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Siderophore affect iron mobility in soil: evidences of iron oxydes dissolution

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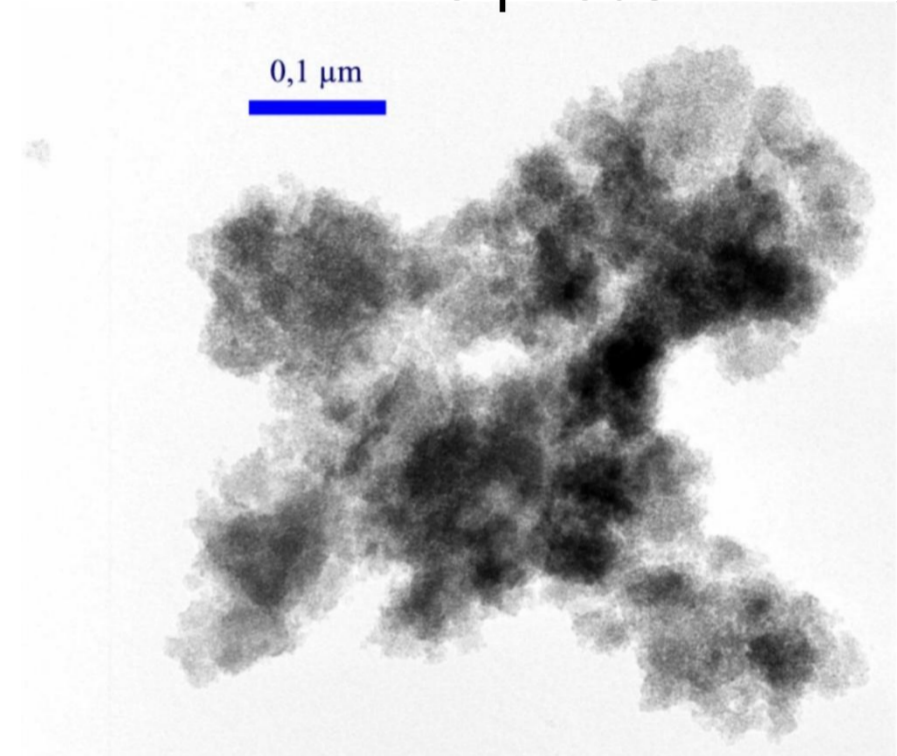
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Objectifs : Iron is an essential element for bacterial growth and metabolic processes. In soil and sediments, iron concentration is low under aerobic conditions because the solubility is controlled by stable hydroxides, oxyhydroxides and oxides. To get access to this crucial element, bacteria produce in their environment siderophore, a high affinity Fe³⁺ chelator. A previous work showed that pyoverdine, the main siderophore produced by fluorescent *Pseudomonas*, was able to promote efficiently iron dissolution of a poorly crystallized ferrihydrite, while the dissolution of the well crystallized goethite was lower. In the present work, we focused on the role of pyocheline on ferrihydrite and goethite alteration process. Moreover, we investigated if both iron acquisition systems, pyoverdine and pyocheline pathway were activated when bacteria were grown in the presence of these iron oxides.

MINERALS

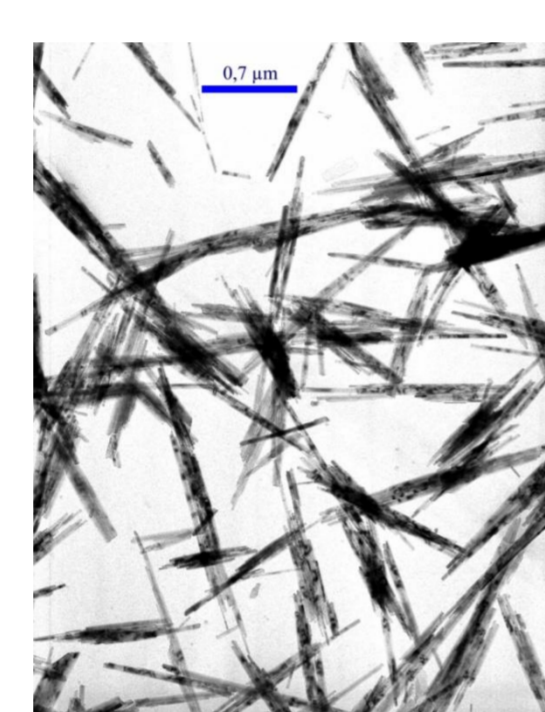
Ferrihydrite

Amorphous



Goethite

Well crystallized



Electron micrographs of synthetic ferrihydrite 2-lines and goethite Iron oxides were prepared according to the methods of Schwertmann and Cornell 1991. A goethite substituted with aluminium was prepared.

PSEUDOMONAS AND SIDEROPHORES

◆ **Bacteria:** fluorescent *Pseudomonas* (*P. aeruginosa* ATCC 15692)

→ a mutant FptX:YFP from O. Cunrath was used

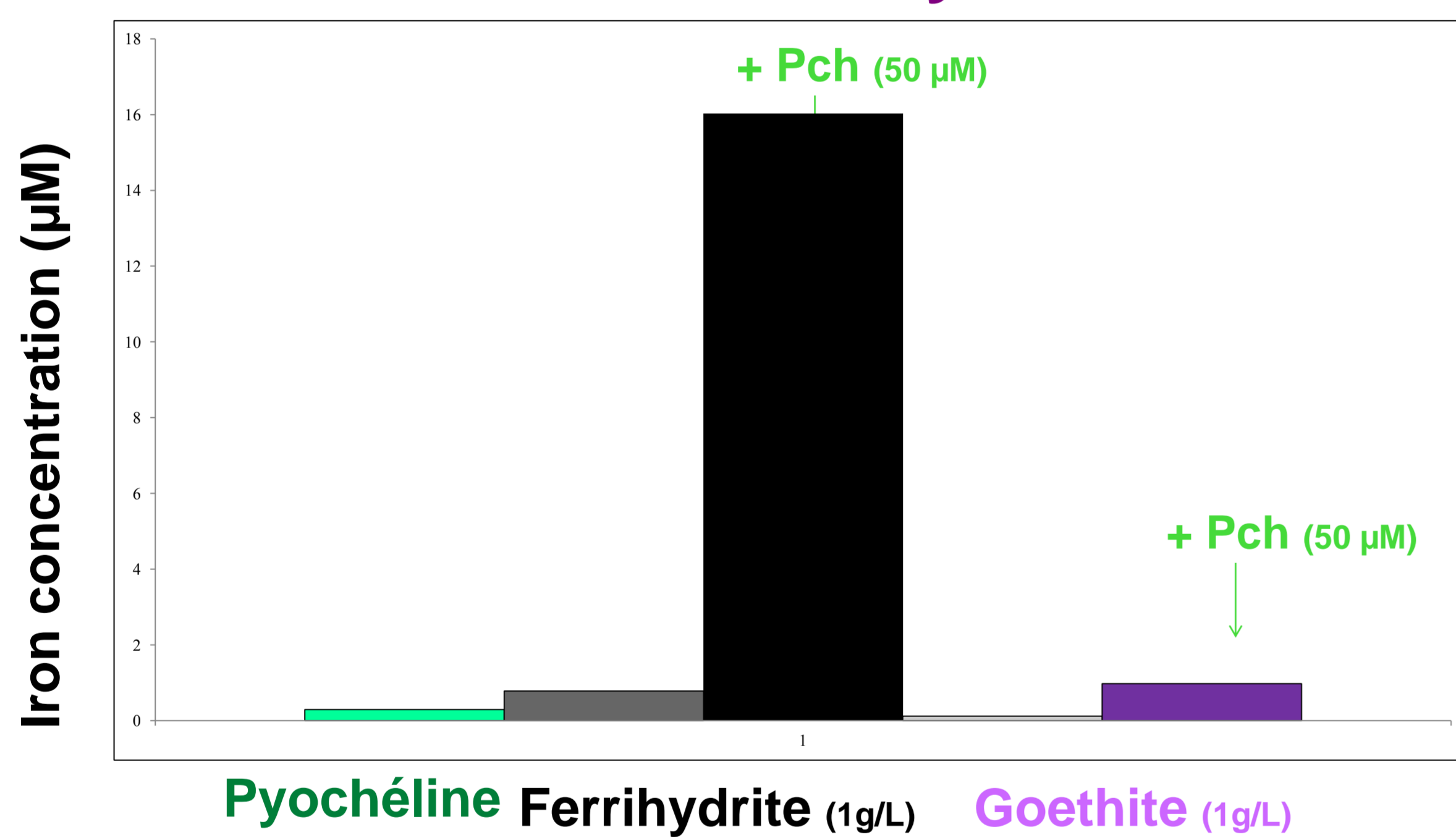
◆ Siderophore produced: pyoverdine (Pvd) and pyocheline (Pch)

◆ **Pyocheline purification**

Pyocheline was extracted from a 1L culture of ΔPvd *P. aeruginosa* strain incubated in succinic minimum medium during 27h at 30°C/200 rpm. After acidification of the supernatant, extraction of pyocheline was proceeded with dichloromethane. After evaporation, pyocheline was resuspended in methanol. Pyocheline was incubated during 1H with the mineral in Tris 50 mM pH8 buffer. Iron concentration was determined by ICP-AES.

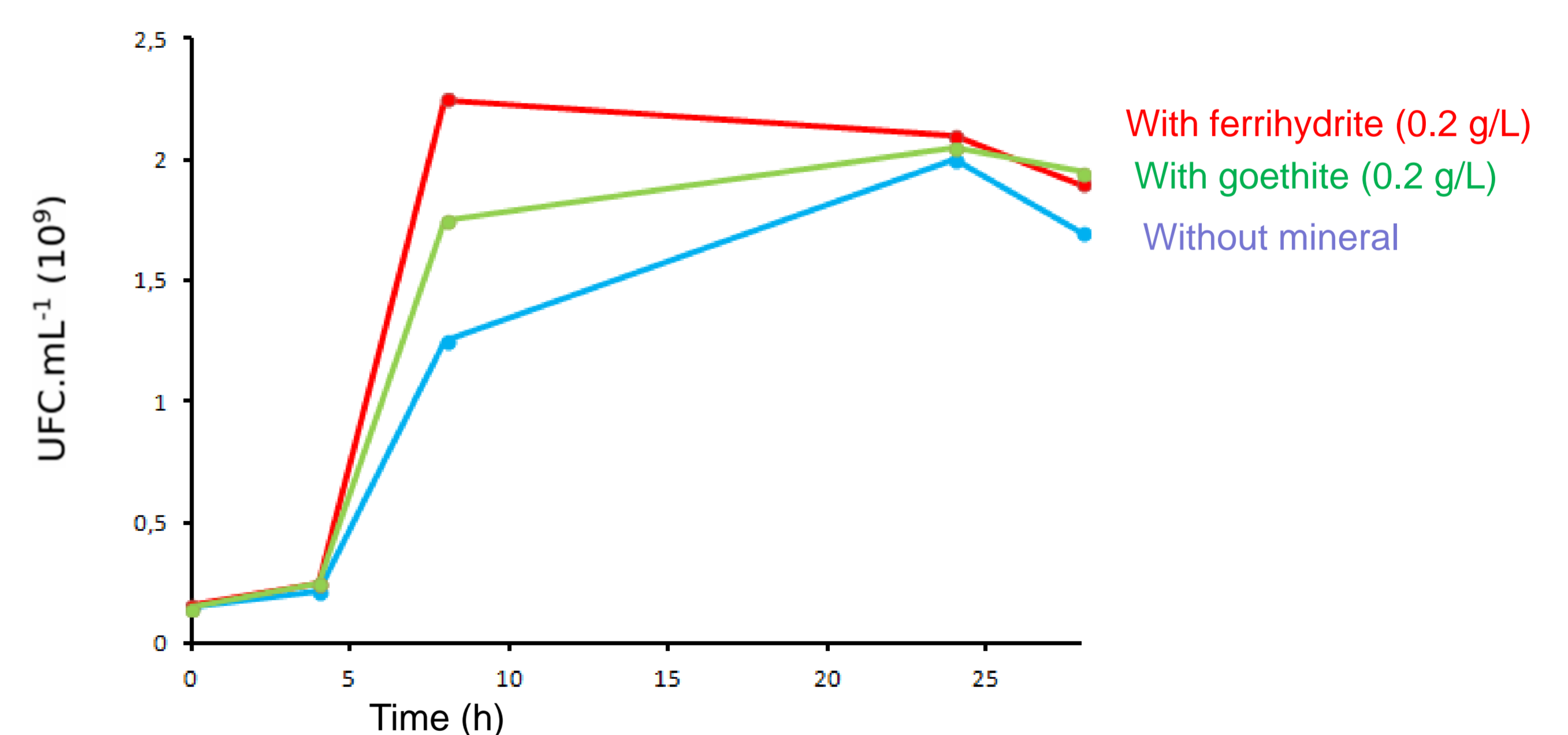
RESULTS

Effect of pyocheline on iron release from iron oxydes



In the presence of pyocheline, iron concentration increased in solution. The amorphous iron oxyde, ferrihydrite, was more easily weathered by pyocheline than the well-crystallized oxyde, goethite.

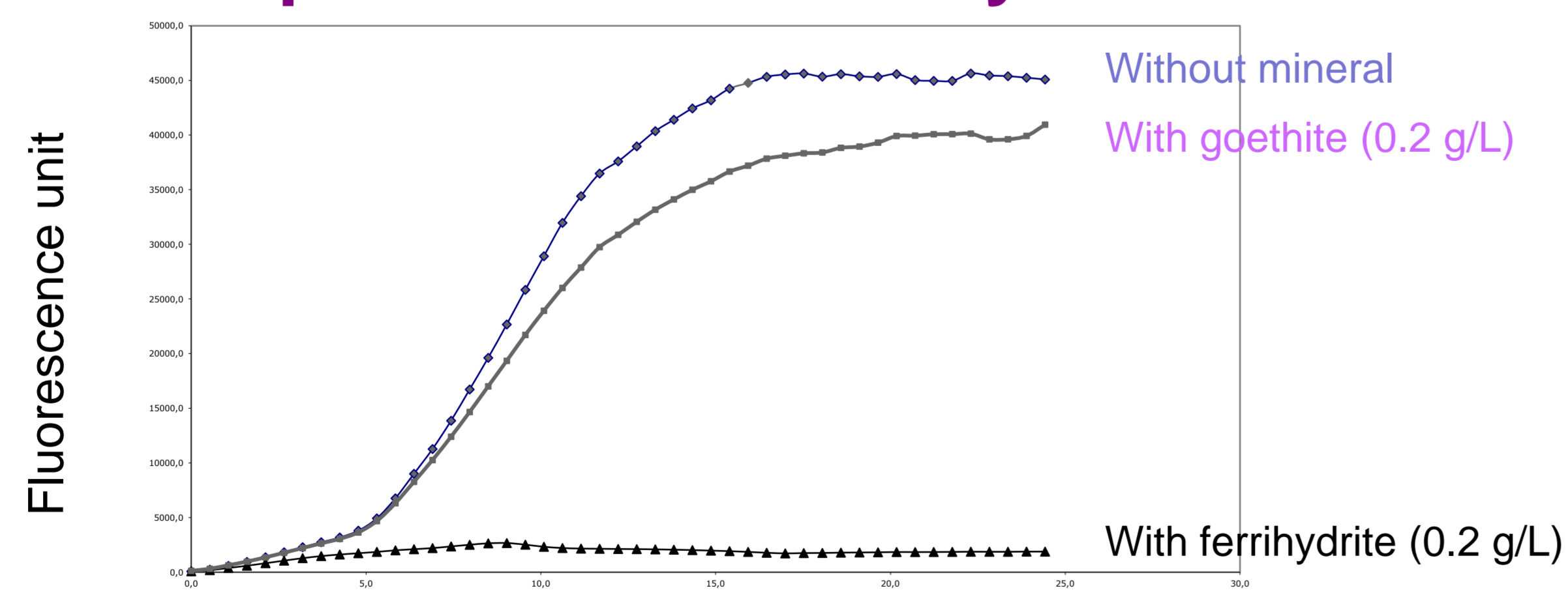
Bacterial growth in the presence of iron oxydes



Bacterial number was enumerated by plating on LB agar, incubation at 30°C/24H.

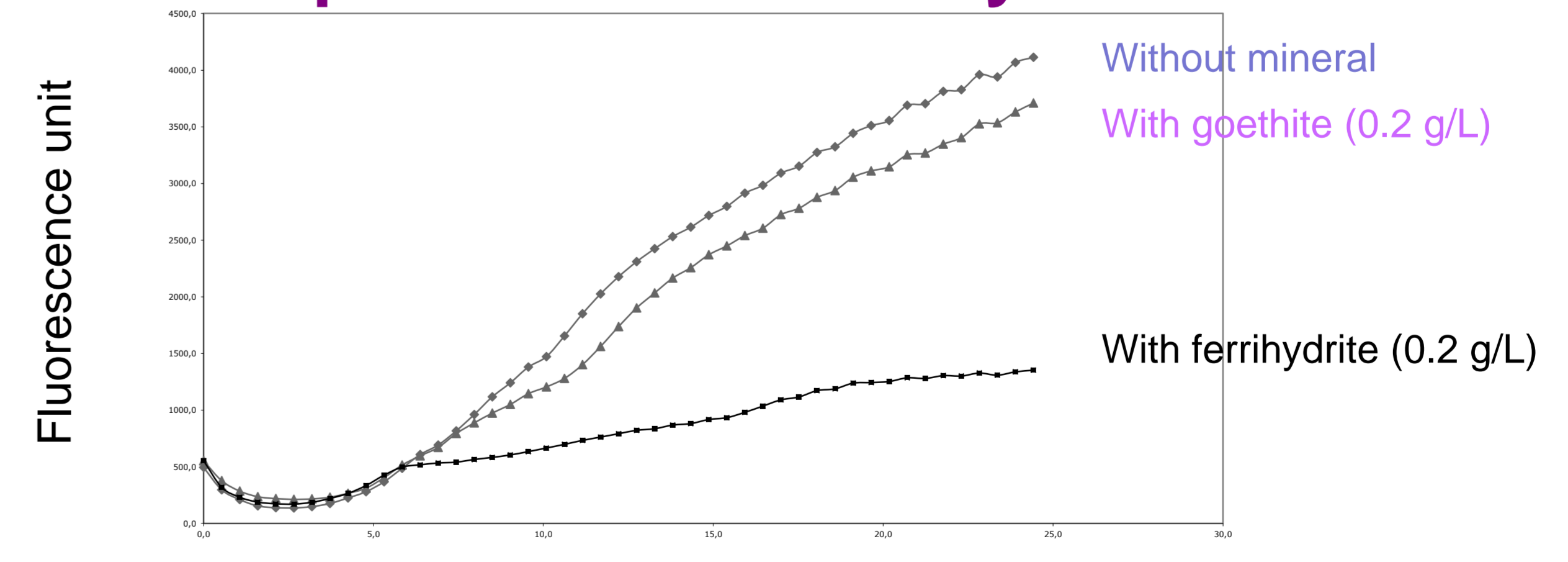
In the presence of minerals, the bacterial growth was stimulated particularly after 8h of growth.

Fluorescence of pyoverdine in the presence of iron oxydes



In the presence of ferrihydrite, the fluorescence of the pyoverdine produced was abolished by the iron released from the iron oxyde, while the fluorescence was only slightly reduced in the presence of the well-crystallized oxyde, goethite.

Induction of the pyochelin pathway in the presence of iron oxydes



In the presence of goethite, FptX, the inner membrane Pch-Fe importer, was highly expressed, while a low level was detected in the presence of ferrihydrite.

Conclusions and perspectives

In conclusion, pyocheline was able to weather more easily ferrihydrite, the amorphous iron oxides than the well cristallized goethite, as we already shown for the pyoverdine siderophore. The iron solubilisation from the iron oxides explained the growth stimulation and siderophore repression observed with *Pseudomonas*. Interestingly, it seemed that the pyocheline pathway was induced only in the presence the goethite, the hardest iron source to dissolve. Further investigations are in process to better understand the interaction between bacteria and minerals. All these data contribute to a better understanding of the iron uptake mechanism in *Pseudomonas aeruginosa* involved in iron acquisition in soil.