LA-UR- 03-0913

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Submitted to:	PLUTONIUM FUTURES CONFERENCE

Los Alamos
NATIONAL LABORATORY

Form 836 (8/00)

Siderophore Production and Facilitated Uptake of Iron and Plutonium in *P. Putida*

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Introduction

Bioremediation is a very attractive alternative for restoration of contaminated soil and ground water. This is particularly true for radionuclide contamination, which tends to be low in concentration and distributed over large surface areas. Microorganisms, through their natural metabolism, produce a large variety of organic molecules of different size and functionality. These molecules interact with contaminants present in the microbe's environment. Through these interactions bio-molecules can solubilize, oxidize, reduce or precipitate major metal contaminant in soils and ground water. We are studying these interaction for actinides and common soil subsurface bacteria. One focus has been on siderophores, small molecules that have great affinity for hard metal ions, and their potential to affect the distribution and mobility of actinide contaminants. The metal-siderophores assembly can be recognized and taken up by micro-organisms through their interference with their iron uptake system. The first step in the active iron transport consists of Fe(III)-siderophore recognition by membrane receptors, which requires specific stereo orientation of the Fe(III)-siderophore complex. Recent investigations have shown that siderophores can form strong complexes with a large variety of toxic metals and may mediate their introduction inside the cell. We have previously shown that a Pu-hydroxamate siderophore assembly is recognized and taken up by the *Microbacterium*

flavescens (JG-9). However, it is not clear if Pu-siderophore assemblies of other siderophores are also recognized.

Results

We have examined siderophore production by *Pseudomonas putida* strain ATCC 33015 grown under iron deficient conditions in minimal succinic media. This micro-organism produces a green fluorescent siderophore with high and specific affinity for iron(III). This siderophore belongs to the pyoverdin family, whose structures composition include a peptide chain and a fluorescent chromophore. Most pyoverdins have the same functional groups responsible for metal binding; however, the structures of the peptide chain and the chromophore vary depending one the strain and the culture media used.

We have followed the production of pyoverdin under iron deficient conditions over a period of 15 days. Although *pseudomonas putida* reaches the growth stationary phase after 24 h, the siderophore production is continued for over the 2 weeks, and most siderophore production (60 %) occurs in the first 4 days. Analysis of the purified culture media by HPLC shows the production of three different siderophores, in which the main siderophore produced (MW : 1091) represents about 70% of the total siderophore production. The two remaining siderophores have molecular weights of 1126 and 1144 and represent 25 and 5 % of the total siderophore produced, respectively. The separation and purification of the three fractions was achieved by using a C18 reverse phase column chromatography. The three fractions were eluted with a 1:1 mixture of acetonitrile/0.05 M pyridine acetic acid buffer at pH 5. The changes in the UV-visible spectrum of the main siderophore produced with pH suggest the presence of two buffer regions are

between pH 2.8 and 4.8 and one between pH 6 and 10. This result is in agreement with literature results suggesting the presence of catecholate and hydroxamate functionalities. Spectral changes upon addition of Fe, Pu and U are very similar to the changes observed in the first buffer region. However, the changes observed between pH 6 and 10 for the free siderophore are no longer observed in the presence of Fe, Pu, and U (Figure 1). This observation indicates that the hydoxamate group binds to metal ions at a much lower pH, in agreement with our previous examination of hydroxamate siderohphore metal binding.

Pyoverdin-mediated uptake of iron(III) experiments show similar results for the three siderophores produced. The experiments were followed by measuring the amount of $^{55}Fe(III)$ accumulated inside the cell as a function of time and the form of added iron. The accumulation of iron inside the cell shows specific recognition of Fe-pyoverdin discriminating other forms, such as Fe-EDTA and Fe-hydroxamates. When Fe-pyoverdin is added to the culture media, the concentration of Fe accumulated inside the cell exponentially reaching saturation after about 30 min. The amount of Fe inside the cell depends on the concentration of Fe added. However, the rate constant of the uptake process was ($k = 6.3 \times 10^{-2}$ min^{-1}) independent of the initial Fe-siderophore concentration over a 10^3 M range. When Fe-EDTA or Fe-DFO were used, the rate of Fe uptake was slow and the amount of intercellular Fe was less than 10% the amount observed with Fe-pyoverdin. We also observed that over time (>60 min) the amount of Fe incorporated inside the cell slowly increased, suggesting ligand exchange between Fe-EDTA or Fe-DFO and receptor bound pyoverdin. Although pyoverdin forms a stable complex with Pu(IV) our uptake experiments showed an insignificant accumulation of plutonium inside

the cell, suggesting that the complex formed is not recognized by Fe-pyoverdin uptake system.

Conclusions

The siderophore produced by *pseudomonas putida* binds plutonium and uranium and will certainly stabilize their IV and VI oxidation states respectively. The complexes formed are not taken up by the micro-organism, which shows that Pu-siderophore complexes are not always recognized and transported by micro-organisms.

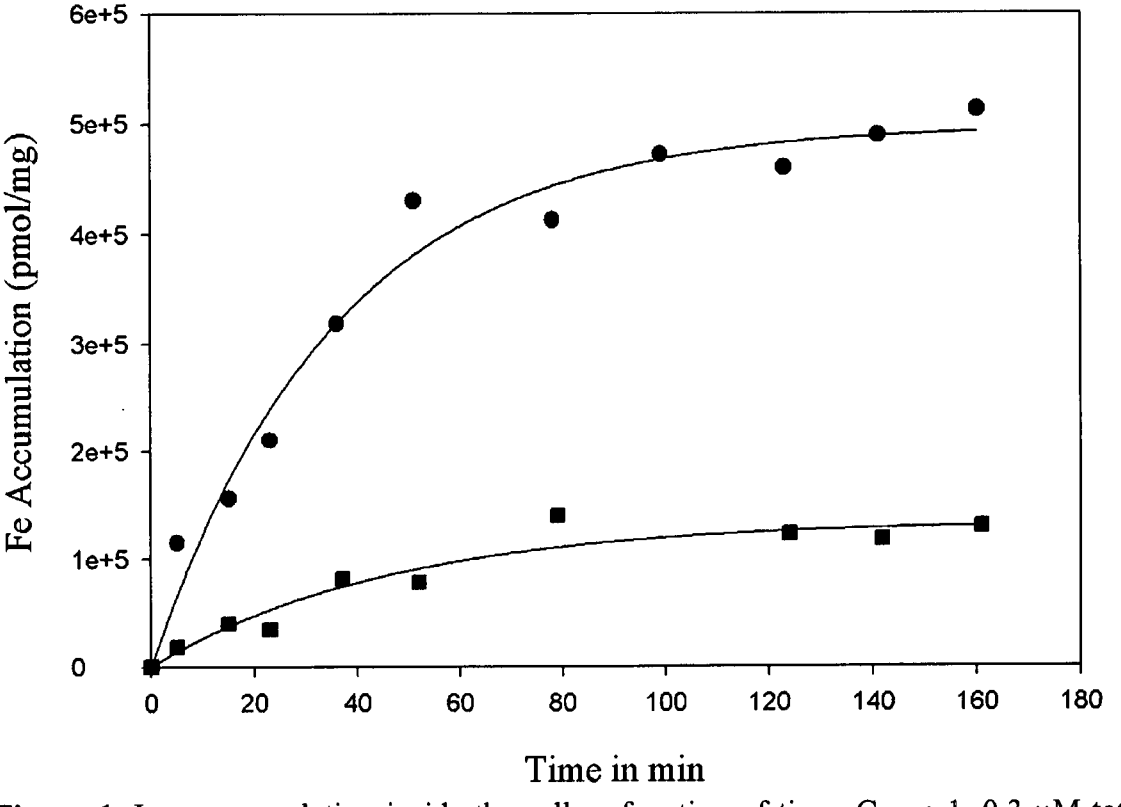

Figure 1. Iron accumulation inside the cell as function of time. Curve 1, 0.3 μM total iron added; Curve 2, 6 μM total iron added.