.

Suggestion

Their phytoremediation potential needs to be further investigated by pot experiment.

Acknowledgements

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THANK YOU
&
Question