The importance of symbiosis of *Mesorhizobium* with *Astralagus sinicus* in the bioremediation of cadmium in organic soils

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1. Introduction

A **heavy metal** is a member of a group of elements not very well defined that exhibits metallic properties. This group mainly include transition metals, some semimetals, lanthanides and actinides. Many different definitions have been proposed based on density, some on atomic number or atomic weight, and some on chemical properties or toxicity. Unlike many other pollutants, heavy metals are difficult to remove from the environment.

Cadmium (Cd) is a heavy metal that produces toxic effects in living organisms, even in very small concentrations. The major sources of cadmium are: air emissions, the use of phosphate fertilizers, sludge from wastewater used in agriculture and accidental pollution. Exposure to cadmium causes the Itai-Itai disease in humans

Bioremediation refers to the process by which living organisms are used to reduce or eliminate environmental hazards resulting from the accumulation of toxic waste and other hazardous compounds [1]

2. Objectives

I. To define “heavy metal”.

II. To understand the importance and the problems that are due to the presence of cadmium in organic soils.

III. To know the bioremediation of cadmium in soils carrying out a symbiosis of a genetically modified *Rhizobium* (*Mesorizhobium huakuii* subsp.rengei B3) with a leguminous plant (*Astralagus sinicus*).

3. Gene constructs

Two recombinant *Rhizobium* have been designed. Each of them have the following genes:

- synthehitc tetrameric metallothionein (MTL4): metallothioneines are low molecular weight proteins rich in cysteine which bind Cd.
- phyochelatin(PCs): this gene comes from *Arabidopsis thaliana* → it inactivates thiol groups of metals

These genes are fused with two promoters: nolB and nifH. (Fig.1)

▪ nolB is very important for the expression of genes that lead to the nodule organogenesis.

▪ nifH encodes enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen assimilated by the plant [1,2]

Experimental constructions
▪ *Mesorhizobium huakuii* subsp. *rengei* B3
▪ NifH: B3(pMPnifHPCS)
▪ NifH: B3::nifHMTL4
▪ NifH: B3(pMPnolBMTL4nifHPCS)
▪ NifH. B3:: nifHMTLA4pMPnifHPCS

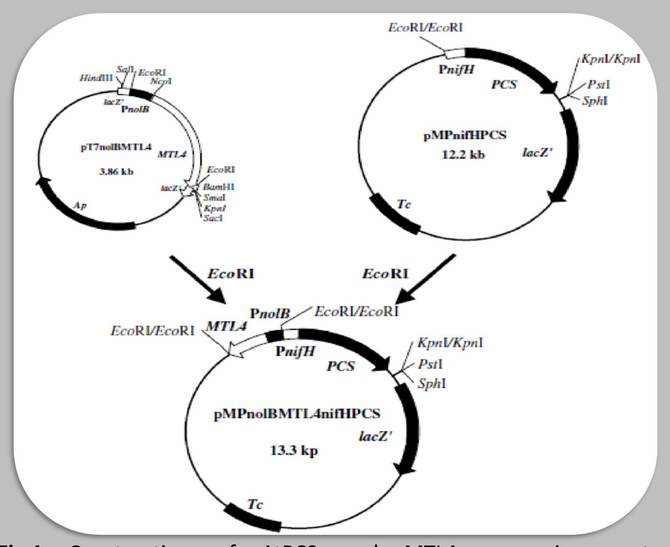

Fig.1 Construction of AtPCS and MTL4 expression vectors pMPnolBMTL4nifHPCS. Abbreviation: PnifH (the promoter of NifH), PnolB (the promoter of NolB), Ap (ampicillin resistant marker gene), Tc (tetracycline resistant marker gene), lacZ (β-galactosidase gene) [1]

4. Plant infection and immobilization of cadmium

After obtaining the construction, plant infection will take place. The infection has two stages (Fig.2)

- **Preinfection**: chemotactic attraction of the bacteria by the plant followed by the induction of structural changes in root hairs. Chemotactic attraction means that the plant exudes compounds.
- **Infection**: bacteria enter in root hairs and form channels that ramify. The cortical cells of the root start to divide to form a primary node. The infection channel is then directed toward the nodule in formation. Concurrently, *Rhizobium* are repeatedly dividing and become bacteroids which are enclosed in an peribacteroidal bacterial membrane. (Fig.3) The presence of recombinant *Rhizobium* in each node allows the expression of genes that help the sequestration of cadmium. [3]

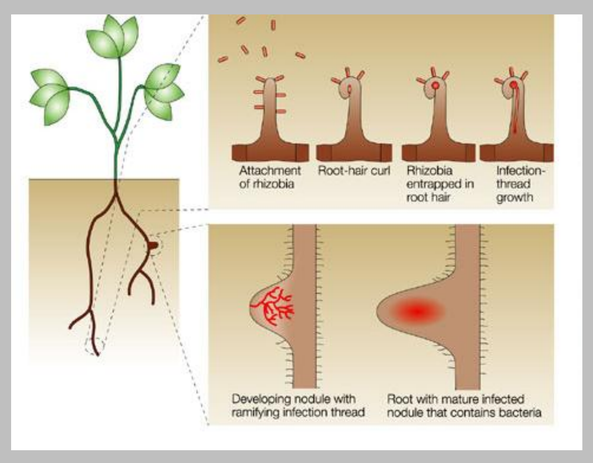

Fig.2 The early stages of the infection of root hairs by *Rhizobium* and growth of a nodule on the roots [4]

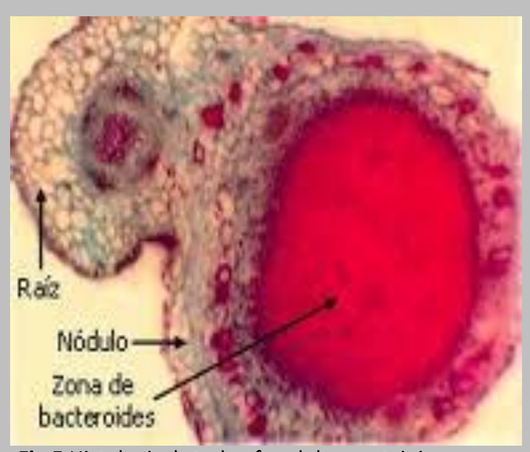

Fig.5 Histological study of nodules containing bacteroids [5]

5. Results

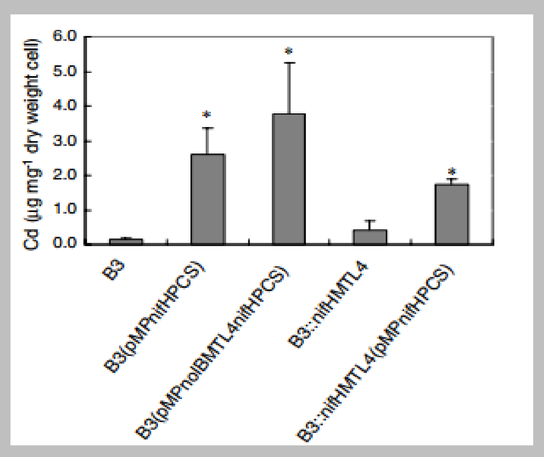

Fig.4 Accumulation of Cd in the recombinant *M. huakuii* subsp. *Rengei* B3 cells that expressed AtPCS and/or MTL4 gene. The asterisks (*) indicate that the values are significantly ($P < 0.05$) higher than those for B3. [1]

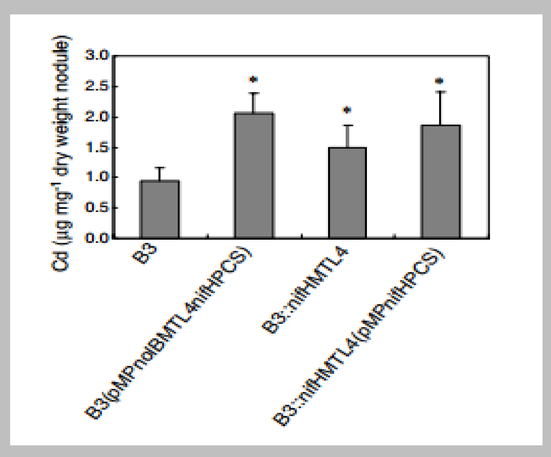

Fig.5 Accumulation of Cd in nodules containing bacteroids from B3 and recombinant strain of B3 carrying AtPCS and/or MTL4 gene. A. sinicus infected by B3 .The asterisks (*) indicate that the values are significantly ($P < 0.05$) higher than those for B3. [1]

6. Conclusions

- The accumulation of cadmium in free-living cells was shown to be significantly increased in B3 (pMPnolBMTL4nifHPCS) compared to the wild type *Rizhobium* B3 and the construction (pMnifHPCS).
- The gradual improvement of the capacity of Cd accumulation in recombinant strains either recombinant simple (a single gene) or recombinant double (with both genes) suggest that both genes binding heavy metals were successfully expressed in free-living cells.
- Nodules containing B3 (pMPnolBMTL4nifHPCS) and B3::nifHMTL4 (pMPnifHPCS) showed a higher concentration of cadmium than the wild *Rizhobium* B3. This suggests that the recombinant bacteroide contribute to a higher concentration of cadmium accumulation not only in culture, but also in the ground.
- Overexpression of MTL4 and AtPCS in *M. huakuii* subsp. *rengei* B3 appears to be a promising method to improve phytoremediation of soils contaminated with multiple heavy metals such as cadmium, mercury and zinc that form complexes with metallothionein and phytochelatin.

7. References

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