Reciprocal interactions between plants and fluorescent pseudomonads in relation with iron in the rhizosphere

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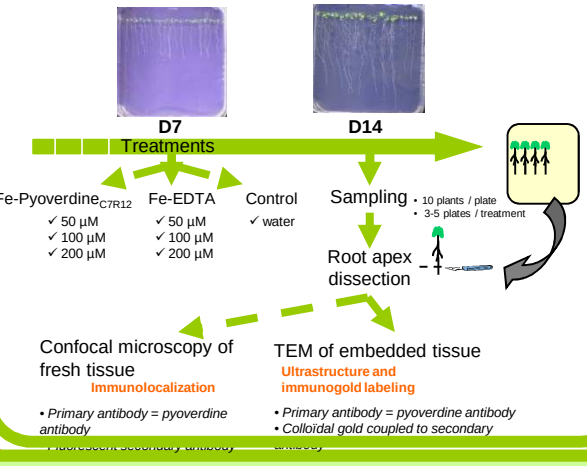
INTRODUCTION

Iron is an essential element for plants and microbes. Iron competition was demonstrated to be an important driver of the interactions between fluorescent pseudomonads and the rhizospheric microflora (Lemanceau *et al*., *in press*). To face this competition, plants and microorganisms have developed active strategies of iron uptake. In non graminaceous plants (strategy I), iron uptake relies on acidification and reduction of Fe(III) to Fe(II) which is incorporated into the roots by iron transporters (*eg*. IRT1). Active iron uptake by microorganisms relies on siderophores showing high affinity for iron.

We have previously shown that plants of *Arabidopsis thaliana* (strategy I) supplemented with Fe-pyoverdine had (i) a higher iron content than those supplemented with Fe-EDTA, (ii) iron incorporation from pyoverdine did not involve IRT1, and (iii) ^{15}N-labeled pyoverdine was incorporated *in planta* (Vansuyt *et al*., 2007). Taken together, these observations suggest that iron from Fe-pyoverdine was incorporated *in planta* not through the strategy I. In the present study, we explored possible mechanisms for incorporation of iron from pyoverdine at the cellular level.

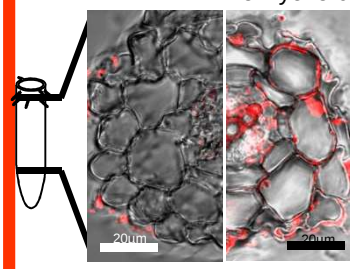
MATERIALS AND METHODS

- ✓ *In vitro* culture of *Arabidopsis* plants
- ✓ Plants cultured for seven days without any iron supplementation and for seven more days after having been supplemented with Fe-pyoverdine or Fe-EDTA or not supplemented
- ✓ Sampling of 14-day old roots for ultrastructural studies with transmission electron microscopy (TEM) and immunolocalization of pyoverdine in roots by confocal microscopy and TEM



RESULTS

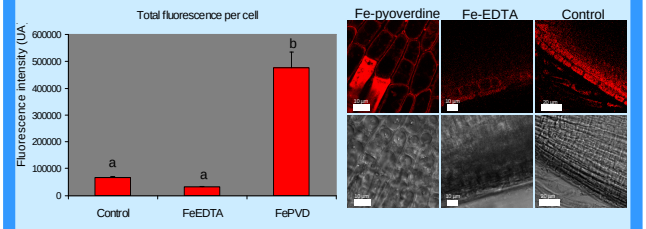
Fe-pyoverdine localization in root



Confocal sections of root labeled with pyoverdine antibody clearly indicated pyoverdine presence *in planta*. Immunolocalization revealed the presence of pyoverdine in the root apoplasmic space.

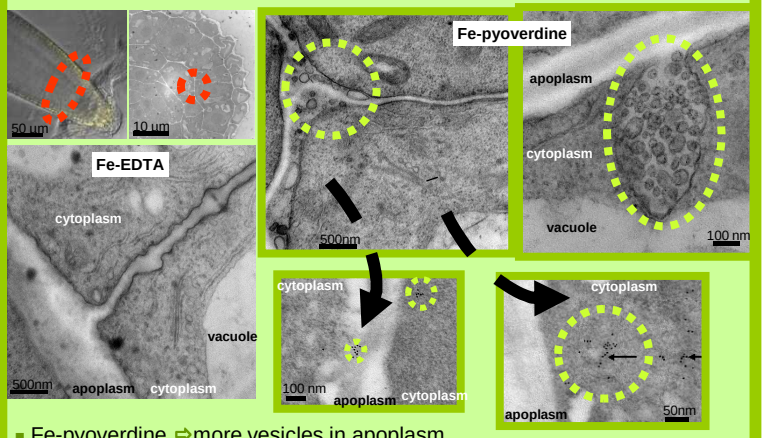
Incorporation of Fe-pyoverdine by endocytosis ?

➢ Monitoring endocytosis by uptake of the endocytosis marker FM4-64 might indicate that incorporation of Fe-pyoverdine relies on endocytosis



Letters indicate values significantly different as measured by the Fisher's least significant difference test (P<0.0001)

Ultrastructural analysis of root cells supplemented with Fe-pyoverdine, Fe-EDTA or not supplemented

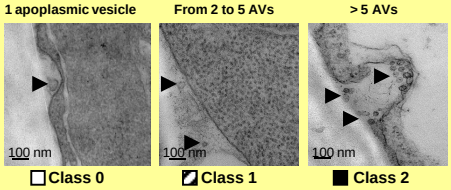


- Fe-pyoverdine ⇨more vesicles in apoplasm
- Presence of immunogold-labeled pyoverdine in cytoplasm and apoplasm
- Not-supplemented did not differ from Fe-EDTA

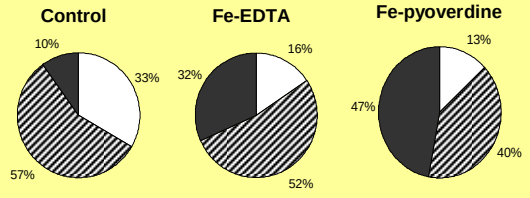
CONCLUSION

Observations and quantification with TEM showed a more abundant presence of vesicles in the root apoplasm of plants when cultured with Fe-pyoverdine than with Fe-EDTA. However pyoverdine immunogold labeling of root sections was not sensitive enough to allow the possible detection of pyoverdine in the vesicles. Altogether, these data confirm the acquisition of iron from Fe-pyoverdine by *A. thaliana* and suggest that iron incorporation from Fe-pyoverdine could be related to endocytosis. Further experimental proof is required to determine if the increase of vesicles in the presence of pyoverdine mediates that process.

Quantification of apoplasmic vesicles in root cells



For each sectioned cell, apoplasmic vesicles (AVs) were classified into three classes and results are expressed in percentage of cells exhibiting 1, 2-5 and >5 AVs per cell



References

Vansuyt G. *et al.*, 2007. Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*. Mol. Plant-Microbes Interact. 20:441-447.

Lemanceau P. *et al.*, 2009. Iron dynamics in the rhizosphere as a case study for analysing interactions between soils, plants and microbes. Plant Soil. 321:513-535.

Lemanceau P., *et al.* Role of Iron in Plant–Microbe Interactions. Advances in Botanical Research, *in press*.

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