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

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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.

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.


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

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

- 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


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

How to get the promising endophytic bacteria

**Introduction**

Pityrogramma calomelanos

**Extraction, Isolation, Characterization & Identification**

Pseudomonas psychrophila
Introduction

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

1. Plants & bacteria preparation
2. Inoculation by pruned-root dip
3. Pb removal test
4. Pb analysis
5. Recovery extraction
Plants & Bacteria preparation

1. Fast-growing trees
   - A. mangium
   - E. camaldulesis

   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

**Inoculation: Pruned root dip**

1. Add **10% of cell suspension** \(10^8\text{ CFU/ml}\) in 300 ml of 25% modified Hoagland’s solution

2. Pruned-root plants were 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. \([\text{Pb}]\) in roots were analyzed by FASS
1. Plant preparation
- Roots were cleaned
- Dried @ 60°C for 3 days
- Ground & Sieved

2. Digestion
- 0.5 g sample + 10 mL HNO₃ in digestion block
- Clear solution

3. Pb analysis
- Solutions were filtrated
- [Pb] was analyzed by FAAS: 217λ, 10 mA
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 dH₂O
   - No colony = success

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/Lagar & Incubate (30°C, 2 d)

*P. psychrophila become the true endophytic bacteria*

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*References*

Luo et al. Chemosphere, 85, 1130-1138 (2011)
Results & Discussion

- Colonization
- Species identification
- Pb accumulation
Survival under metal stress is very important factor to produce the beneficial substances for the activity of endophytic bacteria.

Species identification
16S rRNA gene

Introduction
Methodology
Results & Discussion

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
Result from 16S rRNA => i & J = P. extremaustralis
Inoculated plants show no significant difference from non-inoculated plants.
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 solubilization

Effect of inoculation on metal extraction depends on plant species

Madhaiyan et al. Chemosphere. 69, 220-228 (2007)
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.

Their phytoremediation potential needs to be further investigated by pot experiment.
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THANK YOU & Question