ESRF	**Experiment title:** Speciation of neodynium in the rhizobacteria *pseudomonas putida* and in bacterial ligand candidates	**Experiment number**: eV499
Beamline: BM30	**Date of experiment**: from: 27-10-2022 to: 01-11-2022	**Date of report**:
Shifts: 15	**Local contact(s)**: Denis Testemale	*Received at ESRF:*

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Report:

- Objective & expected results :

Extraction strategies using bacterial proteins specific to rare earth elements (REEs) have recently emerged. In this context, this project aims to study the Nd complexes formed by molecules identified in the model bacterium *Pseudomonas putida*, a versatile soil bacterium known to possess a REE-dependent metabolism. The first objective of this experiment was to acquire EXAFS spectral signature at Nd edge of a library of purified Nd complexes with differents ligands including pyoverdine, organic acids and the protein PedA2. Comparison of the spectral signature of Nd-complexes will determine the potential of EXAFS to discriminate the speciation of Nd in purified complexes. The second objective is to characterize Nd speciation in *P. putida KT2440* whole cells and compare it with the Nd speciation in the pool of soluble proteins

- Results and the conclusions of the study :

1 – Library of reference spectra (organic and inorganic compounds)

EXAFS spectra at Nd LIII-edge (6208 eV) were recorded at 15°K with a He cryostat to limit radiation damage. The k max for the EXAFS is 11.25 A^{-1} before the Nd LII-edge. A large panel of references spectra were succesfully acquiered for 8 Nd complexes formed with low molecular weight organic molecules (Nd-acetate, Nd-asparagine, Nd-citrate, Nd-gluconate, Nd-histidine, Nd-lactate, Nd-malate) and 6 inorganic compounds (Nd_2O_3, $Nd(OH)_3$, $Nd_2(CO_3)_3$, $NdPO_4$, Nd doped Goethite, Nd^{3+} from $Nd(NO_3)_3$). Organic references were prepared in water, at pH 7 with a Nd concentration of 5 g/L an organic compound/Nd ratio of 10. For the organic references, up to 6 scans were collected and merged to improve the signal/noise ratio.

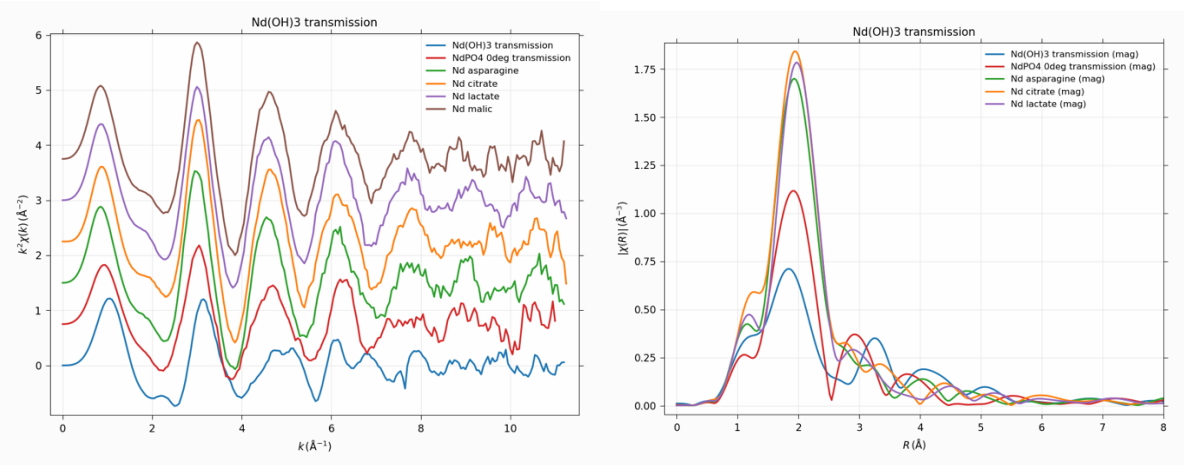

Figure 1 – Nd LIII-edge EXAFS spectra and Fourier transfom on selected references

The EXAFS spectra of the organic references did not show marked differences between the different standards below 8 $Å^{-1}$, and probably correspond to similar Nd coordination involving the carboxylate groups. However, clear differences can be observed between the EXAFS spectra of the organic coumpounds and different inorganic compounds.

2 – Purified complexes : Nd-pyoverdine, Nd-PedA2

Complexes between Nd and a siderophore known to complex Nd, the pyoverdine, were analyzed. Commercialized pyoverdine from *Pseudomonas fluorescense* and pyoverdine isolated and purified from cultures of *Pseudomonas* putida were used to form the Nd-complexes. The Nd-pyoverdine complexes were tested at pH 6 and pH 7 for the pyoverdine from *P. Putida*, to detect a possible pH induced change in Nd coordination.

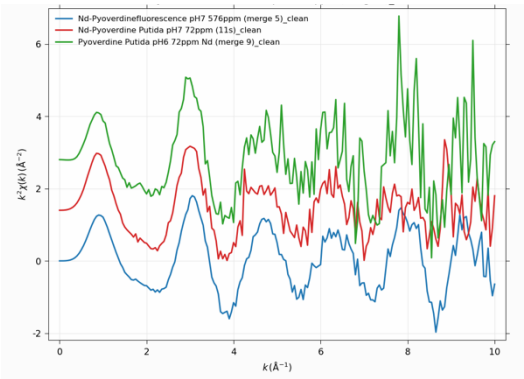

Figure 2 : Nd LIII-edge EXAFS spectra of Nd-pyoverdine from Pseudomonas Fluorescence (blue), and Pseudomonas putida at pH 7 (red) and 6 (green)

This experiment was also the opportunity to test the detection limit of Nd on this beamline. Nd concentration in pyoverdine from *P. fluorescence* and from *P. putida* was 577 ppm and 72 ppm, respectively (Figure 2). Concentrations in Nd greater than 70 ppm seem necessary to have exploitable signals and more than 10 scans recorded in fluorescence mode has to be averaged for each sample.
A Nd complex formed with the purified periplasmic protein PedA2 was also analyzed but the Nd concentration was too low to have EXAFS signal.

3 – Nd speciation in *P. putida* whole cells

The second objective of this project was to characterize Nd speciation in *P. putida KT2440* whole cells and compare it with the speciation of soluble proteins.
P. Putida were cultivated in MM9 media with 5 mM 2-phenyléthanol as carbon source, with 100µM of $NdCl_3$. Three samples were compared : 1) unwashed bacterial pellet, 2) washed bacterial pellet using EDTA, 3) soluble proteins extracted in Tris/NaCl buffer, after cells disruption by sonication. All sampled were frozen immediately in liquid nitrogen and kept frozen

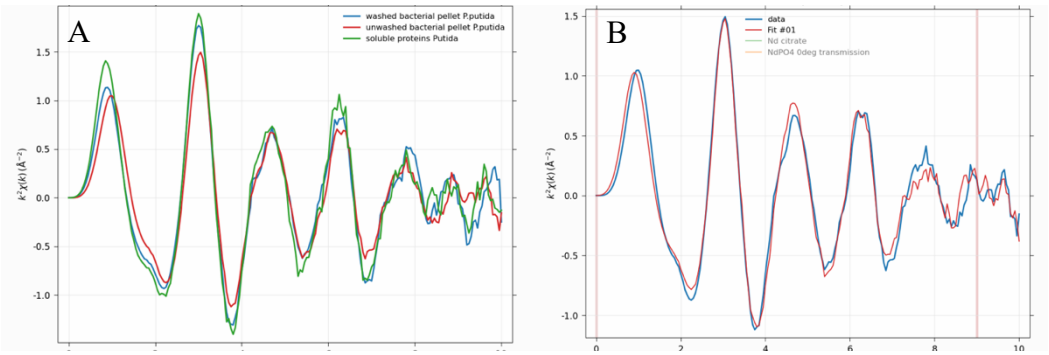

Figure 4 : A - Nd LIII-edge EXAFS spectra of bacterial pellet, unwashed (red), whased (blue) and soluble proteins extract (green). B. example of linear combinaison fitting of unwashed bacterial pellet spectrum (using 2 reference spectra (Nd-citrate complexe (Nd-organic complexe) and $NdPO_4$).

Linear combination fitting (LCF) showed two types of contribution for all the samples : Nd-organic and $NdPO_4$. For the washed bacterial pellet and proteins, best LCF is obtained with a contribution of 50% Nd-organic and 50% $NdPO_4$. LCF of the unwashed pellet indicated a higher contribution of $NdPO_4$ (Figure 5). While the fit of the unwashed pellet is good (R factor 0.03, figure 4-B), the fits of the whashed pellet and proteins are less optimal (R factor 0.05 and 0.06) suggesting missing standards to explain the signal.

The presence of $NdPO_4$ could be due to the precipitation of Nd with the phosphate from the culture media, which could be measured in the unwashed pellet. Alternately, the presence of phosphorylated groups in the extracellular polymeric substances (EPS) of *P. Putida* cell wall could explain in part the analogy with the EXAFS spectra of $NdPO_4$. It would be valuable to complete our database of Nd standards with organic phosphate compounds.

That being said, the fact that the speciation is similar between washed pellet and proteins is really interesting and may indicate that the Nd is mainly associated with proteins in the bacteria. These data have to be confirmed in complementary analyses, and varying the cultivation conditions of *P. putida*.

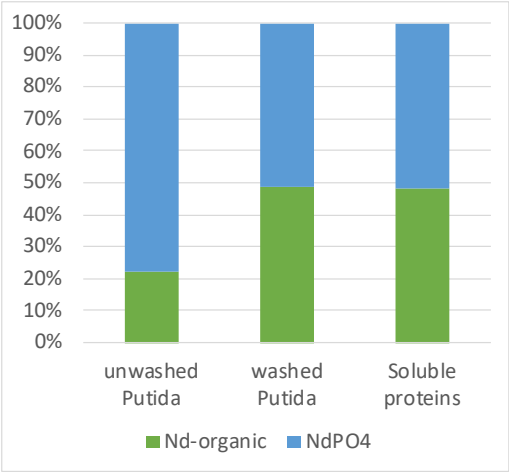

Figure 5 : Linear combination fitting results of Nd LIII-edge of EXAFS spectra : unwashed pellet of P.putida, washed pellet and extracted soluble proteins

- Justification and comments about the use of beam time :

This experiment was one of the first to collect Nd LIII-edge EXAFS spectra in organic compounds. Several shifts were dedicated to the analysis of the Nd reference compounds. A total of 14 reference spectra were acquired combining organic and inorganic compounds. Unfortunately, our database is not complete due to the lack of organic phosphate compounds. Several samples were analysed in fluorescence mode given us a better idea of detection limit for Nd, and the first results of Nd speciation in bacteria pellet and soluble proteins in *P. putida*.